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13 Methyltransferases

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Introduction

The transfer of methyl groups from *S*-adenosyl-L-methionine (AdoMet) to methyl acceptor substrates is one of the most extensive reactions in nature. The consequences of the methyl-transfer reaction are attested to by the great diversity of the methyl-acceptor substrates found in biological systems. These methyl-transfer reactions almost exclusively utilise *S*-adenosyl-L-methionine (AdoMet) as the methyl-donor co-substrate. The transfer of the methyl group occurs to a sulphur-, nitrogen-, or oxygen-nucleophile. The conjugation reactions include, *O*-, *N*- and *S*-methyltransferases. At present more that 100 methyltransferases have been identified. These methyltransferases catalyse the methylation of a diverse group of small molecules, either as drugs, xenobiotics and endogenous hormones and neurotransmitters, as well as macromolecules including proteins, lipids (Hirata, 1982), RNA and DNA. A series of functionally significant, genetic polymorphisms have been discovered for many methyltransferases, some of which have critical importance in clinical therapeutics. The structure and function of selected methyltransferases is described including catechol-*O*-methyltransferase (COMT), phenethanolamine-*N*-methyltransferase (PNMT), histamine-N-methyltransferase (HNMT), and the cytosolic-*S*-methyltransferases, thiopurine-*S*-methyltransferase (TPMT) and thioether-*S*-methyltransferase (TEMT) and finally a membrane bound thiomethyltransferase (TMT).

The co-substrate: *S*-Adenosylmethionine

Any consideration of methyltransferases must begin with an understanding of the nature of the co-substrate AdoMet. AdoMet is the methyl donor of virtually all methyltransferases. Since its discovery in 1953 (Cantoni 1953), AdoMet has been demonstrated in an increasing number of transmethylation reactions, many critical for the survival of cells. AdoMet is formed by the condensation of ATP and L-methionine catalysed by ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6) (MAT). MAT is both a synthetase and a tripolyphosphatase resulting in a complete but asymmetric depho-

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sphorylation of ATP, followed by the release of inorganic pyrophosphate as well as phosphate. The enzyme requires divalent cations for its activity, and is activated by monovalent cations (Green 1969; Lombardini and Talalay 1971). The energy from the dephosphorylation of ATP is transferred to the energy-rich sulphonium complex that contains the reactive methyl group. The product synthesised by the enzymic condensation, AdoMet, has (S) configuration at the sulphonium centre (Conforth *et al*. 1977; de la Haba *et al*. 1959). The same diastereomer with *S*-configuration is required for all the methyl-transfer reactions (de la Haba *et al*. 1959; Zapia *et al*. 1969; Borchardt *et al*. 1976a). Permitted structural changes in AdoMet are limited. Any change in the base, sugar or the amino acid moiety causes a dramatic increase in the K_m values for the methyltransferases (Borchardt *et al*. 1976b).

Upon donating its methyl group, AdoMet is converted to *S*-adenosylhomocysteine (AdoHcy) which then is hydrolysed to adenosine and homocysteine. Homocysteine can undergo remethylation to methionine and re-enter the trans-methylation cycle. It should be noted that AdoHcy, which is a potent, competitive inhibitor of the methyltransferases, has no effect on the MAT reaction. The role of MAT in the level and control of AdoMet synthesis is complex. There are two major mammalian forms of MAT, which differ in both tissue distribution and kinetic properties. The products AdoMet, pyrophosphate, and phosphate regulate the reaction, but the nature of the regulation, feedback inhibition or stimulation varies depending upon the MAT isoenzyme (Kotb and Kredich 1990). For a recent review of the structure and function of MAT see Kotb and Geller (1993).

