Enzyme Systems that Metabolise Drugs and Other Xenobiotics.
Edited by Costas Ioannides

ISBNs: 0-471-894-66-4 (Hardback); 0-470-84630-5 (Electronic)

3 Flavin Monooxygenases

John R. Cashman

Human BioMolecular Research Institute, USA

Introduction

For many years it was assumed that the metabolism of nitrogen- and sulphur-containing chemicals, drugs and xenobiotics to their corresponding *N*-oxides and *S*-oxides was an exclusive property of cytochrome P-450 (CYP) (Cashman 1995). However, after the isolation and purification of the flavin-containing monooxygenase (FMO) from pig liver in the mid-1960s, it was apparent that FMO could catalyse the oxygenation of many nitrogen-, sulphur-, phosphorous-, selenium and other nucleophilic heteroatom-containing chemicals (Ziegler 1980). Today, it is recognised that FMO catalyses the oxygenation of numerous heteroatom-containing drugs (Cashman 1997, 2000), chemicals (Ziegler 1993) and agricultural agents (Hodgson *et al*. 1998; Hodgson and Levi 1992). In this chapter the term 'oxygenation' is used to signify a one-step two-electron substrate oxygenation by FMO as opposed to two sequential one-electron oxidations by CYP. Despite the pioneering studies of Ziegler, Hlavica (Heinze *et al*. 1970; Ziegler 1988) and others, the involvement of the FMO in drug and xenobiotic metabolism has historically been underestimated probably due to a fundamental biochemical property of the enzyme. FMO is considerably more thermally labile than most CYPs and investigations of drug metabolism that did not account for this fact invariably led to the conclusion that FMO was not important in the oxygenation of the chemical studied. Although thermal instability confounds studies with FMO, it also points to a key property of FMO that can be used to distinguish the involvement of FMO in drug metabolism.

In the 1970s, important studies describing the mechanism of molecular oxygen addition to dihydroisoalloxazines and related flavin models by Bruice (Kemal *et al.* 1977), and Balou and Massey (Ballou *et al*. 1969) and others shed considerable light on the chemical basis for FMO action. Studies of the chemistry of 4a-hydroperoxy flavins and the kinetics of reaction with nucleophiles provided insight as to how FMO could accomplish many of the same chemical oxygenations (Ball and Bruice 1983; Kemal and Bruice 1976; Doerge and Corbett 1984; Miller *et al*. 1986). Much of the

Enzyme Systems that Metabolise Drugs and Other Xenobiotics. Edited by C. Ioannides.

current understanding of the mechanism of flavoprotein and FMO catalysis was developed in the late 1970s and early 1980s.

In the mid-1980s several investigators provided evidence that multiple forms of FMO could be present in an animal (Williams *et al*. 1984; Tynes *et al*. 1985; Hlavica and Golly 1991). Thus a 'pulmonary' FMO with properties distinct from that of the 'hepatic' FMO was identified, purified and characterised. Today, we recognise that there are six forms of mammalian FMO and some can be present in multiple tissues of the same organism. The description of multiple forms of FMO was advanced by elucidation of the primary sequences by amino acid and nucleotide analysis (Hines *et al*. 1994; Lawton *et al*. 1994). While the significance of human FMOs had been recognised since the 1960s, it was not until the late 1980s and 1990s that the FMOs were characterised by purification and cDNA cloning (Lomri *et al*. 1992; Lawton and Philpot 1993; Dolphin *et al*. 1992; Phillips *et al*. 1995; Philpot *et al*. 1996).

Today, the number of human FMOs being described in the literature is expected to increase because of the ease of obtaining new sequences with the polymerase chain reaction (PCR) and the availability of the human genome sequence. Numerous allelic variants have been reported and some clinical significance has been associated with the FMO variants. The number of flavoproteins related to FMOs is also likely to increase as investigators become interested in studying FMO in other species including plants, insects and other organisms.

The physiological role of FMO is unknown. However, FMO has been suggested to have evolved to detoxicate nucleophilic heteroatom-containing chemicals and xenobiotics found in foodstuffs by converting them to polar, readily excreted, water-soluble metabolites (Ziegler 1990). FMOs have very broad substrate specificity and, barring steric limitations, accept most nucleophilic heteroatom-containing substrates for oxygenation (Cashman 1995). Recent studies have shown that FMO is capable of oxygenating several endogenous and dietary compounds with significant physiological activities such as biogenic amines (Cashman 2000). It is likely that, as more species are investigated the physiological role of FMO in cellular homeostasis will become clearer.

Nomenclature

FMO is a general definition that may include a number of flavoproteins. For the purposes of describing mammalian FMOs in this chapter, the term FMO applies to flavoproteins among different families that possess a consensus amino acid sequence equal to or greater than 50% identity, and identities among orthologous forms greater or equal to 82% Thus, numerous flavoproteins with similar functional properties (i.e. cyclohexanone monooxygenase (Ryerson *et al*. 1982), yeast FMO (Suh *et al*. 1996) that meet the criteria as a multi-substrate flavoprotein monooxygenase are excluded as an FMO because of low sequence similarity. The prefix '*FMO*' is used to designate the gene or cDNA of an FMO.

As FMOs were discovered and characterised, the common or trivial names assigned to enzymes were formalised and a system of nomenclature was adopted. The nomenclature was developed on the basis of primary amino acid sequence identity. If an FMO has an amino acid sequence identity with $\geqslant$ 82% identity it is grouped within

a family, and the family is indicated by the first numeral of the designation (i.e. 1, 2, 3 . . . .). The order of naming follows the chronology of publication of full-length sequences for each member of the family (Table 3.1). The nomenclature conforms to that approved by the Human Gene Mapping Nomenclature Committee (Dolphin *et al*. 1991). Compared with the CYP gene families, the FMO gene family is relatively simple. Allelic variants have been observed for FMO that usually possess only single base changes. Allelic variation can occur as a function of the population and possibly age and gender and can result in an FMO with altered activity. However, there are other missense, nonsense and deletion or truncation mutants of FMO that can significantly affect enzyme function and these will be discussed below in greater detail.

Gene Organization

The *FMO* genes are localised on chromosome 1q and the human *FMO* gene family may exist as a gene cluster (McCombie *et al*. 1996; Dolphin *et al*. 1997, Gelb *et al*. 1997). The general pattern of intron/exon organisation for *FMO* is assumed to be similar in various animals and humans although this has not been exhaustively examined. Evidence for multiple gene promoters and other regulatory elements have been reported for rabbit *FMO1* (Luo and Hines 1996, 1997) and rabbit *FMO2* (Shehin-Johnson *et al*. 1996). *FMO* cDNA primers can be selected to amplify certain introns on the basis that the junctional sites are conserved across gene family and across species lines. For example, the intron/exon boundaries determined for human *FMO3* (Treacy *et al*. 1998) relied on the gene structure of rabbit *FMO2* (Wyatt *et al*. 1996). After a PCR fragment was obtained and verified by sequencing, the strategy enabled amplification of human *FMO3* introns 1 and 4–8. Introns that are hard to amplify by the above approach can be derived from a consideration of the sequence available in GenBank. For human *FMO3*, sequence analysis indicated that *FMO3* had nine exons ranging in size from 80 to 705 bp. The similarity in exon/intron organisation for the *FMO* genes may suggest that the FMO family members arose from gene

Table 3.1 Summary of mammalian flavin-containing monooxygenases[a]

Designation	Trivial name	Species	Accession number
FMO1	1A1	Rabbit	M32030
FMO1	Ziegler's enzyme	Pig	M32031
FMO1	FMO-1	Human	M64082
FMO2	1B1	Rabbit	M32029
FMO2	Lung enzyme	Rabbit	—
FMO3	1D1	Rabbit	L10037
FMO3	HLFMO	Human	M83772
FMO4	1E1	Rabbit	L10392
FMO4	FMO2	Human	Z11737
FMO5	1C1	Rabbit	L08449
FMO6	—	Human	AL021026

[a]Adapted from Cashman (1995).

duplication and further mutagenesis. Diversification of the *FMO* gene presumably led to selective advantages and new function. Because FMO has been suggested to play a role in detoxicating nucleophilic heteroatom-containing foodstuffs, it is possible that FMO played a role in certain populations to process some biological natural products and protect that population. Further allelic variation of *FMO* (as described below for human *FMO3*) altering the catalytic activity and or substrate specificity could render certain individuals or populations more or less susceptible to the effects of environmental xenobiotics. Human *FMO3*, for example may be another example of an 'environmental gene'. The large allelic variation of codon 158 of human *FMO3* that approaches 50% may represent an example of a protective mechanism of 'animal–plant warfare'. It is possible that evolutionarily conserved allelic variation of human *FMO3* prevalent in certain geographical locations possessing certain plant toxins helps protect humans from plant toxin exposure (Gonzalez and Nebert 1990).

Structural aspects

The primary amino acid sequences of perhaps two to three dozen mammalian FMOs and variants are now known but the three-dimensional structure of FMO is not known. The lack of an X-ray structure probably comes from the fact that mammalian FMOs are highly lipophilic enzymes that are associated with the membrane and are hard to crystallise. Despite the difficulties of working with a membrane-associated enzyme, considerable structural information is known. Most of the sequence information has been deduced from oligonucleotide sequencing (Gasser *et al*. 1990; Lomri *et al*. 1993a; Lawton *et al*. 1993, 1994). For some FMOs, automated Edman degradation sequence and, to a lesser extent, mass spectral sequence analysis has provided substantial sequence information especially for FMO1. Ozols has provided extensive amino acid sequence information of rabbit FMOs (Ozols 1991, 1994). Combined with the sequence deduced from the cDNA data, the amino acid sequence data has provided insight into the cofactor binding domains and the general structural motifs of the protein and has provided some evidence for microheterogeneity (Ozols 1994). It was soon clear that widely studied FMOs such as pig FMO1 and rabbit FMO2 were *N*-terminal blocked (Guan *et al*. 1990). While there were methods available to deacetylate proteins, the most straightforward method to identify post-translational modifications of FMO was by direct peptide sequencing using mass spectrometry. In addition, the cDNA sequence data could not by itself provide information about post-translational modifications, and the cDNA and peptide data suggested that pig *FMO1* had consensus sequences for *N*-glycosylation (Guan *et al*. 1991). By using a combination of biochemical methods and mass spectrometry (i.e. gas chromatography mass spectrometry, HPLC mass spectrometry, electrospray mass spectrometry and matrix-assisted laser desorption mass spectrometry) the site of FMO *N*-glycosylation was identified (Korsmeyer *et al*. 1998). For pig FMO1, the only residue that was *N*-glycosylated was Asn 120. Determination of the site of *N*-glycosylation helped to support construction of molecular models of FMO1. Information about the site of *N*-glycosylation also potentially revealed information as to how pig FMO1 associated with the membrane. However, *N*-glycosylation of FMO1 probably is not essential for

enzyme activity because cDNA-expression in bacteria (that lacks the ability to *N*-glycosylate) nevertheless provides active FMO enzyme.

The amino acid composition of a number of FMOs has been reported (Lawton *et al.* 1994). Although molecular models of FMO based on the crystal structure of other flavoproteins have been proposed, the level of suitability and resolution are not really sufficient to make firm conclusions regarding structure and function. The first FMO model was developed by Ziegler (Ziegler 1999) based on the crystal structure of *E. coli* glutathione reductase (Thieme *et al.* 1981; Mattevi *et al.* 1991). A representation of this model is shown in Figure 3.1(a). In this model, emphasis is placed on a dimer interface formed between the putative FAD and NADPH domains juxtaposed to the dimer interface of the opposite FMO monomer (Christensen 1999). Peptide residues 321–339 that contain the proposed FMO signature sequence FATGY have been proposed as a linkage between the putative FAD domain and active site and residues 473–494. In a different model developed within the author's laboratory in collaboration with Professor Ellie Adman (University of Washington), we used the structure of NADPH-peroxidase to model human FMO3 (Figure 3.1(b)). The NADPH-peroxidase model is supported by some of the data in the literature regarding the site of mutations. For example, in the NADPH-peroxidase model, the nonsense mutation M66I that is associated with trimethylaminuria maps to a region near the proposed FAD and $NADP^+$ domains. In the glutathione reductase model, the M66I maps to a region quite distal to the proposed cofactor domains. Both models show that the site of *N*-glycosylation (discussed below) is in a region remote from the putative cofactor binding domains and this is in accord with other evidence suggesting that *N*-glycosylation is not an essential element of enzyme function.

