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

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

15 Deconjugating Enzymes: Sulphatases and Glucuronidases

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Introduction

The activity of deconjugating enzymes may have a significant impact on the pharmacokinetics of various xenobiotics. Release of the parent compound from a conjugate modifies the concentration and hence the action of a drug. Since conjugation of a drug during phase II metabolism can result in either activation or inactivation, enzymic deconjugation may diminish or augment the efficacy of such a compound. Two of the most important conjugation reactions are glucuronidation and sulphation which are mediated by glucuronosyltransferases and sulphotransferases, respectively. The possible contribution of deglucuronidation and desulphation reactions to the disposition of xenobiotics by β-glucuronidases and sulphatases has not been systematically evaluated. However, these enzymes are well characterised on a biochemical level since genetic deficiency of sulphatases and β-glucuronidase results in a variety of lysosomal storage diseases. This chapter summarises data available on enzyme function regarding metabolism and bioactivation of xenobiotics as well as the underlying mechanisms of action, regulation, tissue and species distribution and genetics.

Sulphatases

OVERVIEW

The sulphatase enzyme family is a group of proteins which catalyse the hydrolysis of sulphate esters from various compounds like steroids, glycosaminoglycans or glycolipids. The physiological role of most sulphatases is lysosomal catabolism of complex

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carbohydrates and lipids. In contrast, sulphatases which are located within the endoplasmic reticulum can be involved in synthetic pathways like that of steroid hormones (Parenti *et al*. 1997; Coughtrie *et al*. 1998). Beside the above-mentioned natural substrates, several sulphate esters of different other compounds like thyroxine, catecholamines, oligo-and mono-saccharides are hydrolysed by sulphatases (Gorham and Cantz 1978, Roy 1979; Roy and Turner 1982; Kung *et al*. 1988; Strobel *et al*. 1990). Members of the subgroup of arylsulphatases (EC 3.1.6.1) are able to hydrolyse sulphate esters of aromatic compounds like *p*-nitrocatechol, *p*-nitrophenol or 4-methylumbelliferone which are used as probes for determination of sulphatase activity (Roy 1979). Hydrolysis of sulphate esters by sulphatases is mediated by a catalytic site containing the unusual amino acid derivative Cα-formylglycine (2-amino-3-oxopropionic acid) which is generated by oxidation of the thiol group of a cysteine that is conserved among all known eukaryotic sulphatases (von Figura *et al*. 1998). Lack of this protein modification in humans results in multiple sulphatase deficiency, a rare lysosomal storage disorder characterised by severely decreased activity of all known sulphatases (Schmidt *et al*. 1995).

Sulphatases are found in lower and higher eukaryotes as well as in prokaryotes (von Figura *et al*. 1998; Schirmer and Kolter 1998). For example, sulphatases have been identified in algae, sea urchins, as well as in mammalian species like rodents, cats, dogs and humans with their amino acid sequences being highly conserved. In addition, the active site of bacterial sulphatases displays a high degree of similarity to eukaryotic sulphatases including the presence of Cα-formylglycine which points to similar catalytic mechanisms and to an evolutionary conservation of the sulphatase gene family.

To date, the role of sulphatases in the metabolism and bioactivation of drugs and other xenobiotics is not clear. However, there are several reports on the influence of mammalian sulphatases on the metabolism of thyroxine and steroids (Kung *et al*. 1988; Pasqualini *et al*. 1996a). In addition, some data are available on the role of bacterial sulphatases in the enterohepatic circulation of thyroxine, bile acids and steroid hormones (Huijghebaert *et al*. 1984; Hazenberg *et al*. 1988; Robben *et al*. 1988). Inhibition of sulphatases by xenobiotics might have consequences for mammalian development causing symptoms comparable to the genetic deficiency of sulphatases as demonstrated for inhibition of arylsulphatase E by warfarin (Franco *et al*. 1995).

ARYLSULPHATASES

Arylsulphatase A

Arylsulphatase A (ARSA; cerebroside 3-sulphatase; EC 3.1.6.8) is a lysosomal enzyme which catalyses the desulphation of cerebroside 3-sulphate and other sulphated glycolipids (Roy 1979; Parenti *et al*. 1997) as well as a wide variety of other substrates like 12-(1-pyrene)dodecanoyl cerebroside sulphate, adrenaline-4-sulphate, noradrenaline-4-sulphate, dopamine-4-sulphate, L-tyrosine *O*-sulphate, ascorbate 2-sulphate, monosaccharide sulphates, some steroid sulphates, adenosine 3′,5′-monophosphate, nitrocatechol sulphate, nitroquinol sulphate, 2-nitropyridinyl 3-sulphate and 4-methy-

lumbelliferyl sulphate with K_m values ranging from 1 to 220 mM (Fluharty *et al*. 1979; Roy and Turner 1982; Marchesini *et al*. 1989; Roy and Mantle 1989; Strobel *et al*. 1990). Among the sulphatase family, ARSA is one of the most extensively studied. However, the role of ARSA in the metabolism and bioactivation of xenobiotics has not been investigated in a systematic manner. To our knowledge there are no data on desulphation of pharmacologically relevant substrates. The hydrolysis of sulphate esters occurs via cleavage of the O–S bond (Roy and Mantle 1989). *In vivo*, the 3-sulphate moiety is hydrolysed by ARSA only if cerebroside 3-sulphate is complexed with the activator protein saposin B (Bierfreund *et al*. 2000). The structure of the active site of the enzyme has been elucidated and a catalytic mechanism involving a trans-esterification step has been proposed (Lukatela *et al*. 1998; Waldow *et al*. 1999). Recent crystal structure data point to an ARSA homooctamer composed of a tetramer of dimers (Lukatela *et al*. 1998). However, the quarternary structure seems to be dependent on enzyme concentration and pH (Roy 1979). The pH optimum has been described to be 4.5 or 5.6 depending on the substrate used (Roy and Mantle 1989) and the enzyme is inhibited by various compounds such as sulphate anions, thiosulphate, potassium ferrate, borate, phosphate, pyrophosphate, arsenate, selenate, silver nitrate and 2-hydroxy-dopamine, (Mercelis *et al*. 1979; Roy 1979; Laidler and Steczko 1986; Roy and Mantle 1989; Cawley and Shickley 1992).