Methionine, the primary initiator and source of AdoMet, is evident from a rapid increase in the levels of AdoMet in various tissues after administration of methionine, either orally or intraperitoneally (Regina *et al*. 1993). It should be noted that MAT activity in various organs of rats does not correlate well with the levels of AdoMet. While the specific activity of MAT (pmole product/min/mg protein) ranged from a low value of 40 in the heart and brain of rats to a high value of 7700 in the liver, an over 180-fold increase, the concentrations of AdoMet ranged from a low of 25 (nmoles/g of tissue) in the brain to a high of 68 in the liver (less than 3-fold increase) (Eloranta 1977). Similarly, despite 13-fold higher specific activity of MAT in the pancreas over that in the heart, the levels of AdoMet in both the tissues were similar. Thus, the complex nature of the MAT isoforms has important roles in controlling the levels of AdoMet in different tissues and organs (Kotb and Geller, 1993). Furthermore, drugs or other xenobiotics that can be methylated by AdoMet-dependent methyltransferases have been shown to affect the levels of AdoMet in various tissues (Fuller *et al*. 1983; Borchardt *et al*. 1976b). While the decreased levels in AdoMet are generally transient, the administration of high levels of quercetin or fisetin, both excellent substrates for COMT, resulted in a decrease of kidney AdoMet levels by 25%. As a consequence of both the reduction in AdoMet levels and the increase in adenosine homocysteine levels, an inhibition of the *O*-methylation of catecholoestrogens could be demonstrated (Zhu and Liehr 1996).

An overview: *O*-methylation

A major AdoMet-dependent methyltransferase is the *O*-methylation reaction. This reaction includes the catechol-*O*-methyltransferase catalysed *O*-methylation of the catechols

dopamine, norepinephrine, epinephrine, L-DOPA and the catecholoestrogens. Another enzyme, hydroxyindole *O*-methyltransferase (EC 2.1.1.4), catalyses the *O*-methylation of the phenolic group of N-acetylserotonin to form melatonin (Axelrod and Weissbach 1961). An important *O*-methylation reaction is catalysed by protein-carboxy *O*-methyltransferase (EC.2.1.1.24). This reaction catalyses the esterification of aspartic and glutamic acid residues on many proteins. Methylation and demethylation may serve as signals to reversibly affect the three-dimensional configuration of proteins and thus their function (Kim *et al*. 1982). Since COMT is the first and, at present the best, described *O*-methyltransferase a more complete review of this methyltransferase is given below.

CATECHOL-O-METHYLTRANSFERASE

Catechol-*O*-methyltransferase (EC 2.1.1.6) (COMT) was first described by Axelrod and Tomchick (1958). At present the enzyme has been cloned, the genetic locus determined, crystallised, the molecular structure of the two isoforms determined, the nature of the active site formulated, effective inhibitors developed, and the tissue localisation, both in the brain and in peripheral tissues, has been described. These developments have been the subject of several extensive reviews (Guldberg and Marsden 1975; Creveling and Hartman 1982; Thakker and Creveling 1990; Creveling and Thakker 1994; Lundstrom *et al*. 1995; Mannisto and Kaakkola 1999).

STRUCTURE OF COMT

COMT is derived from a single gene localised on chromosome 22, band q11.2 (Grossman *et al*. 1992) which codes for both the soluble (S-COMT) and the membrane-bound (MB-COMT) form of the enzyme. S-COMT contains 221 amino acids with a molecular mass of 24.4 kDa. MB-COMT contains an additional 50 amino acids with a molecular mass of 30 kDa. Of the extra amino acids, 20 function as a hydrophobic membrane anchor (Lundstrom *et al*. 1995; Salminen *et al*. 1990). S-COMT is quantitatively the predominant form in peripheral tissues while MB-COMT accounts for 70% in the brain (Tenhunen *et al*. 1994). The MB-COMT is bound by the hydrophobic anchor to the cytoplasmic side of intracellular membranes with the remainder of the enzyme suspended in the cytoplasm (Bertocci *et al*. 1991).

A detailed description of the atomic structure of S-COMT from rat has been derived from the crystallised enzyme at a 1.7 to 2.0-Å resolution (Vidgren and Ovaska 1997; Vidgren *et al*. 1994). COMT has a single domain of eight α-helices around a central β-sheet. The active site is located in a groove on the outer surface of the enzyme. The binding site for the methionine of AdoMet is deeper within the protein and is bound first followed by Mg^{2+}, which promotes the ionisation of one of the hydroxyl groups of the catechol. Mg^{2+} forms an octahedral coordination structure with aspartic acids (Asp141 and 169), asparagine (Asn170), a water molecule and the hydroxyls of the catechol substrate. This configuration controls the orientation of the catechol. At the surface of the protein, leading to the active site is a hydrophobic slot composed of 'gatekeeper' residues Trp38, Trp143 and Pro174. This configuration interacts with the side chains of various substrates and keeps the catechol ring in a planar position. A lysine (Lys144), acting as a general catalytic base, accepts a proton from the ionised

hydroxyl followed by transfer of the methyl group from AdoMet to that hydroxyl group. The methyl transfer results from a direct nucleophilic attack by one of the hydroxyl groups of the catechol on the methyl carbon of AdoMet in a tight SN2 transition state (Woodard *et al*. 1980).