As described above, most of the structural information about FMO comes from studies of pig FMO1. Purified pig FMO1 contains approximately 15 nmol of FAD/mg of protein and is devoid of haem iron or other metals (Ziegler 1980). Highly purified pig FMO1 generally contains variable amounts of lipid and it is generally very difficult to segregate the enzyme from minute amounts of lipid. It is notable that another FMO (i.e. FMO2) is tightly associated with a chaperone protein although it is not known how widely this phenomenon exists for other FMOs from other species or organisms. Rabbit FMO2 is tightly associated with calreticulin (Guan *et al.* 1991), however, for mammalian FMOs it is not known what the physiological role of this association is. The visible spectrum of FMO is similar to other flavoproteins (i.e. λ_{max} of 445 nm and 380 nm and shoulder at 480 nm). In the absence of molecular oxygen, NADPH reduces the FAD prosthetic group to provide reduced $FADH_2$ and the UV-vis spectrum is shifted to shorter wavelength (i.e. λ_{max} of 440 nm and 370 nm with no shoulder) (Poulsen and Ziegler 1979; Beaty and Ballou 1981a, b). As described in greater detail below, addition of molecular oxygen to the fully reduced FMO generates a spectrum similar to peroxy flavins found in other flavoproteins or hydroperoxy isoalloxazines that have been chemically synthesised as models of FMO (Kemal and Bruice 1976; Miller 1982). The formation of a relatively stable hydroperoxy flavin species that represents the 'resting state' of the enzyme is remarkable for at least two reasons. First, FMO somehow stabilises the hydroperoxy species under general cellular conditions that are strongly reducing, and second, stabilisation of the hydroperoxy flavin allows the FMO to oxygenate essentially any nucleophilic substrate that has the appropriate

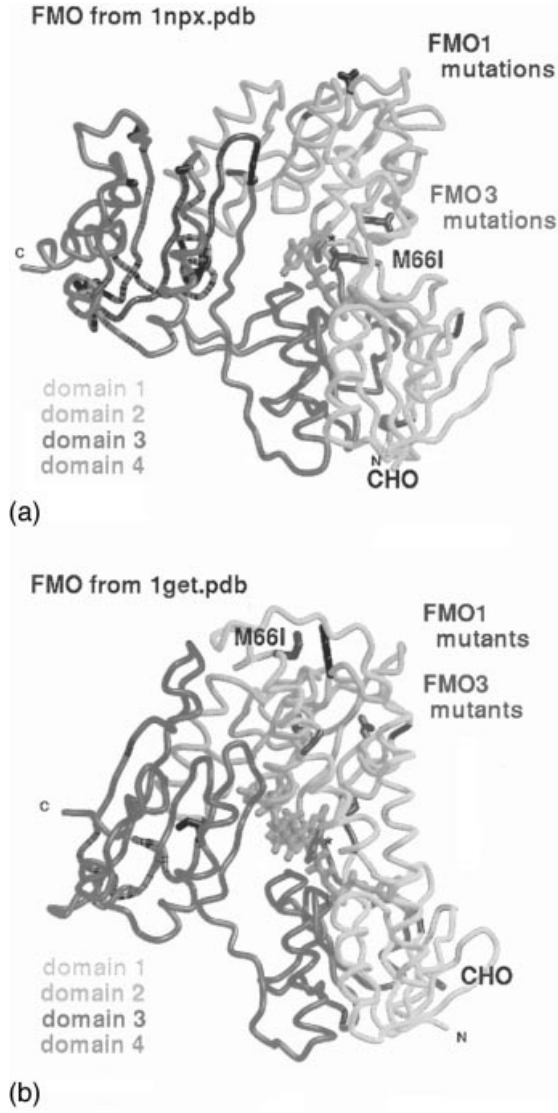

Figure 3.1 (a) Human FMO3 model based on threading the FMO3 sequence onto the X-ray structure of NADPH-peroxidase (npx). (b) Human FMO3 model based on threading the FMO3 sequence onto the X-ray structure of glutathione reductase (get).

steric dimensions to enter the substrate binding channel. It is this fundamental property of FMOs that distinguish them from other monooxygenases and allow FMO to perform its role as an extremely broad-based mixed function monooxygenase.

The cDNAs of FMO reported in the literature encode for active enzymes of

approximately 533–535 amino acids, but examples with 19 or 25 additional *C*-terminal amino acids have been observed. Several regions of FMO contain relatively highly conserved amino acid residues that are presumably important for structure and function (Kubo *et al*. 1997). For example, the FAD- and $NADP^+$-binding domains (i.e. GXGXXG) near amino acid positions 9–14 and 186–196, respectively, share some similarities to other flavoproteins where the crystal structure is known (Vallon 2000). Site-directed mutagenesis studies of the region 9–14 (i.e. GXGXXV) of rabbit *FMO2* gave a cDNA-expressed enzyme that was devoid of activity and a protein that did not bind FAD (Lawton and Philpot 1993). In contrast to many other monooxygenases from the CYP family, the *N*-terminal hydrophobic tail does not help to anchor FMO to the membrane. Other regions must be important. Comparison of FMO hydropathy plots showed numerous regions of conserved hydrophobic segments (Cashman 1997). Although the *N*-terminal region is hydrophobic, the lack of a discernible signal peptide sequence and the obvious importance of the FAD-binding region suggest that this is not a membrane insertion area.

Studies have shown that removal of the hydrophobic amino acid residues of the *C*-terminus still allow the FMO to associate with a bacterial membrane. Although a direct comparison between bacterial and mammalian membranes for FMO association has not been made, nevertheless it is clear that the hydrophobic portion of the C-terminus is not essential for membrane insertion and enzyme function. The conclusion is that FMO likely has an internal sequence that is essential for membrane association.

Genetic aspects

There are a number of points at which the metabolism of a drug or chemical by FMO could be altered. Some of the points are either poorly understood or have not been investigated. For example, for orally administered drugs, the initial pre-systemic metabolism by FMO in the intestine is poorly understood. It appears that FMO1 is the prominent enzyme in the rabbit intestine (Shehin-Johnson *et al*. 1995) but it is unknown whether FMO1 serves to significantly alter human drug bioavailability such as described for CYP 3A4 or the P-glycoprotein systems. A few drugs (i.e. cimetidine, verapamil and albendazol) that are substrates for FMO may have their bioavailability altered as a consequence of FMO action, but this research area is largely unexplored (Piyapolrungroj *et al*. 2000; Redondo *et al*. 1999). Various studies have shown that expression of FMO is both tissue- and species-dependent (Lemoine *et al*. 1991; Wirth and Thorgeirsson 1978; Duffel *et al*. 1981; Cashman *et al*. 1990; Dannan *et al*. 1986). Although it is common for a tissue to have more than one FMO present, the activity is dominated by the most prominent FMO present. For example, in adult human liver, evidence for FMO3, FMO4 and FMO5 has been obtained but it is FMO3 that is by far the most catalytically important species present (Cashman *et al*. 1995). Of course, in a tissue such as the kidney where multiple forms of FMO are more equally present, the oxygenation of a drug or chemical via FMO is probably determined by the kinetic properties of the particular agent (Ripp *et al*. 1999). Of note is the fact that FMO1 is not functionally present in the adult human liver but is the prominent form of FMO in the foetal liver. This represents an intriguing example of regulation of expression of active FMO protein. The observation that FMO1 is not functionally present in adult

human liver underscores another important point: the major form of FMO present in the liver of most commonly used animals is FMO1 and not the form that is the prominent one (i.e. FMO3) found in adult human liver. Thus, some caution should be exercised in comparing preclinical metabolic data obtained in animals with that of adult humans and it is important that an appropriate animal model is used.

In contrast to other monooxygenases of the CYP family, there is little data to suggest that FMOs (other than FMO2) are inducible. FMO2 levels appear to be regulated during development by pregnancy. Gestation increases FMO2 activity in rabbit (Hines *et al*. 1994), mouse placenta (Osimitz and Kulkarni 1982) and pig corpora lutea (Heinze *et al*. 1970). In the rat, some evidence has accumulated that hepatic FMO activity is decreased when animals are placed on a synthetic diet (Kaderlik *et al*. 1991). It is possible that hepatic FMO is maximally present and decreases to a de-induced level in the presence of a synthetic diet or other conditions. Hormones and dietary factors regulate FMO expression but this is done in a species- and tissue-dependent fashion. Traditional receptor-mediated transcriptional regulation that involves ligand binding does not appear to play a significant role in FMO expression as it does for CYP, for example. There is one report in the literature that rat FMO1 is induced by treatment of animals with 3-methylcholanthrene (Chung *et al*. 1997) but this has not been independently confirmed. Expression of FMO is likely to be under the auspices of multiple mechanisms. That expression of FMO is tissue-specific comes from the observation that some tissues contain very high levels of FMO mRNA but very low levels of functional protein or FMO activity. For example, human *FMO2* encodes a truncated non-functional protein (Dolphin *et al*. 1998). Interestingly, while Caucasians apparently do not express human FMO2, some individuals of African descent do possess full-length human FMO2. The expression of active human FMO2 will undoubtedly be highly dependent on the ethnicity of the population examined.

Another way that FMO is regulated is through genetic regulation by polymorphisms. Genetic polymorphisms are defined as allelic variations occurring with a prevalence of at least 1%. Inter-individual variation of enzymic metabolic activity can result in significant population-wide differences in the oxygenation of drugs or xenobiotics. Polymorphisms of monooxygenase genes can exert a dramatic effect on drug metabolism. For example, CYP2D6-mediated debrisoquine 4-hydroxylation is ethnically linked: 'poor metabolisers' make up about 5–10% of the population in the Caucasian population but only about 0.1% of the Asian population (Tucker *et al*. 1977). For those individuals that have the variant gene, the polymorphism causes an exaggerated clinical response to the side effects of debrisoquine. Another example comes from the CYP2C19-mediated 4′-hydroxylation of (*S*)-mephenytoin (Goldstein and de Morais 1994). In Caucasians, the prevalence of the poor (*S*)-mephenytoin metaboliser phenotype is low (approximately 0.2% but quite large in the Asian population (i.e, 15–20%).