ARSA has been characterised in different mammalian species like man, ox, horse, pig, sheep, dog, cat, rabbit, rat and mouse (Waheed and van Etten 1985, Roy and Mantle 1989; Kreysing *et al*. 1994). The enzyme has been found in most tissues investigated so far like liver, kidney, brain, placenta, spleen, testis and oviduct (Waheed and van Etten 1985; Kihara *et al*. 1986; van der Pal *et al*. 1991; Vitaioli *et al*. 1996).

Genetic deficiency of the human enzyme leads to lysosomal accumulation of cerebroside sulphate in the CNS resulting in a disorder called Metachromatic Leukodystrophy (MLD; Barth *et al*. 1994). Pseudodeficiencies of ARSA have been shown to be present in 10–20% of healthy individuals and are due to allelic mutations which result in low ARSA activities without exerting clinical evidence of disease (Thomas 1994).

ARSA has been shown to be regulated by a variety of stimuli. For example, activity of ARSA is inhibited by physiological concentrations of cortisol in myelinogenic cell cultures from embryonic mouse brain (Stephens and Pieringer 1984), whereas oestrogens seem to induce activity of ARSA in rabbit oviduct (Vitaioli *et al*. 1996). In addition, regulation of ARSA activity during menstrual cycle and by endogenous gonadal steroid hormones has been reported in humans (Oner *et al*. 1994; Kamei *et al*. 1997). Suramin has been demonstrated to decrease ARSA activity in mice but to increase it in rats (Marjomaki and Salminen 1986).

Throughout development of rat and mouse brain, and during growth and ageing of human liver cell lines activity of ARSA was increased (Le Gall *et al*. 1979; Bird *et al*. 1981; van der Pal *et al*. 1991). During galactose-induced cataract development in rats ARSA activity has been shown to increase (Harries *et al*. 1985). ARSA activity increases during development of granulomas after infection of mice with *Schistosoma mansoni* (Higuchi *et al*. 1984). In lesions of endodontic origin ARSA activity is elevated compared to healthy periodontal ligament (Aqrabawi *et al*. 1993). Urinary

excretion of ARSA activity was reduced in malnourished children with mild vitamin A deficiency whereas activity was increased in cases of severe vitamin A deficiency (Latif *et al*. 1979).

Arylsulphatase B

Arylsulphatase B (ARSB; *N*-acetylgalactosamine 4-sulphatase; EC 3.1.6.12) is a lysosomal enzyme catalysing the hydrolysis of *N*-acetyl-*D*-galactosamine 4-sulphate moieties from complex molecules like dermatan and chondroitin sulphate as well as from UDP-*N*-acetylgalactosamine 4-sulphate (Fluharty *et al*. 1975; Gorham and Cantz 1978; Habuchi *et al*. 1979). As reported for ARSA, ARSB is able to hydrolyse substrates like catecholamine sulphates, oligosaccharide substrates, nitrocatechol sulphate or 4-methylumbelliferyl sulphate (Daniel 1978; Gibson *et al*. 1987; Strobel *et al*. 1990). A role of ARSB in the hydrolysis of pharmacologically relevant sulphates in *vivo* has not been described so far.

The crystal structure of ARSB has been demonstrated, indicating a monomeric protein consisting of two domains with the active site localised on the larger domain containing cysteine modified to Cα-formylglycine and a calcium ion (Bond *et al*. 1997). Although the geometry and the functional amino acids of the active site of ARSB have been shown to be identical to ARSA, the enzymic mechanism proposed is somewhat different (Bond *et al*. 1997; Lukatela *et al*. 1998). Enzymic activity of ARSB has been shown to be inhibited by compounds like ascorbic acid, sulphidopeptide leukotrienes, metal ions and iodoacetate (Agogbua and Wynn 1976; Weller *et al*. 1986; Selvidge and Verlangieri 1991).

ARSB has been purified or cloned from various mammalian species like man, ox, horse, dog, cat, rabbit, rat and mouse (Murata *et al*. 1975; Wojczyk 1986; Thompson and Daniel 1988; Peters *et al*. 1990; Jackson *et al*. 1992). A wide variety of tissues like liver, kidney, brain, lung, spleen and placenta has been shown to contain ARSB activity (Shapira and Nadler 1975; Daniel 1978; Gibson *et al*. 1987).

Deficiency of ARSB results in the rare lysosomal storage disorder Maroteaux–Lamy disease or mucopolysaccharidosis type VI which is characterised by progressive organ dysfunction with clinical symptoms like skeletal abnormalities and cardiac as well as respiratory failure (Neufeld and Muenzer 1995). About 50 different mutations have been identified pointing to a broad molecular heterogeneity of ARSB deficiency (Isbrandt *et al*. 1994; Villani *et al*. 1999; Wu *et al*. 2000).

A wide variation of ARSB expression among different murine tissues and inbred mouse strains has been described (Daniel 1978, 1987). In rats, ARSB activity of hepatocytes, Kupffer and endothelial cells increases with age (Ferland *et al*. 1990) which is consistent with the data of Le Gall *et al*. (1979) demonstrating a significantly higher ARSB activity in senescent human liver cell lines as compared to actively growing cells. In addition, glycosylation of rat liver ARSB changes depending on age (Przybylo and Litynska 2000).

ARSB has been reported to be inducible by amyloid β-peptide and hydrogen peroxide, compounds which play a central role in the pathogenesis of Alzheimer's disease. Cells resistant to amyloid β express higher levels of ARSB (Li *et al*. 1999). ARSB activity has been detected in lesions of endodontic origin whereas no activity

could be found in the respective control tissue (Aqrabawi *et al*. 1993). Guinea pig natural killer cells (Kurloff cells) show an increased activity of anionic isoforms ARSB during development of acute lymphoblastic leukaemia (Taouji *et al*. 1996). In chronic myelogenous leukaemia, activity of phosphorylated forms of ARSB in leukocytes was increased (Uehara *et al*. 1983). Augmented phosphorylation of ARSB seems to result in elevated enzymic activity in tumours (Gasa *et al*. 1987).