THE SUBSTRATES

COMT catalyses transfer of the methyl group from AdoMet to either the *meta*- or the *para*-hydroxyl group of virtually all substituted catechol derivatives to form a monomethyl ether. The enzyme accommodates catechol substrates with positively charged, negatively charged or neutral substituents (Creveling *et al*. 1970, 1972). Recent studies using recombinant enzyme isoforms have clearly demonstrated that the kinetic differences are due to interactions of the substrate sidechains with COMT (Lotta *et al*. 1992). The preference for *para*-*O*-methylation is exhibited by catechols that contain electronegative substituents like nitro, cyano or fluoro groups. These derivatives are poor substrates but can be potent inhibitors (Thakker *et al*. 1986; Backstrom *et al*. 1989). The *meta/para* ratios of substituted catechols are a consequence of their ability to bind in two dissimilar orientations in the active site (Lan and Bruice 1998).

The endogenous substrates include: the catecholamines, dopamine, norepinephrine and epinephrine and their metabolites which retain the catechol moiety; L-dopa; 2- and 4-hydroxycatechol oestrogens and oestrones (Cavalieri and Rogan 2000; Raftogianis *et al*. 2000) and the dihydroxyindolic intermediates in melanin formation (Smit *et al*. 2000) and the alkaloids, hydroxyisoquinolines and apomorphine. Many pharmacologic agents are substrates for COMT including isoprenaline, α-methyldopa, carbidopa, benserazide, β-lactam antibiotics with a 3,4-dihydroxybenzoyl-functionality, dihydroxyphenylserine and dobutamine. Even drug molecules that contain a single phenolic moiety, which are not subject to *O*-methylation, can become substrates for COMT after hydroxylation *ortho* to the phenolic group by cytochrome P450-dependent hydroxylases. Successive oxidation followed by *O*-methylation results in the metabolism of many phenolic steroids, phenethylamine, and hydroxyindoles (Thakker and Creveling 1990).

Most xenobiotics ranging from dihydroxybenzene to complex polycyclic catechols of plant origin are COMT substrates. Of particular interest are flavonoids containing a catechol moiety. Several polyphenols, water-soluble bearing catechol groups from tea, have been shown to be excellent substrates form COMT (Zhu *et al*. 2000). Of particular interest are flavonoids such as quercetin and fisetin, which are rapidly *O*-methylated by COMT. Such compounds have been shown to be mutagenic *in vivo* by the Ames test and other indicators. However, *in vivo*, these compounds are so rapidly *O*-methylated by COMT and other conjugation reactions that they do not enter a mutagenic pathway (Zhu *et al*. 1994).

THE FUNCTIONS OF COMT IN VIVO

The functions of COMT *in vivo* have become more apparent with the expanding knowledge of the specific cellular localisation of the enzyme (Inoue *et al*. 1977;

Creveling and Hartman 1982; Creveling 1984, 1988; Karhunen *et al*. 1994, 1995).

Several general aspects about COMT can now be appreciated. First, while there is a wide variation in the level of COMT activity in various tissues, among species and strains, the individual level of activity in most tissues and strains show great similarities. In most cases COMT activity increases rapidly from relatively low levels at birth to a characteristic level early in life and remains essentially constant throughout the adult life (Goldstein *et al*. 1980). Second, in certain tissue sites such as the epithelial lining of the uterus and in the ductal epithelium of breast, the level of COMT activity undergoes marked increases in response to pregnancy, lactation and oestrus (Inoue and Creveling 1991, 1995; Amin *et al*. 1983; Creveling 1984).