In humans, polymorphisms of human *FMO3* were recognised and characterised after observations about the abnormal metabolism of trimethylamine (TMA) (Al-Waiz *et al*. 1987; Hadidi *et al*. 1995; Mitchell *et al*. 1997; Thithapandha 1997; Treacy *et al*. 1998; Dolphin *et al*. 1997; Cashman *et al*. 1997). In normal humans, TMA is metabolised to the polar and non-odorous metabolite trimethylamine N-oxide (TMA N-oxide) that is efficiently excreted in the urine (Al-Waiz *et al*. 1988). For normal

individuals, the urinary TMA N-oxide to TMA ratio is 97%:3% (or urinary TMA levels of < 18 μmol/mmol creatinine). For severely affected individuals, the TMA N-oxide:TMA ratio can be as low as 10:90 or almost exactly opposite of the normal condition. Individuals with trimethylaminuria have a diminished capacity to oxidise dietary-derived TMA to its odourless metabolite TMA N-oxide and these people excrete large amounts of TMA in their urine, sweat and breath. Trimethylaminuria patients have been described as suffering from 'fish odour syndrome' because of the fish-like odour (Ayesh *et al*. 1993). Trimethylaminuria is an autosomal recessive inborn error of metabolism that is quite uncommon and non-randomly distributed in the population. It has been relatively well documented in British and Australian populations and it may be more prevalent in North America than currently recognised (Akerman *et al*. 1999). A significant amount of evidence has accumulated that mutations of the human *FMO3* gene are responsible for trimethylaminuria and segregate with the disorder. A genotype–phenotype correlation has emerged. Individuals homozygous or compound heterozygous for the human *FMO3* truncation mutation E305X manifest the most severe phenotype. Another mutation that also causes a severe phenotype is a proline to leucine substitution at codon 153. Homozygotes with this genotype can have a TMA N-oxide:TMA ratio as low as 10%:90% Another causative mutation is a methionine for isoleucine change at codon 66 that causes a more modest change in an individual's ability to metabolise TMA (i.e. TMA levels of 48 μmol/mmol of creatinine) (Treacy *et al*. 1998). There are additional causative mutations and these are listed in Table 3.2.

In addition to the mutations that cause severe trimethylaminuria, evidence for allelic variation of the human *FMO3* gene have been observed that represents pharmacogenetic polymorphisms (Table 3.3). Again, for populations that have a significant number of poor metabolisers it is possible that the individuals may be more susceptible to adverse drug reactions or exaggerated clinical response. Altered substrate activities have been observed for human *FMO3* (Cashman *et al*. 2000) and may be responsible for mild trimethylaminuria (Zschocke *et al*. 1999).

Table 3.2 Nonsense and missense mutations of the human *FMO3* gene associated with trimethylaminuria

Substitution	Location	References
Deletion	Exons 1 and 2	Forrest *et al*. (2000)
A52T	Exon 3	Akerman *et al*. (1999)
N61S	Exon 3	Dolphin *et al*. (1996)
M66I	Exon 3	Treacy *et al*. (1998)
M82T	Exon 3	Dolphin *et al*. (1996)
P153L	Exon 4	Dolphin *et al*. (1997); Treacy *et al*. (1998)
E305X	Exon 7	Treacy *et al*. (1998)
E314X	Exon 7	Akerman *et al*. (1999)
R387L	Exon 7	Akerman *et al*. (1999)
M434I	Exon 9	Dolphin *et al*. (1996)
R492W	Exon 9	Dolphin *et al*. (1996)

Table 3.3 Common polymorphic variation in the Human *FMO3* Gene[a]

Substitution	Location	Prevalence
E158K	Exon 4	K homozygote is present about 17%
V257M	Exon 6	M homozygote is present about 0.5%
E308G	Exon 7	G homozygote is present about 4%

[a] Genotype frequencies determined in a Caucasian population.

The literature is replete with examples of adverse interactions with drugs and/or chemicals mediated by CYP. One such example is the induction of CYP2E1 by ethanol or other related alcohols and ketones that metabolise disulfiram (Antabuse) to toxic species (Guengerich *et al*. 1991). For human FMO evidence for such clear-cut adverse drug interactions has not been reported but some examples have emerged of adverse clinical problems. For example, individuals with trimethylaminuria also suffer from additional metabolic and psychosocial abnormalities including self-esteem, anxiety, clinical depression and addiction to drugs (Todd 1979). Many of these clinical manifestations could arise, at least in part, from abnormal endogenous or xenobiotic metabolism. For example, in an Australian trimethylaminuria cohort several individuals also manifested hypertension and adverse reactions from tyramine, other amines and sulphur-containing medications. Because FMO has been shown to metabolise biogenic amines (Lin and Cashman 1997a,b), deficient FMO metabolism of biogenic amines could contribute to some of the neurochemical effects observed in individuals with trimethylaminuria. One report showed that a trimethylaminuria patient displayed seizures and other behavioural disturbances after subjected to choline loading (McConnell *et al*. 1997). Dietary choline is a major precursor source of TMA. Certain central nervous system drugs that are normally efficiently cleared could produce exaggerated responses for individuals with common polymorphic variants of FMO (Adali *et al*. 1998). For example, the metabolic detoxication of amphetamine and methamphetamine by human FMO3 may be under pharmacogenetic control (Cashman *et al*. 1999b). Anecdotal reports have suggested that tricyclic antidepressants give exaggerated side reactions for individuals suffering from mild or severe trimethylaminuria. Because human FMO3 of the liver is largely responsible for TMA detoxication, hepatic diseases also can exacerbate the trimethylaminuria condition (Fernandez *et al*. 1997; Stransky 1998). In addition, there are some conditions that apparently aggravate the trimethylaminuria condition including menstruation (Zhang *et al*. 1996) and possibly copper deficiency (Blumenthal *et al*. 1980). It is unknown whether a transient trimethylaminuria condition occurs for some children (Mayatepek and Kohlmueller 1998). Associations with such diseases as Prader–Willi syndrome (Chen and Aiello 1993) and Noonon's syndrome (Calvert 1973) have also been linked with trimethylaminuria. Finally, it is possible that small molecules present in *brassica* vegetables can alter the urinary TMA N-oxide to TMA ratio and give a transient trimethylaminuria condition (Fenwick *et al*. 1983). Based on the results of recent studies, it is likely the aggravation of trimethylaminuria by *brassica* vegetables is due to acid condensation products of indole-3-carbinol present in the vegetables. Inhibition of FMO by indole-3-carbinol is discussed in greater detail, below.

Biochemical properties

Below, the biochemical properties of FMO are discussed in some detail to put the monooxygenase system in perspective with other systems. As described above, the mammalian FMOs are a family of gene products that catalyse a remarkable range of oxygenation of nucleophilic nitrogen-, sulphur-, phosphorous- and selenium-containing drugs and xenobiotics to their respective oxides. Although many exceptions are known, generally, formation of polar, oxygenated metabolites can provide a means to terminate the biological activity of a heteroatom-containing compound (Cashman *et al*. 1996). The degree to which a polar, oxygenated metabolite is excreted depends, of course, on further metabolic processes, both oxidative and reductive, and numerous exceptions to the general rule described above have been observed.

There are some biochemical properties unique to the FMO class of monooxygenases. With the possible exception of FMO2, FMOs are unusually sensitive to thermal inactivation and this property often serves as a means to distinguish the contribution to the metabolism of a chemical by FMO from that of other monooxygenases. Thus, procurement of tissue from an animal before the temperature of the animal rises is essential to preserve maximal FMO activity. In the absence of NADPH, about 85% of the activity of most FMOs is lost if the tissue is left standing at 45–55°C for 1–4 minutes. These are conditions sometimes achieved under post-mortem conditions (Ziegler 1980). Thermal lability also represents a practical way to distinguish the contribution of FMO from that of CYP to the N- or S-oxidation of a drug or other chemical. Heat inactivation of microsomes at 55°C for one minute in the absence of NADPH largely destroys FMO activity and retains CYP activity. Addition of NADPH to a preparation treated in this fashion allows for CYP to function normally but generally abrogates FMO activity. Of course, heat inactivation of microsomes tends to produce significant quantities of H_2O_2 and it is always a good idea to destroy any H_2O_2 formed by addition of exogenous catalase. The best way to ensure maximal FMO activity is to add NADPH (or an NADPH-generating system) directly to a freshly thawed preparation of enzyme. Even at the customary incubation temperature of 37°C, if enzyme preparations containing FMO activity are allowed to stand for even a few minutes in the absence of NADPH, significant FMO activity can be lost. This problem may have contributed to the fact that many examples of FMO-mediated metabolism were overlooked. The reason for this is that historically, many metabolic reactions were initiated by the addition of NADPH (or an NADPH-generating system) and this procedure inherently destabilised the FMO. If the pre-incubation phase is conducted in the absence of NADPH at 37°C, significant FMO activity can be lost. This has probably led to a general underestimation of the role of FMO in drug and chemical metabolism. With the advent of cDNA-expressed enzymes in drug metabolism, the challenges of the thermal lability of FMO are less of a problem. Today, the investigator is less dependent on the amount of FMO activity lost during post-mortem inactivation because of the availability of the recombinant enzyme. Another advance has come about from the recognition that some recombinant fusion proteins of FMO are considerably more stable than those of the native enzyme (Brunelle *et al*. 1997).

Another fascinating property of FMO is the formation of a relatively stable

hydroperoxy flavin intermediate. This is important for at least three inter-related reasons. First, the unusually long-lived hydroperoxy flavin species is remarkably stable and resistant to decomposition and disproportionation. This property allows the FMO to be in an oxygenating mode during essentially the entire lifetime of the catalytic cycle. This may account for the second feature of the hydroperoxy flavin: generally (barring steric limitations), almost any strong nucleophile is oxygenated by FMO and, as it will be described in more detail below, for a class of substrate, generally, all are oxygenated at nearly the same rate. This suggests that the formation of product occurs before the rate-limiting step of the enzyme reaction. Regardless of the mechanistic details, FMO has somehow evolved to stabilise and preserve the integrity of a potentially labile oxygenating agent during the catalytic cycle. Although poorly understood, this ingenious mechanism underscores the potential versatility of the catalyst. In addition, it points to some previously undiscovered molecular property of the FMO active site and substrate-binding region construction that allows the hydroperoxy flavin moiety to exist for long periods of time (on a biological time scale) without decomposition. It is possible that the substrate-binding region is constructed of lipophilic, non-nucleophilic amino acid residues that contribute to stabilising this critical species for FMO catalytic function. Evaluation of this suggestion must await further structural information.

Another important feature of FMO is that, under normal conditions, no detectable production of H_2O_2 or other reactive oxygen species is formed during the catalytic cycle of the enzyme, and therefore minimal FMO-mediated autooxidation of substrate is observed. The conclusion is that formation of hydroperoxy flavin is tightly coupled to formation of oxygenated product unless NADPH is not present. Thus, there is generally an excellent stoichiometry between consumption of one mole of molecular oxygen by FMO and formation of one-half mole oxygenated product and one-half mole of water.

Consequently, monitoring consumption of molecular oxygen with an oxygen electrode or some other oxygen-sensing system can provide a method for determining enzyme kinetics. Of course, for multi-step kinetics or where multiple products are formed it is useful to have the authentic synthetic metabolites and quantify the FMO enzyme reaction products by some separation technique such as HPLC. That FMO does not generate copious amounts of H_2O_2 in the absence of substrate suggests that FMO does not expose the cell to untoward effects of oxidative stress. In addition, and as described below, physiological substrates such as glutathione or other cellular nucleophiles appear to be excluded from the substrate binding channel. This is important because if cellular nucleophiles were continuously oxygenated it would be biologically quite wasteful, lead to cellular stress due to loss of NADPH and possibly contribute to proliferation of cellular reactive oxygen species and oxidative stress. This underscores the role of FMO as a xenobiotic detoxication catalyst. However, under severe cellular stress such as that during conditions of post-mortem inactivation, FMO may lose its NADPH cofactor to other apparently more vital cellular function. This may point to the fact that FMO plays an auxiliary role in cellular homeostasis and that under conditions where cellular defence is not essential, FMO participates in cellular survival by providing essential reducing equivalents to important biochemical sites.