Arylsulphatase C

Arylsulphatase C (ARSC, steroid sulphatase, EC 3.1.6.2) is a microsomal, membrane-bound enzyme, which cleaves the sulphate moiety of several endogenous 3-hydroxysteroid sulphates like oestrone sulphate, dehydroepiandrosterone sulphate, pregnenolone sulphate, cholesterol sulphate and testosterone sulphate. Several nonsteroidal compounds are known to be cleaved like the endogenous 3,5,3′-triiodothyronine sulphate and the synthetic compounds *p*-nitrophenyl sulphate and 4-methylumbelliferyl sulphate. K_m values for the endogenous steroid sulphates range from 1 to 70 μM whereas synthetic compounds are desulphated with K_m values between 1 and 5 mM (Hobkirk 1985). For desulphation of 3,5,3′-triiodothyronine sulphate the K_m value was 390 μM (Kung *et al*. 1988). After solubilisation of the enzyme molecular weights from 330 kDa to 533 kDa depending on source and solubilisation method were found. The number of subunits varies between 3 and 8 monomers with molecular weights ranging from 72 to 78 kDa. The pH optimum of the enzyme appears to be substrate-dependent. Oestrone sulphate sulphatase activity exhibited an alkaline pH optimum approximating pH 8.0–8.6 regardless of source. However, sulphatase activity for cholesterol sulphate showed two optima at pH 5.0 and 7.5. The enzyme is inhibited by a variety of steroid sulphates and free steroids. Competitive inhibitors are oestrogens, *p*-nitrophenyl sulphate and nitrophenol. Inhibition by dehydroepiandrosterone sulphate, pregnenolone sulphate, testosterone sulphate, cholesterol sulphate and androgens is noncompetitive. Inorganic anions such as sulphate, sulphite, fluoride, phosphate and cyanide anions moderately inhibit sulphatase, whereas borate is a strong inhibitor (Daniel 1985; Hobkirk 1985). Arylsulphatase C actually consists of two biochemically distinct isozymes, ‘s’ (slow) and ‘f’ (fast), identified by their electrophoretic mobility. Only the s form of arylsulphatase C exhibits steroid sulphatase activity whereas the f form hydrolyses substrates other than 3-hydroxysteroid sulphates (Shankaran *et al*. 1991). While less information on the f form of arylsulphatase C is available, steroid sulphatase has been investigated extensively.

ARSC is present in different mammalian species like man, sheep, guinea pig, rabbit, hamster, rat and mouse (Daniel 1985; Hobkirk 1985). The enzyme has been found in most tissues investigated so far. However, distribution of the two isozymes is not identical. In man the s form is present in spleen, thyroid, heart, skeletal muscle, placenta and adrenal tissue, while the f form is absent or poorly active. In ovary, testis, intestinal and lung tissue both isozymes are present and are usually of equal amount. In contrast, kidney, liver and pancreatic tissue exhibit only the f form of ARSC (Munroe and Chang 1987).

Genetic deficiency of the human enzyme occurs in 1 of 2000 to 6000 newborns (Bradshaw and Carr 1986). In more than 85% of patients with steroid sulphatase

deficiency complete or partial deletions of the sulphatase gene is responsible for the absence of enzymic activity (Hernandez-Martin *et al*. 1999). However, several unique base pair substitutions have been characterised (Alperin and Shapiro 1997; Oyama *et al*. 2000). Steroid sulphatase deficiency syndrome is an X-linked inherited metabolic disease which is characterised by decreased maternal oestriol production due to deficient placental sulphatase activity during foetal life and postnatally by ichthyosis. Placental steroid sulphatase deficiency is manifested by low oestriol levels in urine and plasma, delay in the onset of labour, uterine resistance to oxytocin 1 and increased stillbirth frequency. The liveborn infants are, however, clinically normal at birth. Male infants with steroid sulphatase deficiency develop X-linked ichthyosis early in the first year of life. Prominent skin peeling with a reptilian appearance of the skin is the major clinical feature. Accumulation of undegraded cholesterol sulphate is thought to be responsible for scale-formation in steroid sulphate deficiency. In addition, corneal opacities, cryptorchidism and pyloric stenosis are associated with X-linked ichthyosis (Bradshaw and Carr 1986).

Expression of steroid sulphatase has been reported to depend on age, sex and presence of disease. During mouse ontogeny, expression of steroid sulphatase mRNA was observed in restricted areas of the liver, in cartilage of many tissues, in spleen and skin. Steroid sulphatase mRNA is expressed in the embryonic mouse cortex, hindbrain and thalamus during the last third of gestation (Compagnone *et al*. 1997). At birth, steroid sulphatase levels in brain are clearly higher than those in adult mice (Mortaud *et al*. 1996). The role of pre- and post-pubertal stage and sex on the steroid sulphatase activity has been investigated in human leukocytes. Pre- and post-pubertal females presented a higher sulphatase activity than the comparable male group. Enzymic activity in prepubertal subjects was higher than in postpubertal individuals (Cuevas-Covarrubias *et al*. 1993). Investigations in breast tissue from women with breast fibroadenomas have shown significantly increased steroid sulphatase levels in the fibroadenoma tissue as compared to normal tissue (Pasqualini *et al*. 1997). In addition, human serum albumin has a marked stimulatory effect on steroid sulphatase activity, which is almost completely inhibited by basic fibroblast growth factor in malignant breast tissue *in vitro* (Purohit *et al*. 1999). The cytokines interleukin-6 and tumour necrosis factor α both stimulated steroid sulphatase activity in MCF-7 human breast cancer cells (Purohit *et al*. 1996a). In post-menopausal women the concentration of various oestrogens in cancer tissues is much higher than those found in plasma, suggesting an accumulation of these substances in tumour tissue (Pasqualini *et al*. 1996b). As oestrogens, especially oestradiol, are known to be involved in both the aetiology and maintenance of growth of breast cancer, many steroid sulphatase inhibitors have been developed during the past few years. Oestradiol derivatives include amino oestrones, oestradiol amides and oestrone sulphamates with IC_{50} values of 10 μM, 80 nM and 0.5 nM, respectively (Selcer *et al*. 1996; Woo *et al*. 1998; Boivin *et al*. 1999). Oestrone-3-O-sulphamate is known to be the most potent steroid sulphatase inhibitor, but exhibits strong oestrogenic activity. Structural modifications lead to a variety of potent steroid sulphatase inhibitors lacking any oestrogenicity (Li *et al*. 1998; Purohit *et al*. 1998; Ciobanu *et al*. 1999). In hormone-dependent and hormone-independent mammary cancer cell lines, the androgen danazol and the progestin medrogestone have been observed to inhibit steroid sulphatase activity

(Nguyen *et al*. 1993; Chetrite *et al*. 1999). Several non-steroidal, non-oestrogenic steroid sulphatase inhibitors have been developed including substituted tyramine and coumarin sulphamates (Purohit *et al*. 1996b; Selcer *et al*. 1997). Finally, dietary compounds like natural flavonoids, especially quercetin and naringenin, as well as sulphoconjugates of daidzein, an isoflavone found in leguminosae, are potent inhibitors of steroid sulphatase and thus may offer the potential for breast cancer prevention therapy (Huang *et al*. 1997; Wong and Keung 1997).