Elevated levels of COMT are present in breast adenocarcinomas in women, mouse and rat (Assicot *et al*. 1977; Amin *et al*. 1983; Hoffman *et al*. 1979), and in β-islet insulinomas of rat and hamster. COMT activity is relatively constant and characteristic of the individual at most sites in the adult animal. However, in certain cell types, the epithelial cell of uterus and breast, COMT activity increases in response to hormonal or physiological cues (Inoue and Creveling 1995). These observations strongly suggest that, in addition to the now classical function of COMT in the inactivation of circulating of catecholamines in the liver, originally described by Axelrod and his coworkers (Axelrod and Tomchick 1958), COMT appears to have a much wider role in the control of the level and distribution of substances bearing the reactive catechol moiety.

COMT is an important determinant in the effective use of L-DOPA for the symptomatic therapy of Parkinson's disease. With the discovery of dopaminergic cell loss, the accompanying decrease in striatal dopamine and the introduction of L-DOPA as an effective means for the restoration of central dopamine stores, there has been increasing attention directed towards the COMT-catalysed formation of 3-O-methyl-DOPA (OMD). OMD is a major metabolite of L-DOPA and its formation is increased with higher levels of L-DOPA achieved in the presence of the peripheral decarboxylase inhibitor, carbidopa. OMD formation after the oral administration L-DOPA is of special significance due to the relatively high levels of both S-COMT and MB-COMT in the gut wall (Nissenin *et al*. 1988). OMD competes with L-DOPA with greater affinity for the neutral amino acid transport system in both gut and at the blood-brain barrier, and furthermore OMD has a longer biological half-life than L-DOPA. A new class of inhibitors has been developed which are selective and potent inhibitors of COMT. One derivative, OR-462 [(3-(3,4-dihydroxy-5-nitrobenzylidene)-2,4-pentanedione], effectively inhibits COMT activity in the gut wall for up to 10 hours when given orally to rats. (Nissenin *et al*. 1988). When given with L-DOPA, it produces a long lasting inhibition of OMD formation both peripherally and in the striatum. With this inhibitor, equivalent levels of striatal dopamine were achieved with one-forth as much L-DOPA compared to controls given L-DOPA alone. An extensive review of the current studies on the biochemistry, toxicology and therapeutic applications of this group of nitrocatechols, has been published. This review includes the status of the available therapeutic agents, Tolcapone, Netcapone and Entacpone, and their use in the clinical treatment of Parkinson's disease (Mannisto and Kaakkola 2000).

THE CELLULAR DISTRIBUTION OF COMT

The distribution of COMT in brain is divided between the high-affinity, MB-COMT and the cytosolic S-COMT. MB-COMT accounts for approximately 70% of brain COMT. A specific polyvalent antisera to both MB- and S-COMT has been used for cell-specific immunochemical localisation of COMT (Inoue *et al*. 1977; Grossman *et al*. 1985). COMT is present in the cytoplasm of the ciliated, cuboidal cells of the ventricular ependyma along the borders of the lateral, 3rd and 4th ventricles of the rat brain, perhaps as a barrier between the CSF and the brain parenchyma (Kaplan *et al*. 1979, 1981a). In the brain parenchyma proper, COMT is found primarily in glial elements, oligodendrocytes and fibrous astrocytes. Spatz *et al*. (1986) showed that the endothelium of cerebral capillaries, arterioles, and larger vessels contained COMT. Thus COMT, in conjunction with MAO, may provide cerebral capillaries with an enzymic barrier for the passage of catechols. In the cerebellum, in addition to glial elements, the cell bodies of Bergmann cells, adjacent to S-COMT-negative Purkinje cells, contained S-COMT as did the Bergmann fibres ascending through the molecular layer to the pial surface (Kaplan *et al*. 1981b).

Immunological localisation of COMT in the uterus, oviduct, placenta, mammary gland, and vas deferens and seminal vesicle has led to a greater appreciation of the role of COMT in the reproductive process (Inoue *et al*. 1977; Amin *et al*. 1983; Inoue and Creveling 1986, 1991, 1995).