Catalytic mechanism

The laboratories of Ziegler and Ballou have characterised the detailed steps of the pig FMO1 catalytic cycle (Poulsen and Ziegler 1979; Beaty and Ballou 1981a,b). It is likely that other FMOs also conform to the same general mechanistic picture although as other FMOs from other species are described this question should be re-examined. The prominent steps of the FMO1 catalytic cycle are shown in Figure 3.2. In the first step of the enzyme reaction (step 1), the fully oxidised flavoprotein (FMO-Fl_{ox}) combines with NADPH in a fast step to give the FMO in the reduced form (FMO-FlH_2). The K_m for binding of NADPH is in the low micromolar region and the rate constant (i.e. 53 M^{-1}) suggests that it is among the fastest reactions in the cycle. After delivering the reducing equivalents to the flavin, the $NADP^+$ apparently remains proximal to the reduced flavin moiety and possibly serves as a protector or 'gate-keeper' to the complex. This is important as the reduced flavin is not indefinitely stable and reaction of the reduced flavin with molecular oxygen to form the key hydroperoxy flavin (step 2) may require the presence of the $NADP^+$ cofactor in an as yet poorly understood way. Formation of the hydroperoxy flavoenzyme is also rapid (i.e. 45 M^{-1}) and provides the long-lived hydroperoxy flavin oxygenating species that makes up the vast majority of the resting form of the enzyme. The hydroperoxy flavoenzyme is the form of the enzyme that waits in the ground state until an appropriate substrate comes along. For substrates such as dimethylaniline, oxygenation proceeds very rapidly (i.e. bimolecular rate constant of 4700 M^{-1} s^{-1}) with attack of the nucleophilic nitrogen atom on the terminal hydroperoxy flavin oxygen atom (step 3) to produce the product (S–O) and the hydroxy flavoenzyme species (i.e. the pseudobase FMO–FlHOH). The oxygenated product then leaves the product binding region, again, in a very fast step. The next and final step (step 4) is slow and constitutes the overall rate-limiting step of the catalytic cycle. From kinetic measurements, it is not clear whether dehydration of pseudobase FMO–FlOH or desorption of $NADP^+$ is the rate-limiting step but this step is approximately 20-30-fold slower than any of the other steps in the catalytic cycle

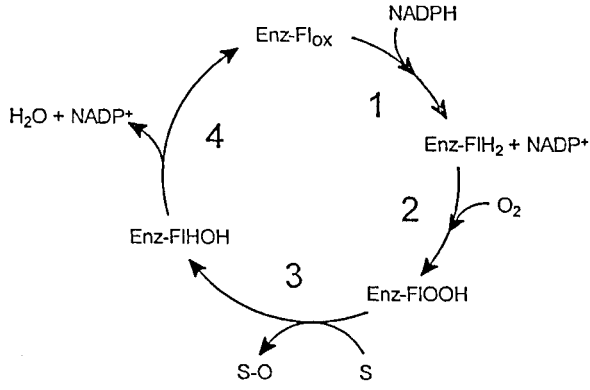

Figure 3.2 Proposed catalytic cycle of FMO. S and S–O represent the substrate and the oxygenated substrate, respectively (adapted from Cashman 1995).

(i.e. 1.9 M^{-1}). It is possible that $NADP^+$comes away last because $NADP^+$ is a competitive inhibitor of the FMO. As discussed above, under normal conditions, formation of the highly protected hydroperoxy flavin species is tightly coupled to substrate oxygenation. The catalytic mechanism also predicts that because release of product comes before the rate-limiting step all good substrates will have similar V_{max} values. Generally, for very good substrates (low K_m, high V_{max} substrates) this is the case but there are exceptions and it is somewhat dependent on the nature of the substrate. Steric factors may play a role in this general conclusion for a given class of FMO substrate. In summary, detailed kinetic studies are in accord with the mechanism of Figure 3.2 and the proposal does not violate the principles of enzyme saturation and Michaelis–Menten kinetics. Ziegler has proposed that FMO operates by providing a single point of attachment for substrate oxygenation and more complex induced fit interactions are not required (Ziegler 1993). However, the prediction that the hydroperoxy flavin of FMO acts similarly to synthetic isoalloxazine hydroperoxides and that the enzyme simply serves as a reactant in a bimolecular reaction is probably not true for all substrates. As described below, however, additional binding interactions must be at work to produce the various degrees of stereoselectivity observed for the FMOs examined.

Substrate specificity, inhibitors and induction

As the drug discovery pipeline has expanded with the advent of combinatorial chemistry, knowledge of monooxygenase-mediated detoxication should become an ever-increasing component in drug development (Cashman 1996). The substrate specificity of most of the FMOs has been summarised previously (Ziegler 1980, 1990, 1993; Cashman 1995). A model of pig FMO1 was proposed to explain much of the structure function information known at the time (Cashman 1995). This was based on the structure–function relations of a series of 10-[(*N*,*N*-dimethylamino)alkyl]-(2-trifluoromethyl)phenothiazine derivatives and other substrates N- and S-oxygenated by FMO1 and FMO3 (Lomri *et al*. 1993 a–c; Overby *et al*. 1995; Nagata *et al*. 1990). Use of the 10-(*N*,*N*-dimethylaminopentyl) derivative provides a highly sensitive means of determining FMO activity and N-oxygenation of this substrate is highly correlated with FMO3 immunoreactivity in human liver preparations (Cashman *et al*. 1993b). Generally, pig FMO1 provides a reasonable starting point for understanding the substrate specificity of animal FMOs because a prominent form of FMO in animal liver is FMO1. However, it appears that human FMO1 is considerably more restricted than pig FMO1 in the size of the nucleophilic heteroatom-containing substrates accepted (Kim and Ziegler 2000). Animal FMO2 differs from pig FMO1 because it is competent to N-oxygenate primary amines. On the other hand, evidence for *stimulation* of pig FMO1 by primary amines has been observed. However, careful examination of FMO1 shows that the enzyme can *N*-oxygenate primary amines but it does so more than 100-fold less efficiently than FMO3 (Lin and Cashman 1997a,b). Both chlorpromazine and imipramine have been reported to activate human FMO3 toward the oxygenation of good substrates such as methimazole (Overby *et al*. 1997; Wyatt *et al*. 1998). As described above, animal FMOs 1, 2, and 3 oxygenate a wide variety of nucleophilic tertiary and secondary amines as well as sulphur-containing compounds. Little is

known about FMO4 and FMO6 substrate specificity because of the difficulties associated with cDNA-expression and characterisation. FMO5 is an unusual FMO and evidence for selective N-oxygenation of long-chain aliphatic primary amines has been reported (Overby *et al*. 1995), but FMO5 does not apparently S-oxygenate the widely used substrate methimazole.

Because FMO3 is probably the most functionally important FMO from the standpoint of human drug and xenobiotic metabolism, most of the discussion in this section will focus on this form of the enzyme. It is notable that the hepatic form of FMO present in rats is FMO1. Thus, rats represent a poor choice to use as FMO models for human hepatic drug development. For mice and dogs where FMO3 levels are more nearly similar to human liver caution should be exercised as there is a gender effect for FMO3 in these species (Ripp *et al*. 1999). Rabbit liver preparations may overpredict the contribution of FMO3 to drug metabolism because the hepatic levels appear to be present to a greater extent than in human liver. In designing a small animal strategy useful as a small animal model of human hepatic FMO3, the female mouse or dog may be more suitable but gender effects on other pharmacodynamic properties of the drug or xenobiotic to be evaluated may confound the picture.

Traditionally, human FMO3 activity has been described based on studies in human liver microsomes. Today, more studies are emerging that report on human FMO3 activity in other systems including hepatocytes (Fischer and Wiebel 1990; Sherratt and Damani 1989; Rodriguez *et al*. 1996) and other propagated cells (DiMonte *et al*. 1991). With the widespread use of recombinant human FMO3 enzyme preparations, a fuller description of substrate specificity is available. When practical, an important approach is to utilise substrates both *in vitro* and *in vivo* to characterise human FMO3. Thus, (*S*)-nicotine (Park *et al*. 1993), TMA (Treacy *et al*. 1998), cimetidine (Cashman *et al*. 1993a), clozapine (Sachse *et al*. 1999) and ranitidine (Kang *et al*. 2000) have been used to phenotype various populations for human FMO3 activity. All five chemicals have been shown to be relatively selective probes of human FMO3 activity *in vitro*. A non-invasive approach is to utilise the tertiary amine TMA that arises from the dietary precursor choline. Each substrate has some advantages and disadvantages. (*S*)-Nicotine is selectively *N*-1′-oxygenated by human FMO3 to form exclusively the *trans N*-1′-oxide both *in vitro* and *in vivo*. Animal FMO1 forms a 50:50 mixture of *cis* and *trans* nicotine *N*-1′-oxide. Therefore, the stereochemistry of the product reveals whether FMO1 or FMO3 N-oxygenates nicotine. That only the *trans N*′1′-oxide diastereomer is formed in humans *in vivo* suggests that only FMO3 metabolises (*S*)-nicotine. Despite the usefulness of nicotine as a stereoselective *in vitro* probe of human FMO3, its use *in vivo* is somewhat limited due to the relatively high K_m of nicotine. TMA is an excellent substrate for FMO3 *in vitro* (Cashman *et al*. 1997; Lang *et al*. 1998) but because it arises from dietary choline and other sources the TMA levels may vary *in vivo* and it is therefore important to establish *in vivo* TMA N-oxide:TMA ratios. Cimetidine S-oxygenation is another selective substrate for human FMO3 presumably because of the nucleophilicity of the sulphur atom and because the imidazole nucleus serves to inhibit CYP-mediated oxidation (Cashman *et al*. 1993a). Urinary cimetidine S-oxide is mainly present as the (-)-isomer (i.e. (-)−75%: (+)−25% cimetidine S-oxide). The *in vivo* result was in good agreement with that observed studying the S-oxygenation of cimetidine *in vitro*. The conclusion is that

human FMO3 largely forms (-)-cimetidine S-oxide and human FMO1 forms (+)-cimetidine S-oxide. The bimodal profile of formation of plasma cimetidine S-oxide may be due to absorption differences or due to the action of different FMOs. Another possibility is that presystemic metabolism of cimetidine in the intestine contributes to the variability of cimetidine pharmacokinetics (Lu *et al*. 1998). However, the role of presystemic FMO in the metabolism of drugs and other xenobiotics is an understudied area. Clozapine is a cyclic tertiary amine that is efficiently N-oxygenated by human FMO3 (Tugnait *et al*. 1997). Clozapine and caffeine have been studied *in vivo* as probes of human FMO3 phenotype and genotype (Sachse *et al*. 1999). While clozapine is an effective *in vitro* probe, three common polymorphisms of human FMO3 were not linked to either clozapine or caffeine metabolism. For clozapine, it is possible that the K_m value is too high to serve as a useful *in vivo* marker. The lack of correlation of caffeine with FMO3 genotype and the reported lack of substrate activity for human FMO3 (Rettie and Lang 2000) brings up the issue as to whether caffeine is a useful probe for human FMO3. In view of the lack of a nucleophilic nitrogen atom and the lack of substrate activity, this suggests that caffeine metabolism is not dependent on the human FMO3. Ranitidine N-oxygenation has found use in correlating phenotype with genotype in a Korean population (Park *et al*. 1999). Other nucleophilic heteroatom-containing compounds have been shown to be selectively oxygenated by human FMO3. For example, tamoxifen (Kupfer and Dehal 1996), benzydamine (Ubeaud *et al*. 1999), xanomeline (Ring *et al*. 1999), *N*-deacetyl ketoconazole (Rodriguez *et al*. 1999) and sulindac sulphide (Hamman *et al*. 2000) are substrates for human FMO3 oxygenation.