Other arylsulphatases

Arylsulphatases D (ARSD), E (ARSE) and F (ARSF) are non-lysosomal enzymes localised in the endoplasmic reticulum (ARSD, ARSF) or the golgi apparatus (ARSE), respectively (Parenti *et al*. 1997). These enzymes have been first identified on genomic level by their high DNA sequence homology to the sulphatase gene family (Franco *et al*. 1995; Puca *et al*. 1997). The physiological role of these enzymes as well as their natural substrates have not yet been identified. However, ARSE and ARSF have been demonstrated to be heat-labile and to hydrolyse the synthetic substrate 4-methylumbelliferyl sulphate with the maximal activity of ARSF being at a pH of 8 (Franco *et al*. 1995; Puca *et al*. 1997). ARSE activity is inhibited by warfarin which seems to be responsible for warfarin embryopathy (Franco *et al*. 1995).

To date, ARSD, ARSE and ARSF homologues have not been identified in species other than man. ARSD mRNA has been detected in various tissues as pancreas, kidney, liver, lung, placenta, brain and heart whereas ARSE mRNA seems to be exclusively expressed in pancreas, liver and kidney (Franco *et al*. 1995).

Mutations in the ARSE gene have been shown to cause an X-linked recessive defect of bone and cartilage development called chondrodysplasia punctata which is characterised by abnormal calcium deposition in regions of enchondral bone formation and shares striking phenotypic similarities with warfarin embryopathy. However, the physiological role of ARSE in bone development remains unknown (Franco *et al*. 1995). Up to now less than ten different point mutations in the ARSE gene have been identified, four of them resulting in a complete loss of enzymic activity (Franco *et al*. 1995; Daniele *et al*. 1998; Sheffield *et al*. 1998; Dahl *et al*. 1999).

OTHER SULPHATASES

A wide variety of other non-arylsulphatases has been described, some of them being associated with genetic deficiencies resulting in lysosomal storage diseases. However, their role in the metabolism and bioactivation of xenobiotics has not been investigated.

N-Acetylgalactosamine-6-sulphatase (Chondroitinsulphatase, Galactose-6-sulphatase, EC 3.1.6.4) hydrolyses *N*-acetyl-*D*-galactosamine 6-sulphate and *D*-galactose 6-sulphate residues from chondroitin sulphate and keratan sulphate, respectively (Habuchi *et al*. 1979; Nakanishi *et al*. 1979). In addition, several other substrates like sulphated tetrasaccharide or UDP-*N*-acetylgalactosamine 6-sulphate have been identified (Singh *et al*. 1976; Nakanishi *et al*. 1979). Compounds like chondroitin 6-sulphate, keratan sulphate, heparin, heparan sulphate, hyaluronic acid, sulphated

pentasaccharides and several inorganic ions including sulphate anions have been shown to inhibit the enzyme (Glössl *et al*. 1979; Lim and Horowitz 1981). *N*-Acetylgalactosamine-6-sulphatase activity has been identified in different tissues of man, dog, rat and in bacteria (Hayashi 1978; Glössl *et al*. 1979; Habuchi *et al*. 1979; Salyers and O'Brien 1980). Human deficiency of this enzyme leads to Morquio A syndrome (Mucopolysaccharidosis IV A) and is based on a great number of different mutations reflecting an excessive allelic heterogeneity (Singh *et al*. 1976; Bunge *et al*. 1997; Tomatsu *et al*. 1998).

Iduronate-2-sulphatase (Chondroitinsulphatase, EC 3.1.6.13) is a lysosomal enzyme acting on L-iduronate 2-sulphate units of dermatan sulphate, heparan sulphate and heparin (Yutaka *et al*. 1982) and is inhibited by compounds like inorganic and organic sulphates, heparan sulphate, chondroitin 4- and 6-sulphate, suramin and phosphate (Constantopoulos *et al*. 1980; Lissens *et al*. 1984). The enzyme exists as multiple molecular forms and has been identified in various human tissues as well as in rat liver (Constantopoulos *et al*. 1980; Di Natale and Ronsisvalle 1981; Archer *et al*. 1982; Yutaka *et al*. 1982). In addition, the cDNA of murine iduronate-2-sulphatase has been cloned (Daniele *et al*. 1993). Deficiency of human iduronate-2-sulphatase, which can be caused by several different types of gene mutations (Karsten *et al*. 1998, 1999), leads to lysosomal accumulation of heparan and dermatan sulphate resulting in a clinical disorder called Hunter syndrome (Mucopolysaccharidosis II), one of the most common mucopolysaccharidoses (Neufeld and Muenzer 1995)

N-Acetylglucosamine-6-sulphatase (Glucosamine-6-sulphatase, Chondroitinsulphatase, EC 3.1.6.14) catalyses the hydrolysis of *N*-acetyl-*D*-glucosamine 6-sulphate units from heparan and keratan sulphate (Freeman and Hopwood 1987). Additionally, other substrates like glucose 6-sulphate have been identified (Freeman and Hopwood 1987; Freeman *et al*. 1987). *N*-Acetylglucosamine-6-sulphatase has been found in different human tissues like placenta, liver, heart, spleen and kidney (Freeman *et al*. 1987). Deficiency of this enzyme has been reported to result in mucopolysaccharidosis III D (Sanfilippo D), the rarest of the mucopolysaccharidoses known (Neufeld and Muenzer 1995).

N-Sulphoglucosamine-3-sulphatase (Glucosamine-3-sulphatase, Chondroitinsulphatase, EC 3.1.6.15) acts on *N*-2-sulpho-*D*-glucosamine 3-sulphate residues of heparin as well as on methyl-2-deoxy-2-sulphamino-alpha-*D*-glucopyranoside 3-sulphate. The enzyme is inhibited by sulphate and phosphate anions (Leder 1980). In addition to the human enzyme, *N*-sulphoglucosamine-3-sulphatase activity has been observed in bacteria (Bruce *et al*. 1985).