Of interest is the presence of elevated levels of COMT in mammary glands, human breast tumours and the apparent positive relationship between the COMT activity and the grade of malignancy in primary carcinomas (Assicot *et al*. 1977; Creveling and Inoue, 1994), and the demonstration of *de novo* synthesis of catecholsteroids in breast tumours (Hoffman *et al*. 1979; Raftogianis *et al*. 2000).

POLYMORPHISM OF COMT

The levels of COMT activity were shown to be controlled by a common genetic polymorphism over 20 years ago (Weinshilboum and Raymond 1977). The phenotypic trait of low COMT was detected as present in approximately 25% of a caucasian population. Subsequent molecular pharmacogenetic studies have identified a single nucleotide polymorphism in the COMT gene that results in a Val 108/Met substitution in S-COMT. This amino acid substitution results in a enzyme with low activity. The frequency occurrence of each allele is approximately 50%. The variation in these alleles has been determined in a variety of racial groups and various pathologies (Weinshilboum *et al*. 1999). For example, of great interest is the possible relationship of the low activity form of COMT and the pathophysiology of breast cancer (Lavigne *et al*. 1977; Thompson *et al*. 1998; Millikan *et al*. 1998; Thompson and Ambrosone 2000; Yager 2000).

CONCLUSION

In conclusion, it should be emphasised that methyltransferases and the methyl-donor cosubstrate, SAM, play a pivotal role in diverse biological systems. In this chapter, the

role of one such methyltransferase, COMT has been discussed in considerable detail. It appears that the role of COMT is more extensive than just the inactivation of catechol xenobiotics, circulating catecholamines, and catecholamine neurotransmitters. The function of COMT in the reproductive system, the presence of sexual dimorphism with regard to COMT, the physiological and neoplastic alterations in the activity of COMT, and the extensive localisation of COMT, clearly point to a significant role in the inactivation of catechol oestrogens, as a barrier for the passage of catechols between tissue compartments, and the control of other, as yet unrecognised, catechol-mediated functions. The recent development by Orion Pharmaceutica of Finland of a series of derivatives of 3-nitrocatechol appears to have provided the research community with a selection of specific, long-lasting, essentially irreversible inhibitors of COMT.

N-Methyltransferases: an overview

N-Methylation is a prominent pathway for the metabolism of many endogenous hormones and neurotransmitters. It is an important metabolic step in the transformation of molecules containing primary, secondary, or tertiary amino groups. As is the case with *O*-methylation, *N*-methylation also occurs by the transfer of a methyl group from AdoMet to nucleophilic amino groups. The resultant products are the *N*-methylated metabolites and AdoHcy. Amine-*N*-methyltransferase catalyses the *N*-methylation of amines with a very wide variety of structures. Despite its broad substrate selectivity, it does not catalyse such important molecules as histamine and norepinephrine. Interestingly, the highly specific enzyme, phenethanolamine-*N*-methyltransferase, a biosynthetic enzyme, is responsible for the *N*-methylation of norepinephrine to form epinephrine. Equally specific is histamine-*N*-methyltransferase, which catalyses the *N*-methylation of histamine. It is of interest that histamine may exhibit genetic polymorphism. There are many *N*-methyltransferases in the liver and gastrointestinal mucosa which *N*-methylate both exogenous compounds as well as endogenous compounds. Nicotinamide-*N*-methyltransferase activity exhibits wide variations in both human and animal liver and is of interest in that it may have genetic polymorphisms. The *N*-methylation reaction plays an important role in the metabolic inactivation of drugs since a large number of drug molecules contain an amino functionality. It should be noted that in selected cases, *N*-methylation may result in the formation of active metabolites. This is best illustrated by the conversion of apomorphine to the pharmacologically active morphine. *N*-methylation of 4-aminoazobenzene followed by biological oxidation results in the formation of the *N*-hydroxyarylamine that is a proximate carcinogen (Ziegler *et al*. 1988).