To date, few examples of true competitive inhibition of human FMO3 have been reported. Most of the inhibitory effects on FMO have been examples of alternate substrate competitive inhibition. This is the case where a chemical is a better substrate for the compound being studied and addition of the chemical decreases the apparent oxygenation of the compound. Chemicals with low K_m, high V_{max} kinetic parameters for human FMO3 such as methimazole or thiobenzamide generally show alternate substrate competitive inhibition of human FMO3. Recently, a true competitive inhibitor based on dimethylamino stilbene carboxylate was reported (Clement *et al*. 1996). Another compound, indole-3-carbinol and its acid condensation products are potent, competitive inhibitors of human FMO3 (Cashman *et al*. 1999a). Indole-3-carbinol is a dietary constituent of cruciferous vegetables and is degraded to dimers and trimers upon reaching the acidic contents of the stomach. In a study comparing the *in vitro* inhibitory potency with the *in vivo* inhibitory potency, it was shown that the dimer was a potent inhibitor of human FMO3. Rat hepatic and intestinal FMO1 are also inhibited by dietary indole-3-carbinol (Larsen-Su and Williams 1996) and the down-regulation of FMO coupled with the induction of CYP may predispose animals to potential drug–drug interactions (Katchamart *et al*. 2000).

Stereochemical considerations

A number of reports have presented evidence on the stereoselective oxygenation of heteroatom-containing drugs and chemicals by the FMO. Some of this work was recently summarised (Cashman 1998). For FMO, advantage can be taken of the fact

that many S- and N-oxides formed by FMO are sufficiently stable to spontaneous racemisation to allow the determination of optical activity after the monooxygenase reaction. For example, sulphoxides are relatively stable to stereomutation and racemise at elevated temperatures (i.e. above 200°C). In general, tertiary amines are more prone to thermal stereomutation than sulphoxides. However, cyclic tertiary amine N-oxides and even some linear amine N-oxides are sufficiently stable to thermal racemisation to assess FMO-mediated stereoselectivity. Because racemisation or decomposition of S- or N-oxides can occur by a number of routes (i.e. photochemical, acid-promoted or elimination reactions), in any studies of quantification of FMO stereoselectivity, it is advisable to chemically synthesise the product and do stability studies on the material that possesses a centre of chirality. Therefore, an important step in the determination of FMO-dependent stereoselectivity is to establish a bioanalytical method to measure the optical purity of the enzyme-catalysed reaction. ^{1}H-NMR spectroscopy in the presence of a chiral auxiliary (i.e. a europium shift reagent, Eu(hfc)$_3$) was used to probe the FMO-mediated stereoselectivity of aryl-1,3-dithiolane S-oxygenation formation (Cashman and Olsen, 1990; Cashman *et al*. 1990) and the optical purity was established by correlating the circular dichroism absorbance spectra by use of the Cotton effect (Mislow *et al*. 1965). Another approach is to use gas–liquid chromatography with a chiral stationary phase to separate lipophilic S-oxides. Because of the relatively low volatility and susceptibility to thermal decomposition, chiral phase gas–liquid chromatography is not generally useful in quantifying FMO-mediated stereoselectivity reactions. One possible exception is where an S-oxide or N-oxide can be quantitatively converted into a material that is efficiently chromatographed by gas-liquid chromatography. Two such examples are the thermal rearrangement of FMO metabolites (*S*)-nicotine *N*-1′-oxide to an oxazine (Jacob *et al*. 1986) and the formation of 3,4-dimethoxy styrene from verapamil N-oxide (Cashman, 1989). Of course, the most unambiguous method to determine FMO product stereoselectivity is to determine the X-ray crystal structure of the S- or N-oxide. However, the requirement for significant amounts of high-quality crystals and the amount of time and expense associated with this technique has limited its usefulness.

Commercially available chiral stationary phase HPLC (CSP HPLC) has allowed rapid advances in the characterisation of the stereoselectivity of enzyme-mediated reactions especially when used in conjunction with other methods to determine the absolute configuration. A summary of some of this technology has been presented previously (Cashman 1998). Generally, use of CSP HPLC is the method of choice for the determination of the absolute configuration of FMO products due to the speed, relative lack of expense and accuracy. Only 1–2 μg of FMO metabolite is required in each chromatographic run and this is easily obtained from typical small-scale incubations. In addition, synthetic chemical or enzymic methods are available to stereoselectively form the desired metabolite in enantiomerically enriched form. For example, by use of nonchiral oxidising agents in the presence of a chiral macromolecule, stereoselective S-oxygenation is easily achieved. Treatment of sulphides with sodium meta periodate in the presence of bovine serum albumin often provides multi-milligram quantities of S-oxide with great enantioselectivity (Cashman 1998). With the development of milder and more selective oxidising agents (Boyd *et al*. 1989), additional reagents should continue to become available to provide increasingly

efficient means of obtaining authentic chiral standards. One of the best ways to chemically synthesise small amounts of S- or N-oxides with enantioenriched centres of chirality is with the modified Sharpless chiral oxidation reagent (Pitchen *et al*. 1984). The author's laboratory has had success by using this procedure to synthesise authentic metabolites useful as a standard in developing CSP HPLC for FMO-mediated chemical oxygenations (Cashman 1998). Another useful method for generating FMO-mediated metabolites possessing a centre of chirality is the use of other enzymes. Today, many monooxygenases are available commercially in pure form. Use of different monooxygenases can also provide information whether other enzyme systems give the same product stereochemistry, and this can be valuable in determining the contribution of other monooxygenases in substrate probe stereoselectivity. For example, alkyl-substituted *p*-tolyl sulphides have been shown to be stereoselectively S-oxygenated to S-oxides by FMOs (Rettie *et al*. 1995) (Table 3.4). CYPs or other haemoproteins also S-oxidise these same substrates and in some cases give the same product stereochemistry, in other cases give a distinct one (Pike *et al*. 1999). Thus, FMO1, 2, and 3 give a predominance of the (+)-(*R*) S-oxide but FMO5 and CYP2B and 2C6 mainly produce the (-)-(*S*) S-oxide (Rettie *et al*. 1994) (Table 3.4). This example points out the advantage of using stereochemistry to identify the contribution of a particular enzyme to the formation of a product, but it also suggests that caution should be exercised when multiple enzyme systems can oxidise the same probe substrate, depending on metabolic reaction conditions and substrate concentrations.

Role of FMO in toxicological aspects

In contrast to the CYP field of monooxygenases where some key advances have been made based on the observation that CYP bioactivated a chemical or drug to a toxic metabolite, fewer examples of FMO-mediated bioactivation are available. Rather, as discussed above, FMO has been associated with detoxication processes that convert nucleophilic heteroatom-containing chemicals or drugs into relatively polar, readily excreted metabolites. As described previously by Ziegler, it is possible that FMO evolved to inactivate many chemicals present in plants that would otherwise inhibit

Table 3.4 Stereoselective *S*-oxidation of ethyl *p*-tolyl sulphides and related compounds

Enzyme system	Absolute configuration	Enantiomeric excess	References
Horseradish Peroxidase[a]	(+)-(*R*)	100	Grayson and Rous, (1987).
Hog FMO1[b]	(+)-(*R*)	95	Light *et al*. (1982)
Rabbit FMO1[b]	(+)-(*R*)	99	Rettie *et al*. (1994)
Rabbit FMO2[b]	(+)-(*R*)	91	Rettie *et al*. (1990)
Rabbit FMO3[b]	(+)-(*R*)	v. low	Rettie *et al*. (1994)
Rabbit FMO5[b]	(+)-(*S*)	92	Fisher *et al*. (1995)
Cyclohexanone[b] Monooxygenase	(-)-(*S*)	80	Boyd *et al*. (1989)

[a]The substrate used was methyl *p*-tolyl sulphide.
[b]The substrate used was ethyl *p*-tolyl sulphide.

and destroy CYPs (Ziegler 1990). Thus, conversion of a sulphur-containing chemical to a polar S-oxide that might otherwise be oxidised by and inactivate CYP would constitute a chemoprotective strategy. The evidence for this postulate is that FMO is primarily localised where CYP resides and FMO is recalcitrant to inactivation by many chemicals that inhibit CYP. For example, depending on the structure, thiones (i.e. thioamides, mercaptoimidazoles, thiocarbamides, etc.) are metabolised by both CYP and FMO (Decker *et al*. 1991, 1992; Decker and Doerge 1991). In the case of 2-mercaptoimidazole (i.e. methimidazole) S-oxidation by CYP or FMO leads to a metabolite (i.e. a sulphenic acid) that covalently modifies CYP (Kedderis and Rickert 1985).

There are numerous examples of reactive metabolites produced by FMO- or CYP-mediated S-oxidative bioactivation that results in CYP inactivation without much effect on FMO. Of course, the presence of thiophiles such as glutathione to form disulphides after reaction with sulphenic acids can attenuate the relative toxicity of sulphenic acids formed by FMO. Once formed, the disulphides can undergo disulphide exchange and produce the parent thione and oxidised glutathione. This is an example of a futile metabolic cycle whereby the substrate is oxidised and after a reductive step is returned to its parent oxidation state. Oxidation of glutathione and consumption of NADPH may make the cell more susceptible to the toxic properties of other reactive metabolites especially if the thione is a low K_m high V_{max} substrate and depletes the cell of glutathione (Mizutani *et al*. 2000). In summary, judging whether an FMO-dependent oxygenation is a detoxication or bioactivation process is not always a simple exercise. However, on the basis of information available in the literature, the majority of the data suggests that FMO is a detoxication catalyst.

There are a few examples in the literature of how the toxicity of certain chemicals may be different under certain experimental conditions where the expression of FMO is altered. For example, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is efficiently N-oxygenated by FMO in an apparent detoxication process (Cashman and Ziegler 1986). In a species where low FMO activity is present, the majority of MPTP is metabolised by monoamine oxidase (MAO) to the neurotoxic metabolite 1-methyl-4-phenyl-2,3-dihydropyridinium ion ($MPDP^+$) and *N*-methyl-4-phenylpyridinium ion (MPP^+). In different strains of mice, it is likely that FMO-mediated N-oxygenation of MPTP is a detoxication process leading to a non-toxic, readily excreted product whereas MAO-mediated oxidation results in highly electrophilic metabolites that participate in interruption of cellular function (Chiba *et al*. 1988). In the 1970s there was the view that the FMO system could *N*-hydroxylate procarcinogenic arylamines. This is likely to be true for some arylamines but human FMO is likely to be only a minor contributor to the overall *N*-oxygenation of these types of compounds. However, aliphatic primary amines avoid potentially toxic hydroxylamine formation by efficient *N*-oxygenation by human FMO3 (Cashman 2000) and this may lead to significant cytoprotection (Clement *et al*. 2000).

There are a few clinical examples of individuals with deficient FMO activity that result in toxic sequelae. However, to date, aside from the inherited defect of metabolism called trimethylaminuria discussed above, there is no direct link between altered FMO and a human disease state. However, drug–drug interactions may pose a problem for individuals with common variants of FMO.

Acknowledgments

I appreciate the collaborative work of the many co-workers cited in the references from my laboratory. I thank Professor Ellie Adman (University of Washington) for the valuable contribution of Figure 3.1. The author thanks the National Institutes of Health (Grants GM36426 and DK59618) and the TRDRP (Grant 9RT-0196) for their financial support.

References

Adali O, Carver GC and Philpot RM (1998) Modulation of human flavin-containing monooxygenase 3 activity by tricyclic antidepressants and other agents: importance of residue 428. *Arch. Biochem. Biophys.*, **358**, 92–97.

Akerman BR, Lemass H, Chow LM, Lambert DM, Greenberg C, Bibeau C, Mamer OA and Treacy EP (1999) Trimethylaminuria is caused by mutations of the FMO3 gene in a North American population. *Mol. Genet. Metab.*, **68**, 24–31.

Al-Waiz M, Ayesh R, Mitchell SC, Idle JR and Smith RL (1987) A genetic polymorphism of the N-oxidation of trimethylamine in humans. *Clin. Pharmacol. Ther.*, **42**, 588–594.

Al-Waiz M, Ayesh R, Mitchell SC, Idle JR and Smith RL (1988) Trimethylaminuria ('fish-odour syndrome'): a study of an affected family. *Clin. Sci.*, **74**, 231–236.