Glucuronate-2-sulphatase (EC 3.1.6.18) hydrolyses the 2-sulphate groups of the 2-O-sulpho-*D*-glucuronate residues of glycosaminoglycans like chondroitin sulphate or heparan sulphate (Shaklee *et al*. 1985). The enzyme is stimulated by Cu^{2+} and Zn^{2+} and inhibited by compounds like sulphate anions, sodium hydrogen phosphate, EDTA, glucuronic acid 2,5-anhydro-*D*-mannose 6-sulphate and glucuronic acid 2-sulphate-2,5-anhydro-*D*-mannose 6-sulphate (Freeman and Hopwood 1989). Glucuronate-2-sulphatase has been purified from chicken embryo and from human skin fibroblasts as well as from liver, lung and kidney (Shaklee *et al*. 1985; Freeman and Hopwood 1989).

BACTERIAL SULPHATASES

Sulphatase activities have been identified in rat and human intestinal bacteria like *Peptococcus*, *Chlostridium*, *Lactobacillus*, *Eubacterium*, *Peptostreptococcus* and *Bacteroides* (Hazenberg *et al*. 1988; Van Eldere *et al*. 1988, 1991). Sulphates of endogenous and xenobiotic compounds have been shown to be hydrolysed by these enzymes pointing to an enterohepatic circulation of these substances. For example, *Lactobacillus* and *Eubacterium* strains as well as *Peptostreptococcus productus* isolated from rat and human faecal suspensions, respectively, hydrolysed different iodothyronine sulphates (Hazenberg *et al*. 1988). Reabsorption of 3,3′,5-triiodothyronine released from its sulphate was significantly reduced in germ-free as compared to conventional rats (Rutgers *et al*. 1989). Contamination of germ-free rats with sulphatase-producing bacteria like *Chlostridium sp*. resulted in enhanced circulation of sulphated bile salts (Robben *et al*. 1988). As shown by Huijgebaert *et al*. (1984), oestrone sulphate is absorbed from the small intestine after deconjugation by the intestinal microflora. Reduced levels of contraceptive steroids in women treated with antibiotics have been attributed to impairment of the intestinal flora which resulted in interruption of the enterohepatic circulation (Orme and Back 1979). Bacterial strains from species like *Clostridium*, *Lactobacillus*, *Eubacterium*, and *Peptococcus* have been identified as producers of steroid sulphatase activity with a broad substrate specificity. For example, oestrone 3-sulphate, β-oestradiol-3-sulphate, the 3β-sulphates of 5α-androstane-17-one and 5β-androstane-17-one, the 3β-sulphates of 5α-androstane-17-one, Δ^5-androstane-17-one, 5α-pregnane-20-one and Δ^5-pregnene-17-one, the 3α and 3β-sulphates of 5α and 5β-bile acids, as well as *p*-nitrocatechol sulphate, *p*-nitrophenyl sulphate and phenolphthalein disulphate have been shown to be hydrolysed by bacterial steroid sulphatases (Van Eldere *et al*. 1987, 1988). Three types of steroid sulphatases have been characterised in *Peptococcus niger* H4, two of them being inducible by their substrates with the exception of bile acid sulphates. The K_m values vary from 180 to 643 μM and their activity is competitively inhibited by various substrates. In addition, sulphite, sulphate, taurine, cyanide and fluoride have been demonstrated to inhibit bacterial steroid sulphatase activity under certain conditions (Van Eldere *et al*. 1991).

An example of activation of xenobiotics by bacterial sulphatases is conversion of cyclamate (cyclohexylamine *N*-sulphonate) to the bladder carcinogen cyclohexylamine in the intestine (Goldin 1990). Further examples for desulphation by intestinal bacteria have been reviewed by Scheline (1973).

Glucuronidases

OVERVIEW

β-Glucuronidase (β-gluc; EC 3.2.1.31) is one of the most extensively studied glycosidases active in the metabolic hydrolysis of conjugated compounds. The activity of β-gluc has been measured in tissue extracts of mammalians and other vertebrates, molluscs and bacteria (Fishman 1974).

β-Gluc is an exoglycosidase that cleaves β-*D*-glucuronic acid residues from the nonreducing termini of glycosaminoglycans such as chondroitin sulphate and hyaluro-

nic acid, and is an essential enzyme for the normal restructuring and turnover of these extracellular matrix components (Paigen 1989). It has been suggested that β-gluc plays a role in the enzymic hydrolysis of glucuronides of endogenous compounds and xenobiotics in humans because it is capable of hydrolysing glucuronide conjugates *in vitro* (Schöllhammer *et al*. 1975; Kauffman 1994; Sperker *et al*. 1997). This could have clinical consequences, especially if glucuronides are accumulated during long-term therapy as described for oxazepam, imipramine or propranolol (Walle *et al*. 1979; Sisenwine *et al*. 1982; Sutfin *et al*. 1988). Even if glucuronides are not accumulated, the enzyme may alter the net rate of glucuronide formation because it can catalyse the reverse reaction.

In addition, many substances that undergo glucuronidation and secretion into the bile are hydrolysed through the action of bacterial and perhaps of enteric β-gluc and subsequently reabsorbed (Rollins and Klaassen 1979). This enterohepatic circulation can influence disposition and action of xenobiotics, endogenous compounds and toxic chemicals. The extended enterohepatic circulation of morphine is an example for such an effect (Plaa 1975). Furthermore, the enzyme plays an important role in the bioactivation of certain prodrugs (Sinhababu and Thakker 1996). Several glucuronide prodrugs of anticancer agents, from which the active drug is released by the action of β-gluc, are currently under development (Bosslet *et al*. 1994; Houba *et al*. 1999; Guerquin-Kern *et al*. 2000). The aim of the approach is to selectively activate the prodrug at the tumour site by utilising β-gluc.

In contrast to β-gluc, there are no reports on the relevance of other glucuronidases, such as hyaluronidase, glycyrrhizinate β-glucuronidase, glucuronosyl-disulphoglucosamine glucuronidase and α-glucuronidase for drug metabolism.