PHENETHANOLAMINE-*N*-METHYLTRANSFERASE (PNMT)

PNMT is the terminal enzyme in the biosynthetic pathway for the catecholamines and catalyses the *N*-methylation of norepinephrine to yield epinephrine. It is located primarily in the adrenal medulla but smaller amounts are also present in small intensity fluorescent cells of sympathetic ganglia and sensory nuclei of the vagus nerve. Peripheral PNMT is responsive to glucocorticoids. In the brain, PNMT is found

in small cell groups in the medulla oblongata, hypothalamus, amygdala, and in retinal and amacrine cells of the retina. PNMT is highly specific for phenethanolamines and does not accept phenethylamines not bearing a β-hydroxyl group (Rafferty and Grunewald 1982). The endogenous substrates are limited to norepinephrine and epinephrine. Curiously, certain compounds in which the aromatic ring is replaced by cyclohex-3-enyl, cyclohexyl or cyclooctyl rings are good substrates for PNMT. An extended series of investigations seeking effective substrates or inhibitors have been published. The goal of these investigations is through inhibition of central PNMT to determine the possible function of the enzyme in brain (Liang *et al*. 1982).

The nucleotide sequence and the deduced amino acid sequence of bovine PNMT was reported and a full-length clone isolated using PNMT mRNA (Joh *et al*. 1983; Baetge *et al*. 1986). Subsequently, the PNMT-coding sequence and amino acid sequence for human PNMT were published (Kane *et al*. 1988). The human enzyme consists of 282 amino acids with a molecular mass of 30.9 kDa. The gene for human PNMT is assigned to a single gene on chromosome 17. The protein has binding sites for glucocorticoid regulatory elements. The central PNMT is not regulated by glucocorticoids. It should be noted that the limited amounts and substrate specificity makes it unlikely that PNMT plays an important role in the *N*-methylation of drugs or dietary xenobiotics.

HISTAMINE-*N*-METHYLTRANSFERASE (HNMT)

HNMT is a cytosolic, monomeric, AdoMet-dependent enzyme. A cDNA clone of 1.3 kb has been derived from rat kidney consisting of a coding region of 876 nucleotides and an amino acid sequence of 292 residues for a molecular mass of 34 kDa. Expression in *E. coli* yielded a catalytically active transferase (Takamura *et al*. 1992). Northern Blot analysis established that HNMT is widely expressed in human tissues. HNMT is approximately 34 kb in length and mapped to human chromosome 2 (Aksoy *et al*. 1996). HNMT is a very specific methyltransferase and will *N*-methylate only histamine derivatives in which positions 1, 2, and 3 are unsubstituted (Ansher and Jakoby 1990). Further substrates must have a positive charge on the side chain. Histamine derivatives with a negative charge on the side chain are not substrates. The methyl group transfer from AdoMet to histamine is by direct transfer to the histamine nitrogen and is accompanied by inversion of the configuration (Asano *et al*. 1984).

Histamine is exclusively metabolised in the brain by transfer of the methylgroup from AdoMet to form *N*-methylhistamine, followed by oxidative deamination by monoamine oxidase B. The enzyme is localised primarily in neurons and the vascular walls of vessels in the brain. The localisation of HNMT in the blood vessel walls suggests that vascular levels of histamine and histamine released from mast cells associated with blood vessels are metabolised locally. Within the brain, the cell bodies of histaminergic neurons are present in the tuberomannillary nucleus of the posterior hypothalamus. Fibres from this nucleus are distributed widely throughout the brain in a manner similar to the distribution of catecholamine fibres from the locus ceruleus (Watanabe *et al*. 1984). In peripheral sites, primarily the bronchi, HNMT has been shown to be a major metabolic pathway. This localisation may be important since histamine is an important factor in asthma and allergies (Wasserman 1983).

S-Methylation: an overview

Unlike *O*- and *N*-methylation reactions which have many biosynthetic roles as well as interactions with xenobiotics, the AdoMet-dependent *S*-methylases appear to be associated primarily with the metabolic inactivation function. The cytosolic sulphotransferases catalyse sulphoconjugation of relatively small lipophilic endogenous compounds and xenobiotics (see Chapter 10). Recent reviews suggest that there are at least forty-four cytosolic sulphotransferases, which have been identified in mammalian tissues. The identification of the sulphotransferases is based upon their amino acid sequences, and the enzymes constitute five different families which are localised on at least five different chromosomal sites. Most sulphotransferases are active in the sulphation of various xenobiotics and well as such endogenous compounds like oestrogens, corticoids, and thyroxin. An understanding of sulphotransferases is complicated both by the overlapping nature of the substrate specificity and a non-uniform nomenclature. The relationship between the cytosolic and membrane bound sulphotransferases is at present unclear. The biochemistry and pharmacogenetics have been extensively reviewed (Weinshilboum 1989a, 1989b) and more recently by Nagata and Yamazoe (2000).