Ayesh R, Mitchell SC, Zhang A and Smith RL (1993) The fish odour syndrome: biochemical, familial, and clinical aspects. *Br. Med. J.*, **307**, 655–657.

Ball SS and Bruice TC (1983) Oxidation of amines by a 4a-hydroperoxyflavin. *J. Am. Chem. Soc.*, **102**, 6498–6503.

Ballou D, Palmer G and Massey V (1969) Direct demonstration of superoxide anion production during the oxidation of reduced flavin and of its catalytic decomposition by erythrocuprein. *Biochem. Biophys. Res. Commun.*, **36**, 898–904.

Beaty NB and Ballou DP (1981a) The oxidative half-reaction of liver microsomal FAD-containing monooxygenase. *J. Biol. Chem.*, **256**, 4619–4625.

Beaty NB and Ballou DP (1981b) The reductive half-reaction of liver microsomal FAD-containing monooxygenase. *J. Biol. Chem.*, **256**, 4611–4618.

Blumenthal I, Lealman GT and Franklyn PP (1980) Fracture of the femur, fish odour, and copper deficiency in a preterm infant. *Arch. Dis. Child.*, **55**, 229–231.

Boyd DR, Walsh CT and Chen YC (1989) In *Sulfur-Containing Drugs and Related Organic Compounds*, Part A, Volume 2, Damani LA (ed.), Ellis Horwood, Chichester, pp. 67–99.

Brunnelle A, Bi YA, Lin J, Russell B, Luy L, Berkman C and Cashman JR (1997) Characterization of two human flavin-containing monooxygenase (form 3) enzymes expressed in *Escherichia coli* as maltose binding protein fusions. *Drug Metab. Dispos.*, **25**, 1001–1007.

Calvert GD (1973) Trimethylaminuria and inherited Noonan's syndrome. *Lancet*, **1**, 320–321.

Cashman JR (1989) Enantioselective N-oxygenation of verapamil by the flavin-containing monooxygenase. *Mol. Pharmacol.*, **36**, 497–503.

Cashman JR (1995) Structural and catalytic properties of the mammalian flavin-containing monooxygenase. *Chem. Res. Toxicol.*, **8**, 165–181.

Cashman JR (1996) Drug discovery and drug metabolism. *Drug Discovery Today*, **1**, 209–216.

Cashman JR (1997) Monoamine oxidase and flavin-containing monooxygenase. In *Comprehensive Toxicology, Volume 3*, Ch. 6, Guengerich FP (ed.), Elsevier Science, New York, pp. 69–96.

Cashman JR (1998) Stereoselectivity in S- and N-oxygenation by the mammalian flavin-containing monooxygenase and cytochrome P-450 monooxygenases. *Drug Metab. Rev.*, **30**, 675–708.

Cashman JR (2000) Human flavin-containing monooxygenase: substrate specificity and role in drug metabolism. *Curr. Drug Metab.*, **1**, 1037–1045.

Cashman JR and Olsen LD (1990) Stereoselective S-oxygenation of 2-aryl-1,3-dithiolanes by hog liver flavin-containing monooxygenase. *Mol. Pharmacol.*, **38**, 573–585.

Cashman JR and Ziegler DM (1986) Contribution of N-oxygenation to the metabolism of MPTP

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by the flavin-containing monooxygenase by various liver preparations. *Mol. Pharmacol.*, **29**, 163–167.

Cashman JR, Olsen LD, Lambright CE and Presas MJ (1990) Enantioselective S-oxygenation of *p*-methoxy phenyl-1,3-dithiolane by various tissue preparations: effect of estradiol. *Mol. Pharmacol.*, **37**, 319–327.

Cashman JR, Park SB, Yang ZC, Washington CB, Gomez DY, Giacomini KM and Brett CM (1993a) Chemical, enzymic, and human enantioselective S-oxygenation of cimetidine. *Drug Metab. Dispos.*, **21**, 587–597.

Cashman JR, Yang Z, Yang L and Wrighton SA (1993b) Stereo- and regioselective N- and S-oxidation of tertiary amines and sulphides in the presence of adult human liver microsomes. *Drug. Metab. Dispos.*, **21**, 492–501.

Cashman JR, Park SB, Berkman CE and Cashman LE (1995) Role of hepatic flavin-containing monooxygenase 3 in drug and chemical metabolism in adult humans. *Chem. Biol. Interact.*, **96**, 33–46.

Cashman JR, Perotti BY and Berkamn CE (1996) Lin J (1996) Pharmacokinetics and molecular detoxication. *Environ. Health Perspect.*, **104**, 23–40.

Cashman JR, Bi YA, Lin J, Youil R, Knight M, Forrest S and Treacy E (1997) Human flavin-containing monooxygenase form 3: cDNA expression of the enzymes containing amino acid substitutions observed in individuals with trimethylaminuria. *Chem. Res. Toxicol.*, **10**, 837–841.

Cashman JR, Xiong Y, Lin J, Verhagen H, van Poppel G, van Bladeren PJ, Larsen-Su S and Williams DE (1999a) *In vitro* and *in vivo* inhibition of human flavin-containing monooxygenase form 3 in the presence of dietary indoles. *Biochem. Pharmacol.*, **58**, 1047–1055.

Cashman JR, Xiong YN, Xu L and Janowsky A (1999b) N-Oxygenation of amphetamine and methamphetamine by the human flavin-containing monooxygenase (form 3): role in bioactivation and detoxication. *J. Pharmacol. Exper. Ther.*, **288**, 1251–1260.

Cashman JR, Akerman BR, Forrest SM and Treacy EP (2000) Population-specific polymorphisms of the human FMO3 gene: signifcance for detoxication. *Drug Metab. Dispos.*, **28**, 169–173.

Chen H and Aiello F (1993) Trimethylaminuria in a girl with Prader-Willi syndrome and del(15)(q11q13). *Am. J. Med. Genet.*, **45**, 335–339.

Chiba K, Kubota E, Miyakawa T, Kato Y and Ishizaki T (1988) Characterization of hepatic microsomal metabolism as an *in vivo* detoxication pathway of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. *J. Pharmacol. Exp. Ther.*, **246**, 1108–1115.

Christensen D (1999) What's that smell? *Science News*, **155**, 316–317.

Chung WG, Park CS, Roh HK and Cha YN (1997) Induction of flavin-containing monooxygenase (FMO1) by a polycyclic aromatic hydrocarbon, 3-methylcholanthrene, in rat liver. *Mol. Cells*, **7**, 738–741.

Clement B, Weide M and Ziegler DM (1996) Inhibition of purified and membrane-bound flavin-containing monooxygenase by (*N*,*N*-dimethylamino)stilbene carboxylates. *Chem. Res. Toxicol.*, **9**, 599–604.

Clement B, Behrens D, Moller W and Cashman JR (2000) Reduction amphetamine hydroxylamine and other aliphastic hydroxylamines by benzamidoxime reductase and human liver microsomes. *Chem Res. Toxicol.*, In press.

Dannan GA, Guengerich FP and Waxman DJ (1986) Hormonal regulation of rat liver microsomal enzymes. Role of gonadal steroids in programming, maintenance and suppression of Δ-4-steroid-5-α-reductase, flavin-containing monooxygenase and sex-specific cytochrome P-450. *J. Biol. Chem.*, **261**, 10728–10735.

Decker CJ and Doerge DR (1991) Rat hepatic microsomal metabolism of ethylenethiourea. Contributions of the flavin-containing monooxygeanse and cytochrome P-450 isozymes. *Chem. Res. Toxicol.*, **4**, 482–489.

Decker CJ, Cashman JR, Sugiyama K, Maltby D and Correia MA (1991) Formation of glutathionyl-spironolactone disulphide by rat liver cytochromes P450 or hog liver flavin-containing monooxygenases: a functional probe of two-electron oxidations of the thiosteroid? *Chem. Res. Toxicol.*, **4**, 669–677.

Decker CJ, Doerge DR and Cashman JR (1992) Metabolism of benzimidazoline-2-thiones by rat

hepatic microsomes and hog liver flavin-containing monooxygenase. *Chem. Res. Toxicol.*, **5**, 726–733.

DiMonte D, Wu EY, Irwin I, Delanney LE and Langston JW (1991) Biotransformation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in primary cultures of mouse astrocytes. *J. Pharmacol. Exper. Therap.*, **258**, 594–600.

Doerge DR and Corbett MD (1984) Primary arylamine oxidation by a flavin hydroperoxide. *Biochem. Pharmacol.*, **33**, 3615–3619.

Dolphin CY, Shephard EA, Povey S, Palmer CN, Ziegler DM, Ayesh R, Smith RL and Phillips IR (1991) Cloning, primary sequence, and chromosomal mapping of a human flavin-containing monooxygenase (FMO1). *J. Bio. Chem.*, **266**, 12379–12385.

Dolphin CT, Shephard EA, Povey S, Smith RL and Phillips IR (1992) Cloning, primary sequence and chromosomal localizator of human FMO2, a new member of the flavin-containing monooxygenate family. *Biochem. J.*, **287**, 261–267.

Dolphin CT, McCombie RR, Smith RL, Shephard EA and Phillips IA (1996) Molecular genetics of human flavin-containing monooxygenases. *ISSX Proceed.*, **10**, 217.

Dolphin CT, Janomohamed A, Smith RL, Shephard EA and Phillips IR (1997a) Missense mutation in flavin-containing monooxygenase 3 gene, FMO3, underlies fish-odour syndrome. *Nat. Genet.*, **17**, 491–494.

Dolphin CT, Riley JH, Smith RL, Shephard EA and Phillips IR (1997b) Structural organisation of the human flavin-containing monooxygenase 3 gene, (FMO3), the favored candidate for fish-odour syndrome, determined directly from genomic DNA. *Genomics*, **46**, 260–267.

Dolphin CT, Beckett DT, Janmohamed A, Cullingford TE, Smith RL, Shephard EA and Phillips IR (1998) The flavin-containing monooxygenase 2 gene (FMO2) of humans, but not of other primates, encodes a truncated, nonfunctional protein. *J. Biol. Chem.*, **273**, 30599–30607.

Duffel MW, Graham JM and Ziegler DM (1981) Changes in dimethylaniline N-oxidase activity of mouse liver and kidney induced by steroid sex hormones. *Mol. Pharmacol.*, **19**, 134–139.

Fenwick GR, Butler EJ and Brewster MA (1983) Are brassica vegetables aggravating factors in trimethylaminura (fish odour syndrome)? [letter]. *Lancet*, **2**, 916.

Fernandez MS, Gutierrez C, Vila JJ, Lopez A, Ibanez V, Sanguesa C, Lluna J and Barrios JE (1997) Congenital intrahepatic portocaval shunt associated with trimethylaminuria. *Pediatr. Surg. Int.*, **12**, 196–197.

Fischer V and Wiebel FJ (1990) Metabolism of fluperlapine by cytochrome P450-dependent and flavin-dependent monooxygenases in continuous cultures of rat and human cells. *Biochem. Pharmacol.*, **39**, 1327–1333.

Fisher MB, Lawton MP, Atta-Asafo-Adjei E, Philpot RM and Rettie AE (1995) Selectivity of flavin-containing monooxygenase 5 for the (*S*)-sulfoxidethon of short-chain arakyl sulpfides. *Drug Metab. Dispos.*, **23**, 1431–1433.

Forrest SM, Knight M, Akerman BR, Cashman JR and Treacy EP (2000) A novel deletion in the flavin-containing monooxygenase gene (FMO3) in a Greek patient with trimethylaminuria. *Pharmacogenetics*, **11**, 169–174.

Gasser R, Tynes RE, Lawton MP, Korsmeyer KK, Ziegler DM and Philpot RM (1990) The flavin-containing monooxygenase expressed in pig liver: Primary sequence, distribution, and evidence for a single gene. *Biochemistry*, **29**, 119–124.