MAMMALIAN β-GLUCURONIDASES

Human β-glucuronidase

β-Gluc (exo-β-*D*-glucuronidase, β-glucuronide glucuronosylhydrolase) is an acid lysosomal hydrolase expressed at variable levels by virtually every cell in the body. The main metabolic role of β-gluc is thought to be glycosaminoglycan degradation in lysosomes by removing terminal β-glucuronosyl residues from dermatan sulphate and heparan sulphate (Stahl and Fishman 1984; Paigen 1989). The classical substrates of β-gluc are β-*D*-glucopyranosiduronic acids containing an aglycone from one of the following groups:

(1) drugs and other xenobiotics
(2) steroids
(3) endogenous non-steroid compounds

Release of the parent compound from glucuronide conjugates has been described for xenobiotics such as clofibric acid (Meffin *et al*. 1983), lorazepam (Herman *et al*. 1989), glycyrrhizin (Kanaoka *et al*. 1986), paracetamol (Bohnenstengel *et al*. 1999), doxycycline (Pedersen and Miller 1980), ranitidine (Miller 1984) and diflunisal (Brunelle and Verbeeck 1996). Three synthetic substrates are commonly used for determination of β-gluc activity: 4-nitrophenyl-β-*D*-glucuronide and phenolphthalein-

β-glucuronide for colorimetric tests and 4-methylumbelliferyl-*β*-*D*-glucuronide (MUG) for a more sensitive fluorometric assay (Fishman *et al*. 1967; Szasz 1967; Sperker *et al*. 1996). For *in situ* localisation of *β*-gluc by enzyme histochemical methods a number of substrates like 8-hydroxy-quinoline-*β*-*D*-glucuronide, naphthol AS-BI *β*-*D*-glucuronide (6-bromo-2′-hydroxy-3-naphthoyl-o-anisidine *β*-*D*-glucuronide) and 5-bromo-4-chloro-3-indolyl-*β*-*D*-glucuronide (X-gluc) have been described (Fishman and Baker 1956; Hayashi *et al*. 1964).

The function of *β*-gluc is dependent on several factors, such as pH and Ca^{2+} concentration at the enzyme site (Wakabayashi 1970; Foster and Conigrave 1999). The human enzyme has a pH optimum of 4 to 5 and at physiological tissue pH about 10% of the maximum activity of the enzyme is retained (Wakabayashi 1970).

β-Gluc has been shown to catalyse glycoside bond hydrolysis, presumably via a covalent glucuronyl-enzyme intermediate (Wong *et al*. 1998). Recently, the active site responsible for this mechanism and the crystal structure of the enzyme have been characterised (Jain *et al*. 1996; Islam *et al*. 1999a). Human *β*-gluc is a tetrameric glycoprotein of about 310 to 380 kDa with a dihedral symmetry resulting from disulphide-linked dimers consisting of two identical monomers. After cleavage of the signal peptide, the unglycosylated protein contains 629 amino acids and is modified during or after the transport of the enzyme to lysosomes (Oshima *et al*. 1987; Tanaka *et al*. 1992; Islam *et al*. 1993). Following glycosylation and *C*-terminal processing within the endoplasmic reticulum and the Golgi complex, the enzyme is directed to lysosomes via the mannose 6-phosphate receptor (Shipley *et al*. 1993). Recent data from Islam *et al*. (1999b) point to a dual localisation of the human enzyme in lysosomes as well as in the endoplasmic reticulum. This seems to be due to binding of the enzyme to a human functional homologue of the murine esterase egasyn.

β-Gluc is present in most cell types such as macrophages and most other blood cells except erythrocytes, in organs like liver, spleen, kidney, intestine, lung, muscle, reproductive organs, lymph nodes and pancreas as well as in body fluids including bile, intestinal juice, urine, seminal plasma and serum (Platt and Platt 1970; Wakabayashi 1970; Gupta and Singh 1983; Heinert *et al*. 1983; Paigen 1989; Sperker *et al*. 2000). A large interindividual variability in its activity and expression has been described for tissues like liver and kidney as well as in serum (Fishman *et al*. 1967; Corrales-Hernandez *et al*. 1988; Sperker *et al*. 1997). *β*-Gluc activity in plasma is about 500–1000 times lower than in liver or kidney (Sperker, unpublished data).

The autosomal recessive deficiency of *β*-gluc activity results in progressive accumulation of undegraded glycosaminoglycans in the lysosomal compartment of many tissues and organs, leading to the lysosomal storage disease mucopolysaccharidosis type VII (MPS VII). Lysosomal accumulation of glycosaminoglycans impairs normal function of several tissues, resulting in clinical abnormalities which include glycosaminoglycan excretion, bone deformities, growth and mental retardation, hepatosplenomegaly, corneal clouding and abdominal organ enlargement (Sly *et al*. 1973). About 50 cases with heterogeneous phenotypes have been reported. From these cases, more than 20 different point mutations in the coding region of the *β*-gluc gene have been identified (Tomatsu *et al*. 1990, 1991; Vervoort *et al*. 1996). In addition, several pseudogenes and a pseudodeficiency allele have been described (Shipley *et al*. 1993; Vervoort *et al*. 1995, 1998; Speleman *et al*. 1996). Since analysis of the 5′ flanking

region of the human β-gluc gene revealed properties commonly associated with 'housekeeping' genes the human enzyme was assumed to be unregulated (Shipley *et al*. 1991). In contrast, Sperker *et al*. (2001) have found that the calcium ionophore A23187 and the calcium ATPase inhibitor thapsigargin downregulate β-gluc expression in HepG2 cells, respectively. Several other reports have demonstrated that endogenous compounds or xenobiotics affect β-gluc release and activity. Triggiani *et al*. (2000) demonstrated that secretory phospholipase A2 and *p*-aminophenyl-mannopyranoside, a mannose-receptor-ligand, release β-gluc from human macrophages. 17β-Oestradiol decreases secreted levels of β-gluc in a dose-dependent manner (Kremer *et al*. 1995) and cyclosporin increases serum β-gluc after treatment for 8 to 16 weeks (Falkenbach *et al*. 1993).

Furthermore, clinical relevant concentrations of benzodiazepines, such as midazolam and flunitrazepam increased the activity of β-gluc released from polymorphonuclear neutrophil leukocytes (Krumholz *et al*. 2000). Interestingly, increased tissue and serum β-gluc activity has been observed in certain disease states like cancer, inflammatory joint and urinary tract diseases (active pyelonephritis, acute renal necrosis), dermatosis, some hepatic diseases, Type 1 diabetes mellitus, multiple sclerosis, periodontal disease and AIDS (Stephens *et al*. 1975; Camisa *et al*. 1988; Saha *et al*. 1991; Ohta *et al*. 1992; Waters *et al*. 1992; Boyer and Tannock 1993; Goi *et al*. 1993; Zenser *et al*. 1999; Layik *et al*. 2000). For example, β-gluc activity has been reported to be higher in breast, prostate, kidney and lung tumours as compared to peritumoral tissues (Albin *et al*. 1993; Mürdter *et al*. 1997). A significantly lower concentration of β-gluc has been observed in epithelial cells of the renal cortex under pathological conditions (Heinert *et al*. 1983). In addition, cataractous lenses exhibited decreased β-gluc activity compared to normal lenses (Kamai 1995). Serum levels of β-gluc are 16-fold higher in HIV-infected patients than in healthy individuals (Saha *et al*. 1991).