THIOPURINE-*S*-METHYLTRANSFERASE (TPMT)

A major group of cytosolic sulphotransferases catalyses the methylation sulphydryl groups to form methyl thioether products. A characteristic thiopurinemethyltransferase (TPMT, 2.1.1.67) catalyses the methylation of the sulphur atom in aromatic and heterocyclic thiols such as 6-mercaptopurine. TPMT exhibits genetic polymorphism where approximately 5–10% people are heterozygotic at the TPMT gene locus and have intermediate enzyme activity, while less than 1% inherit two mutant TPMT alleles and are TPMT deficient. The role of TPMT is clinically important in the treatment of acute lymphoblastic leukaemia with 6-mercaptopurine. Patients with homozygous and heterozygous deficiency for TPMT have extreme sensitivity to 6-mercaptopurine due to the accumulation of thioguanine nucleotides. Unless such patients are treated with lower doses of 6-mercaptopurine, they develop profound haematopoietic toxicity that can be fatal. Thus determination of the patients TPMT status is essential (Relling *et al*. 1999).

THIOETHER-*S*-METHYLTRANSFERASE (TEMT)

An interesting cytosolic mammalian *S*-methylase, thioether *S*-methyltransferase (TEMT, 2.1.1.96), was discovered in a search for the biosynthetic source of a urinary metabolite of selenium, trimethyl selenonium ion. The enzyme was originally known as a selenoether methyltransferase (Mozier and Hoffman 1988). The purified enzyme has a molecular mass of 28 kDa and has a broad substrate specificity, methylating the sulphur atom as well as selenium and telluride atoms in a variety of thio ethers, and thus was termed TEMT.

THIOMETHYLTRANSFERASE (TMT)

A microsomal, membrane-bound thiomethyltransferase (TMT, EC 2.1.1.9) TMT has a broad specificity towards thiol-containing compounds and catalyses the methylation of sulphur atoms in many drugs like captopril, thiopurines and cephalosporins, thiols like D-penicillamine and other aliphatic sulphydryl compounds like 2-mercaptoethanol. The distribution of TMT activity is highest in intestinal mucosa suggesting a primary role in the detoxification of hydrogen sulphide formed by anaerobes in the intestinal tract (Weisinger and Jakoby 1980). It should be noted that many substrates for thiol methylation are generated by initial conjugation with glutathione followed by enzymic degradation to the cysteine conjugates, and finally cleavage by various β-lyases (see Chapter 1). *S*-Methylation of the thiol metabolites generated by β-lyase action is known as the thiomethyl shunt. This pathway diverts conjugates to the formation and excretion of mercapturic acids (Stevens and Bakke 1990).

Conclusions

Our knowledge of the methyltransferases has expanded rapidly over the last three decades so that at present at least 100 AdoMet-dependent methyltransferases have been described. One can expect that the variety and functions of *O*-, *N*- and *S*-methyltransferases will continue to be discovered. The appreciation of the complexity of the genetic diversity resulting in the polymorphisms, from the apparent simplicity of COMT to the growing number of forms of *S*-methyltransferases, is now clearly recognised. Many of these studies have made serious additions to clinical therapeutics. The role of the level of TPMT in the toxicity of 6-mercaptopurine in the treatment of acute lymphoblastic leukaemia is a clear example of the knowledge of the polymorphism of this enzyme. Studies of the risk factor for breast cancer in women with the low activity form of COMT, while not yet fully understood are an active area of research. The methods of molecular biology, the cloning of cDNAs and genes have enhanced the definitive discovery of genetic polymorphisms. With the availability of access to the human genome, one challenge for the research community will be to discover signature sequences and polymorphisms for methyltransferases. Knowledge of the genetic variation in methyltransferases will lead to an enhanced understanding of individual variation and the pathophysiology of disease, and guides for clinical therapeutics.

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