Gelb BD, Zhang J, Cotter PD, Gershin IF and Desnick RJ (1997) Physical mapping of the human connexin 40 (GJA5), flavin-containing monooxygenase and natriuretic peptide receptor a genes on 1q21. *Genomics*, **39**, 409–411.

Goldstein A and de Morais SM (1994) Biochemistry and molecular biology of the human CYP2C subfamily. *Pharmacogenetics*, **4**, 285–299.

Gonzalez FJ and Nebert DW (1990) Evolution of the P450 gene superfamily: animal–plant 'warfare', molecular drive and human genetic differences in drug oxidation. *Trends Genet.*, **66**, 182–186.

Grayson DH and Rous AJ (1987) Enzymetic preparation of optically-active suphoxides. In *RSC Meeting on Synthesis and Biosynthesis of Natural Products*, Edinburgh.

Guan S, Falick AM and Cashman JR (1990) *N*-terminus determination: FAD-and NADP+-binding domain mapping of hog liver flavin-containing monooxygenase by tandem mass spectrometry. *Biochem. Biophys. Res. Commun.*, **170**, 937–943.

Guan S, Falick AM, Williams DE and Cashman JR (1991) Evidence for complex formation between rabbit lung flavin-containing monooxygenase and calreticulin. *Biochemistry*, **30**, 9892–9900.

Guengerich FP, Kim DH and Iwasaki M (1991) Role of human cytochrome P450 IIE1 in the oxidation of several low molecular weight cancer suspects. *Chem. Res. Toxicol.*, **4**, 168–179.

Hadidi HF, Cholerton S, Atkinson S, Irshaid YM, Rawashdeh NM and Idle JR (1995) The N-oxidation of trimethylamine in a Jordanian population. *Br. J. Clin. Pharmacol.*, **39**, 179–181.

Hamman MA, Haehner-Daniels BD, Wrighton SA, Rettie AE and Hall SD (2000) Stereoselective sulfoxidation of sulindac sulphide by flavin-containing monooxygenase: Comparison of human liver and kidney microsomes and mammalian enzymes. *Biochem Pharmacol.*, **60**, 7–17.

Heinze E, Hlavica P and Kiese M (1970) N-oxygenation of arylamines in microsomes prepared from corpra lutea of the cycle and other tissues of the pig. *Biochem. Pharmacol.*, **19**, 641–649.

Hines RN, Cashman JR, Philpot RM, Williams DE and Ziegler DM (1994) The mammalian flavin-containing monooxygenases: molecular characterisation and regulation of expression. *Toxicol. Appl. Pharmacol.*, **125**, 1–6.

Hlavica P and Golly L (1991) On the genetic polymorphism of the flavin-containing monooxygenase. In *N-Oxidation of Drugs*, Hlavica P and Damani LA (eds), Chapman and Hall, London, pp. 71–90.

Hodgson E and Levi PE (1992) The role of the flavin-containing monooxygenase (EC 1.14.13.8) in the metabolism and mode of action of agricultural chemicals. *Xenobiotica*, **22**, 1175–1183.

Hodgson E, Cherrington N, Coleman SC, Liu S, Falls JG, Cao Y, Goldstein JE and Rose RL (1998) Flavin-containing monooxygenase and cytochrome P-450-mediated metabolism of pesticides: from mouse to human. *Rev. Toxicol.*, **6**, 231–243.

Jacob P III, Benowitz NL, Yu L and Shulgin AT (1986) Determination of nicotine N-oxide by gas chromatography following thermal conversion of 2-methyl-6-(3-pyridyl)tetrahydro-1,2-oxazine. *Anal. Chem.*, **58**, 2218–2221.

Kaderlik RF, Weser E and Ziegler DM (1991) Selective loss of liver flavin-containing monooxygenases in rats on chemically defined diets. *Prog. Pharmacol. Clin. Pharmacol.*, **3**, 95–103.

Kang JH, Chung WG, Lee KH, Park CS, Shin IC, Roh HK, Dong MS and Baek HM (2000) Phenotypes of flavin-containing monooxygenase activity determined by ranitidine oxidation are positively correlated with genotypes of linked FMO3 gene mutations in a Korean population. *Pharmacogenetics*, **10**, 67–78.

Katchamart S, Stresser DM, Dehal SS, Kupfer DD and Williams DE (2000) Concurrent flavin-containing monooxygenase down-regulation and cytochrome P-450 induction by dietary indoles in rat: implications for drug-drug interaction. *Drug Metab. Dispos.*, **28**, 930–936.

Kedderis GL and Rickert DE (1985) Loss of rat liver microsomal cytochrome P-450 during methimazole metabolism. Role of the flavin-containing monooxygenase. *Drug Metab. Dispos.*, **13**, 58–61.

Kemal C and Bruice TC (1976) Simple synthesis of a 4a-hydroperoxy adduct of a 1,5-dihydroflavine: Preliminary studies of a model for bacterial luciferase. *Proc. Natl. Acad. Sci. USA*, **73**, 995–999.

Kemal C, Chan TW and Bruice TC (1977) Reaction of $^3O_2$ with dihydroflavins. 1. $N^{3,5}$-dimethyl-1,5-dihydrolumiflavin and 1,5-dihydroisoalloxazines. *J. Am. Chem. Soc.*, **99**, 7272–7286.

Kim YM and Ziegler DM (2000) Size limits of thiocarbamides accepted as substrates by human flavin-containing monooxygenase 1. *Drug Metab. Dispos.*, **28**, 1003–1006.

Korsmeyer KK, Guan S, Yang ZC, Falick AM, Ziegler DM and Cashman JR (1998) N-Glycosylation of pig flavin-containing monooxygenase form 1: determination of protein modification by mass spectrometry. *Chem. Res. Toxicol.*, **11**, 1145–1153.

Kubo A, Itoh S, Itoh K and Kamataki T (1997) Determination of FAD-binding domain in flavin-containing monooxygenase. *Arch. Biochem. Biophys.*, **345**, 271–277.

Kupfer D and Sehal SS (1996) Tamoxifen metabolism by microsomal cytochrome P-450 and flavin-containing monooxygenase. *Methods Enzymol.*, **272**, 152–163.

Lang DH, Yeung CK, Peter RM, Ibarra C, Gasser R, Itagaki K, Philpot RM and Rettie AE (1998) Isoform specificity of trimethylamine N-oxygenation by human flavin-containing monooxygenase (FMO) and P450 enzymes: selective catalysis by FMO3. *Biochem. Pharmacol.*, **56**, 1005–1012.

Larsen-Su S and Williams DE (1996) Dietary indole-3-carbinol inhibits FMO activity and the expression of flavin-containing monooxygenase form 1 in rat liver and intestine. *Drug Metab. Dispos.*, **24**, 927–931.

Lawton MP and Philpot RM (1993) Functional characterisation of flavin-containing monooxygenase 1B1 expressed in *Saccharomyces cerevisiae* and *Escherichia coli* and analysis of proposed FAD- and membrane binding domains. *J. Biol. Chem.*, **268**, 5728–5734.

Lawton MP, Cashman JR, Cresteil T, Dolphin C, Elfarra A, Hines RN, Hodgson E, Kimura T, Ozols J, Phillips I, Philpot RM, Poulsen LL, Rettie AE, Williams DE and Ziegler DM (1994) A nomenclature for the mammalian flavin-containing monooxygenase gene family based on amino acid sequence identities. *Arch. Biochem. Biophys.*, **308**, 254–257.

Lemoine A, Williams DE and Cresteil T (1991) Hormonal regulation of microsomal flavin-containing monooxygenase: tissue-dependent expression and substrate specificity. *Mol. Pharmacol.*, **40**, 211–217.

Light DR, Waxman DJ and Walsh C (1982) Studies on the chirality of sulfoxidation catalysed by bacterial flavoenzyme cyclohexanone monooxygenase and hog liver flavin-adenine dinucleotide containing monooxygenase. *Biochemistry*, **21**, 2490–2498.

Lin J and Cashman JR (1997a) Detoxication of tyramine by the flavin-containing monooxygenase: stereoselective formation of the *trans* oxime. *Chem. Res. Toxicol.*, **10**, 842–852.

Lin J and Cashman JR (1997b) N-oxygenation of phenethylamine to the trans-oxime by adult human liver flavin-containing monooxygenase and retroreduction of phenethylamine hydroxylamine by human liver microsomes. *J. Pharmacol. Exp. Ther.*, **282**, 1269–1279.

Lomri N, Thomas J and Cashman JR (1993a) Expression in *Escherichia coli* of the cloned flavin-containing monooxygenase from pig liver. *J. Biol. Chem.*, **268**, 5048–5059.

Lomri N, Yang Z-C and Cashman JR (1993b) Expression in *Escherichia coli* of the flavin-containing monooxygenase from adult human liver. Determination of a distinct tertiary amine substrate specificity. *Chem. Res. Toxicol.*, **6**, 425–429.

Lomri N, Yang Z-C and Cashman JR (1993c) Regio- and stereoselective oxygenations by adult human liver flavin-containing monooxygenase 3. Comparison with forms 1 and 2. *Chem Res. Toxicol.*, **6**, 800–807.

Lu X, Li C and Fleisher D (1998) Cimetidine sulfoxidation in small intestinal microsomes. *Drug Metab. Dispos.*, **26**, 240–242.

Luo Z and Hines RN (1996) Identification of multiple rabbit flavin-containing monooxygenase form 1 (FMO1) gene promoters and observation of tissue-specific DNase I hypersensitive sites. *Arch. Biochem. Biophys.*, **336**, 251–260.

Luo Z and Hines RN (1997) Further characterisation of the major and minor rabbit FMO1 promoters and identification of both positive and negative distal regulatory elements. *Arch. Biochem. Biophys.*, **346**, 96–104.

Mattevi A, Schierbeek A and Hol W (1991) Refined crystal structure of lipoamide dehydrogenase from *Azobacter vinelandii* at 2.2 resolution. A comparison with the structure of glutathione reductase. *J. Mol. Biol.*, **220**, 975–994.

Mayatepek E and Kohlmuller D (1998) Transient trimethylaminuria in childhood. *Acta Paediatr. Int. J. Paediatr.*, **87**, 1205–1207.

McCombie RR, Dolphin CT, Povey S, Phillips IR and Shephard EA (1996) Localization of human flavin-containing monooxygenase genes FMO2 and FMO5 to chromosme 1q. *Genomics*, **15**, 426–429.

McConnell HW, Mitchell SC, Smith RL and Brewster M (1997) Trimethylaminuria associated with seizures and behavioural disturbance: a case report. *Seizure*, **6**, 317–321.

Miller A (1982) A model for FAD-containing monooxygenase: the oxidation of thioanisole derivatives by an isoalloxazine hydroperoxide. *Tetrahedron Lett.*, **23**, 753–756.

Miller A, Bischoff JJ, Bizub C, Luminoso P and Smiley S (1986) Electronic and steric effects in oxidations by isoalloxazine 4a-hydroperoxides. *J. Am. Chem. Soc.*, **108**, 7773–7778.