Plasma levels of β-gluc in males from 24 to 60 years were significantly higher (about 30%) than in females of the same age (Lombardo *et al*. 1981). Additionally, plasma level of β-gluc was highest in the umbilical cord blood, then dropped slowly to reach the absolute minimum at the age of 10–14 years. Thereafter the enzymic activity increased reaching a maximum around 20–24 years followed by a decrease (30–34 years) and a second slow increase up to the old age. Similarly, age-dependent variations of the specific β-gluc activities have been described for different tissues. For example, the level of total β-gluc activity in childhood liver was higher than in foetal liver (Minami *et al*. 1979). Platt (1970) has demonstrated that the enzyme activity increased significantly in liver and adrenal glands from birth to 80 years. The increase in β-gluc activity with age was also shown in the retinal pigment epithelium (Verdugo and Ray 1997). In contrast, activity in the kidney decreased from birth to adult age (Platt 1970).

In addition to glycosaminoglycan degradation, human β-gluc plays a role in the deconjugation of some other endogenous substances. For example, deconjugation of glucuronides of bilirubin in human bile and development of cholelithiasis is dependent on biliary β-gluc activity (Ho *et al*. 1986). Neonatal intestinal β-gluc antagonises the net clearance of bilirubin by increasing enterohepatic circulation and hence causing increased serum bilirubin levels (Poland and Odell 1971). In addition,

intestinal β-glucuronidase originating from breast milk may cause hyperbilirubinaemia in infants of mothers with diabetes mellitus (Sirota *et al*. 1992).

Drug glucuronides can be susceptible to enterohepatic circulation since many glucuronides are secreted into bile. In the intestine, the glucuronides may be hydrolysed by the action of endogenous (biliary or enteric) and bacterial β-gluc and subsequently reabsorbed. This mechanism has been suggested for a variety of compounds like digitoxin, some benzodiazepines, morphine and indomethacin (Caldwell and Greenberger 1971; Rollins and Klaassen 1979; Herman *et al*. 1989). However, the relative influence of enteric β-gluc on enterohepatic circulation is not clear. Brunelle and Verbeeck (1996) could show that the glucuronidation rate of diflunisal is affected by the microsomal β-gluc activity.

Cleavage of glucuronides by β-gluc also plays a role in drug toxicity and chemical carcinogenesis. It has been suggested that non-toxic metabolites of aromatic hydrocarbons are exported from the liver as glucuronides which are subsequently hydrolysed to mutagenic metabolites in peripheral tissues. For example, it has been demonstrated that β-gluc deconjugates benzidine- and 4-aminobiphenyl-glucuronides as well as 3-benzo(α)pyrenyl glucuronide resulting in the release of the carcinogenic aromatic compounds. These aromatic amines may cause bladder cancer in humans (Moore *et al*. 1982; Zenser *et al*. 1999). Furthermore it has been suggested that inhibitors of β-gluc could be used to reduce the rate of deglucuronidation and to protect against carcinogenesis. Such inhibitors could be ascorbic acid (Young *et al*. 1990), silymarin and silybin (Kim *et al*. 1994) as well as cortisone and gold-I-complexes (Chang *et al*. 1993). In human liver and kidney homogenates, *D*-glucaro-1,4-lactone, glycyrrhizin, oestradiol 3-glucuronide and paracetamol glucuronide competitively inhibit the β-gluc-mediated cleavage of 4-methylumbelliferyl-*D*-glucuronide (Ho *et al*. 1985; Sperker *et al*. 1997).

An important approach in the alleviation of toxic adverse effects in cancer chemotherapy is the development of prodrugs of anticancer agents which are less reactive or less cytotoxic than the respective parent compounds. A desired objective of prodrug therapy is the selective delivery of the cytotoxic agent to the tumour tissue, which can be achieved by antibody-directed enzyme prodrug therapy (ADEPT) or by prodrug therapy based on elevated tumour activities of bioactivating enzymes. In both approaches, β-gluc can be used as the activating enzyme. Recently, fusion proteins consisting of a human β-gluc moiety and humanised antibody fragment directed against tumour specific antigens have been designed for use in ADEPT (Bosslet *et al*. 1992; Haisma *et al*. 1998). Furthermore, it was demonstrated that endogenous β-gluc mediates tumour-selective release of doxorubicin from a glucuronide prodrug (HMR1826) at the tumour site, using an isolated, perfused human lung tumour model (Mürdter *et al*. 1997). A reason for the tumour selective activation of the glucuronide prodrug is the localisation of β-gluc. In contrast to normal tissue, tumoral β-gluc is localised extracellularly probably due to secretion by inflammatory cells and disintegrating tumour cells (Bosslet *et al*. 1998). Weyel *et al*. (2000) have shown that transduction of human tumour cells with a secreted form of human β-gluc resulted in conversion of HMR1826 to doxorubicin and in enhanced tumour cell killing. Recently, an improved tumour targeting of daunorubicin and 5-fluorouracil in nude mice bearing

human cancer xenografts has been demonstrated by using the respective glucuronide prodrugs (Houba *et al*. 1999; Guerquin-Kern *et al*. 2000).

Rodent β-glucuronidase

Rodent β-gluc is an unusual protein in the class of hydrolases because it is localised not only in lysosomes but also in the endoplasmic reticulum (ER) of liver parenchymal cells, kidney and lung, but not in spleen, brain, heart, erythrocytes, testis and skin (Fishman *et al*. 1967; Himeno *et al*. 1976; Lusis and Paigen 1977). Liver lysosomal and microsomal β-gluc are products of a single structural gene. Both enzymes are catalytically identical and show the same immune reactivity. They are similar in molecular weight, but differ in both sugar and amino acid composition (Wang and Touster 1975; Himeno *et al*. 1976; Owens and Stahl 1976; Lusis and Paigen 1977). The ER localisation of β-gluc is due to its interaction with the esterase egasyn (Lusis and Paigen 1977; Medda *et al*. 1986, 1987). This binding results in the retention of a fraction of β-gluc in the lumen of the ER I (Zhen *et al*. 1993). Inhibitors of egasyn-esterase activity like organophosphates caused rapid dissociation of egasyn-microsomal β-gluc complex as a result of a massive increase of microsomal β-gluc in plasma (Medda *et al*. 1987). Furthermore, mouse strains lacking egasyn also lack ER glucuronidase (Lusis and Paigen 1977).