Mislow K, Green MM, Laur P, Melilillo JT, Simmons T and Ternay Jr AL (1965) Absolute

configuration and optical rotary power of sulphoxides and sulfinate esters. *J. Am. Chem. Soc.*, **87**, 1958–197.
Mitchell SC, Zhang AQ, Barrett T, Ayesh T and Smith RL (1997) Studies on the discontinuous N-oxidation of trimethylamine among Jordanian, Ecuadorian and New Guinean populations. *Pharmacogenetics*, **7**, 45–50.
Mizutani T, Yoshida K, Murakami M, Shirai M and Kawazoe S (2000) Evidence for the involvement of N-methylthiourea, a ring cleavage metabolite in the hepatotoxicity of methimazole in glutathione-depleted mice: structure-toxicity and metabolic studies. *Chem. Res. Toxicol.*, **13**, 170–176.
Nagata T, Williams DE and Ziegler DM (1990) Substrate specificities of rabbit lung and porcine liver flavin-containing monooxygenases: Differences due to substrate specificity. *Chem. Res. Toxicol.*, **3**, 372–376.
Osimitz TG and Kulkarni AP (1982) Oxidative metabolism of xenobiotics during pregnancy: significance of microsomal flavin-containing monooxygenases. *Biochem. Biophys. Res. Commun.*, **109**, 1164–1171.
Overby LH, Buckpitt AR, Lawton MP, Atta-Asafo-Adjei E, Schulze J and Philpot RM (1995) Characterization of flavin-containing monooxygenase 5 (FMO5) cloned from human and guinea pig: Evidence that the unique catalytic properties of FMO5 are not confined to the rabbit ortholog, *Arch. Biochem. Biophys.*, **317**, 275–284.
Overby LH, Carver GC and Philpot RM (1997) Quantitastion and kinetic properties of hepatic microsomal and flavin-containing monooxygenates 3 and 5 from humans. *Chem. Biol. Interact.*, **106**, 29–45.
Ozols J (1990) Covalent structure of liver microsomal flavin-containing monooxygenase form 1. *J. Biol. Chem.*, **265**, 10289–10299.
Ozols J (1990) Multiple forms of liver microsomal flavin-containing monooxygenases. *Arch. Biochem. Biophys.*, **290**, 103–115.
Ozols J (1994) Isolation and structure of a third form of liver microsomal flavin monooxygenase. *Biochemistry*, **33**, 3751–3757.
Park SB, Jacob P, Benowitz NL and Cashman JR (1993) Stereoselective metabolism of (*S*)-(-)-nicotine in humans: formation of *trans*-(*S*)-(-)-nicotine *N*-1′-oxide. *Chem. Res. Toxicol.*, **6**, 880–888.
Park CS, Chung WG, Kang JH, Roh HK, Lee KH and Cha YN (1999) Phenotyping of flavin-containing monooxygenase using caffeine metabolism of FMO3 gene in a Korean population. *Pharmacogenetics*, **9**, 155–164.
Phillips IR, Dolphin CT, Clair P, Hadley MR, Hutt AJ, McCombie RR, Smith RL and Shephard EA (1995) The molecular biology of the flavin-containing monooxygenases of man. *Chem. Biol. Interact.*, **96**, 17–32.
Philpot R, Overby L and Wyatt M (1996) The flavin-containing monooxygenase gene family: structure and function. *ISSX Proceed.*, **10**, 3.
Pike MG, Martin YN, Mays DC, Benson LM, Naylor S and Lipsky JJ (1999) Roles of FMO and CYP450 in the metabolism in human liver microsomes of S-methyl-*N*,*N*-diethyldithiocarbamate, a disulfiram metabolite. *Alcohol Clin. Exp. Res.*, **23**, 1173–1179.
Pitchen PE, Dunach MN, Deshmukh E and Kagan HB (1984) An efficient asymmetric oxidation of sulphides to sulphoxides. *J. Am. Chem. Soc.*, **106**, 8188–8193.
Piyapolrungroj N, Zhou YS, Li C, Liu G, Zimmermann E and Fleisher D (2000) Cimetidine absorption and elimination in rat small intestine. *Drug Metab. Dispos.*, **28**, 65–72.
Poulsen LL and Ziegler DM (1979) The liver microsomal FAD-containing monooxygenases. Spectral characterisation and kinetic studies. *J. Biol. Chem.*, **254**, 6449–6455.
Redondo PA, Alvarez AI, Garcia JL, Larrode OM, Merino G and Prieto JG (1999) Presystemic metabolism of albendazole: experimental evidence of an efflux of albendazole sulfoxide to intestinal lumen. *Drug Metab. Dispos.*, **27**, 736–740.
Rettie AE and Lang DH (2000) Can caffeine metabolism be used as an in-vivo probe for human flavin-containing monooxygenase activity? *Pharmacogenetics*, **10**, 275–277.
Rettie AE, Bogucki BD, Lim I and Meier GP (1990) Stereoselective sulfoxidation of a series of alkyl *p*-tolyl sulphides by microsomal and purified flavin-containing monooxygenases. *Mol. Pharmacol.*, **37**, 643–651.

Rettie AE, Lawton MP, Sadeque AJ, Meier GP and Philpot RM (1994) Prochiral sulfoxidation as a probe for mulitple forms of the microsomal flavin-containing monooxygeanse: studies with rabbit FMO1, FMO2, FMO3, and FMO5 expressed in *Escherichia coli*. *Arch. Biochem. Biophys.*, **311**, 369–377.

Rettie AE, Meier GP and Sadeque AJ (1995) Prochiral sulphides as in vitro probes for multiple forms of the flavin-containing monooxygenase. *Chem. Biol. Interact.*, **96**, 3–15.

Ring BJ, Wrighton SA, Aldridge SLK, Hansen K, Haehner B and Shipley LA (1999) Flavin-containing monooxygenase-mediated *N*-oxidation of the M(1)-muscarinic agonist xanomeline. *Drug Metab. Dispos.*, **27**, 1099–1103.

Ripp SL, Itagaki K, Philpot RM and Elfarra AA (1999) Species and sex differences in expression of flavin-containing monooxygenase and kidney microsomes. *Drug Metab. Dispos.*, **27**, 46–52.

Rodriguez RJ, Davila JC and Acosta D (1996) Establishment of flavin-containing monooxygenases in hepatic microsomes obtained from primary cultures of rat hepatocytes. *Fund. Appl. Toxicol.*, **30**, 215.

Rodriguez RJ, Proteau PJ, Marquez BL, Heatherington CL, Buckholz CJ and O'Connell KL (1999) Flavin-containing monooxygenase-mediated metabolism of N-deacetyl ketoconazole in hepatic microsomes. *Drug Metab. Dispos.*, **27**, 880–886.

Ryerson CC, Ballou DP and Walsh C (1982) Mechanistic studies on cyclohexanone oxygenase. *Biochemistry*, **21**, 2644–2655.

Sachse C, Ruschen S, Dettling M, Schley J, Bauer S, Muller-Oerlinghausen B, Roots I and Brockmoller J (1999) Flavin monooxygenase 3 (FMO3) polymorphism in a white population: all mutation linkage, functional effects on clozapine and caffeine metabolism. *Clin. Pharmacol. Ther.*, **66**, 431–438.

Shehin-Johnson SE, Williams DE, Larsen-Su S, Stresser DM and Hines RN (1995) Tissue-specific expression of flavin-containing monooxygenase (FMO) forms 1 and 2 in the rabbit. *J. Pharmacol. Exper. Therap.*, **272**, 1293–1299.

Shehin-Johnson SE, Palmer KC and Hines RN (1996) Identification of tissue-specific DNase I hypersensitive sites in the flavin-containing monooxygenase form 2 gene. *Drug Metab. Dispos.*, **24**, 891–898.

Sherratt AJ and Damani LA (1989) Activities of cytosolic and microsomal drug oxidases of rat hepatocytes in primary culture. *Drug Metab. Dispos.*, **17**, 20–25.

Stransky L (1998) Fishy odour syndrome after acute hepatitis B. *Dermatosen Beruf Umwelt*, **46**, 79–80.

Suh JK, Poulsen LL, Ziegler DM and Tobertus JD (1996) Molecular cloning and kinetic characterisation of a flavin-containing monooxygenase from *Saccharomyces cerevisiae*. *Arch. Biochem. Biophys.*, **336**, 268–274.

Thieme R, Pai E, Schrimer R and Schulz G (1981) Three-dimensional structure of glutathione reductase at 2Å resolution. *J. Mol. Biol.*, **152**, 763–782.

Thithapadha A (1997) A pharmacogenetic study of trimethylaminuria in Orientals. *Pharmacogenetics*, **7**, 497–501.

Todd WA (1979) Psychosocial problems as the major complication of an adolescent with trimethylaminuria. *J. Pediatr.*, **94**, 936–937.

Treacy EP, Akerman BR, Chow LML, Youil R, Bibeau C, Lin J, Bruce AG, Knight M, Danks DM, Cashman JR and Forrest SM (1998) Mutations of the flavin-containing monooxygenase gene (FMO3) cause trimethylaminuria, a defect in detoxication. *Hum. Mol. Genet.*, **7**, 839–845.

Tucker GT, Silas JH and Iyun AO (1977) Polymorphic hydroxylation of debrisoquine. *Lancet*, **2**, 718.

Tugnaut M, Haives EM, McKay G, Rettie AE, Harning RL and Midha KK (1997) *N*-oxygenation of dozapine by flavin-containing monooxygenase. *Drug metab. Dispos.*, **25**, 524–527.

Tynes RE, Sabourin PJ and Hodgson E (1985) Identification of distinct hepatic and pulmonary forms of microsomal flavin-containing monooxygenase in the mouse and rabbit. *Biochem. Biophys. Res. Commun.*, **126**, 1069–1075.

Ubeaud G, Schiller CD, Hurbin F, Jaeck D and Coassolo P (1999) Estimation of flavin-containing monooxygenase activity in intact hepatocytes of rat, hamster, rabbit, dog and human by using N-oxidation of benzydamine. *Eur. J. Pharm. Sci.*, **4**, 255–260.

Vallon O (2000) New sequence motifs in flavoproteins: evidence for common ancestry and structure. *Proteins*, **38**, 95–114.
Williams DE, Ziegler DM, Nordin DJ, Hale SE and Masters BSS (1984) Rabbit lung flavin-containing monooxyenase is immunologically and catalytically distinct from the liver enzyme. *Biochem. Biophys. Res. Commun.*, **125**, 116–122.
Wirth PJ and Thorgeirsson SS (1978) Amine oxidase in mice-sex difference and developmental aspects. *Biochem. Pharmacol.*, **19**, 601–603.
Wyatt MK, Philpot RM, Carver G, Lawton MP and Nikbakht KN (1996) Structural characteristics of flavin-containing monooxygenase genes one and two (FMO1 and FMO2). *Drug Metab. Dispos.*, **24**, 1320–1327.
Wyatt MK, Overby LH, Lawton MP and Philpot RM (1998) Identification of amino acid residues associated with modulation of flavin-containing monooxygenase (FMO) activity of imipramine: structure/function studies in pig and rabbit. *Biochemistry*, **37**, 5930–5938.
Zhang AQ, Mitchell SC and Smith RL (1996) Exacerbation of symptoms of fish-odour syndrome during menstruation [letter]. *Lancet*, **348**, 1740–1741.
Ziegler DM (1980) Microsomal flavin-containing monooxygenase: oxygenation of nucleophilic nitrogen and sulfur compounds. In *Enzymatic Basis of Detoxication, Volume 1*, Jakoby WB (ed.), Academic Press, New York, pp. 201–277.
Ziegler DM (1988) Flavin-containing monooxygenase: catalytic mechanism and substrate specificities. *Drug Metab. Rev.*, **19**, 1–31.
Ziegler DM (1990) Flavin-containing monooxygenases: enzymes adapted for multisubstrate specificity. *Trends Pharmacol. Sci.*, **11**, 321–324.
Ziegler DM (1993) Recent studies on the structure and function of multisubstrate flavin-containing monooxygenases. *Ann. Rev. Pharmacol. Toxicol.*, **33**, 179–199.
Ziegler DM (1999) Flavin-containing monooxygenase family of isozymes. Abstract, Proc. of the First International Workshop on Trimethylaminuria, Bethesda, MD, March 29–30.
Zschocke J, Kohlmueller D, Quak E, Meissner T, Hoffmann GF and Mayatepek E (1999) Mild trimethylaminuria caused by common variants in FMO3 gene. *Lancet*, **354**, 834–835.