Murine β-gluc gene complex (*Gus*) has been studied extensively because it provides a useful system for understanding mammalian gene regulation. Three common alleles of *Gus-s* (*-s*^{a}, *-s*^{b} and *-s*^{h}) specify allozymes which differ in electrophoretic mobility and heat stability (Paigen 1961; Swank and Paigen 1973; Lalley and Shows 1974). Specific alleles of each of three GUS regulatory elements (*Gus-r, Gus-t and Gus-u*) are associated with specific alleles of *Gus-s*.

The combination of *Gus-s* with the regulatory elements define three common haplotypes (Gus^a, Gus^b, Gus^h) with different effects on the expression of GUS. *Gus-r* controls the androgen responsiveness of kidney GUS mRNA (Palmer *et al*. 1983). The second element, *Gus-u*, controls the levels of constitutive GUS synthesis in all tissues (Lusis 1983). In addition, the third regulatory element, *Gus-t*, exerts an additional temporal control over GUS synthesis in certain tissues (Meredith and Ganschow 1978; Lusis 1983). Mouse and rat β-gluc are identical in length and share 88.2% sequence identity. Mouse and human β-gluc are 75.3% identical (Funkenstein *et al*. 1988). Like the human enzyme, rodent β-gluc is a tetramer of identical subunits formed from a single gene product (Delvin and Granetto 1970).

Heringova *et al*. (1965) found that rat β-gluc activity in the intestinal mucosa is age-dependent. Relatively high activity was measured during the first two weeks of life and decreased to adult levels at the end of the third week. Activity of β-gluc in murine lungs increased markedly with advancing age (Traurig 1976). Significant age-dependent decrease of the total activity of β-gluc has been found in kidney, rib cartilage and skin whereas significant age-dependent increase of this enzyme activity has been demonstrated in the spleen and liver of rats (Lindner *et al*. 1986). In the studies by Watson *et al*. (1985), the enzyme has been shown to be both developmentally and hormonally regulated in rats and mice. In the mouse kidney, β-gluc is subject to induction by androgens, which results in an 120-fold elevation of mRNA concentra-

tion (Palmer *et al*. 1983). In female rat preputial glands β-gluc is regulated by oestrogen (Levy *et al*. 1958). The administration of oestradiol produced an increase of β-gluc activity (Briggs 1973; Gallagher and Sloane 1984) and glycyrrhizin significantly inhibited an increase of uterine β-gluc activity by oestradiol-17β (Kumagai *et al*. 1967). Furthermore, low levels of vitamin A caused an increase in β-gluc activity in rats and mice (Kostulak 1974; Rundell *et al*. 1974). In contrast, cortisone acetate (Baglioni *et al*. 1978), cortisone (Koldovsky and Palmieri 1971; Horowitz *et al*. 1978), thyroxine (Horowitz *et al*. 1978), ganoderenic acid A (Kim *et al*. 1999) and dexamethasone (Hicks *et al*. 1994) decreased β-gluc activity in rats.

Hepatic microsomal β-gluc can influence biliary excretion and hepatic elimination of bilirubin-IXα as well as other endogenous and exogenous compounds. Whiting *et al*. (1993) found an increased excretion of glucuronides of bilirubin-IXα in a strain of mice lacking hepatic microsomal β-gluc. In rat liver microsomes, the production of diflunisal glucuronide has been shown to increase when β-gluc is inhibited by *D*-saccharic acid 1,4-lactone (Brunelle and Verbeeck 1993). Furthermore, a mechanism of enterohepatic circulation in rodents has been suggested for digitalis glycosides (Caldwell and Greenberger 1971) and lorazepam (Ruelius 1978).

β-Glucuronidases in other species

In addition to humans and rodents, the enzyme has been characterised in many mammalian species like dog, monkey, cow, pig and cat. The optimal pH for β-gluc activity in the retinal pigment epithelium was found to range from 4.0 to 4.5. Except for the very unstable bovine enzyme, β-gluc activity displays a very high resistance to heat inactivation at pH 5.0 (Ray *et al*. 1997). Canine and feline β-gluc show a high degree of homology to the human enzyme (Fyfe *et al*. 1999; Ray *et al*. 1999). Schuchman et al. (1989) reported that about 65% of total β-gluc in canine liver is membrane associated and can be solubilised by detergent.

β-Gluc from bovine liver and porcine kidney catalysed the hydrolysis of some glucopyranosiduronamides and glucopyranosides as well as of oestrone-3-glucuronide (Gowers and Breuer 1980; Parker *et al*. 1981). Recently, some new compounds with β-gluc inhibitory activity have been synthesised and tested on bovine liver homogenate, such as glucuronic acid-type 1-*N*-iminosugar of *D*-arabinose (Igarashi *et al*. 1996), *D*-glucaro-δ-lactam, an oxidation product of nojirimycin (Niwa *et al*. 1972), nojirimycin A (Tsuruoka *et al*. 1996) and sistatin B analogues (Satoh *et al*. 1996).

Both, dogs and cats are useful animal models of human diseases, such as pancreatitis and mucopolysaccharidosis type VII (Dlugosz *et al*. 1977; Haskins *et al*. 1991; Gitzelmann *et al*. 1994). Dogs with acute experimental pancreatitis showed an increase of free β-gluc activity in pancreas homogenates. This increased β-gluc level was antagonized by glucagon (Dlugosz *et al*. 1977). Mucopolysaccharidosis type VII (MPD VII) was first described in a mixed-breed dog which was homozygous for a single G to A missense mutation in the β-gluc gene (Haskins *et al*. 1984, 1991). Ray *et al*. (1998) could demonstrate that the mutation reduces the canine β-gluc activity by more than 100-fold. Pathological lesions of MPS VII in dog and man are similar (Haskins *et al*. 1991; Neufeld and Muenzer 1995). In 1994, Gitzelmann *et al*. have

described a case of feline MPS VII in a domestic male cat. Like dogs, a single G to A missense mutation of β-gluc cDNA reduced β-gluc activity to background levels.

BACTERIAL β-GLUCURONIDASE

Most *E. coli* strains, including many pathogenic serogroups, produce β-gluc (GUS) and are positive with the 4-methylumbelliferyl-β-*D*-glucuronide assay (Feng and Hartman 1982; Hartman 1989). In addition, Ralovich *et al*. (1991) found, that other gram-negative bacteria such as *Salmonella*, *Shigella* and *Yersinia* strains show a positive β-gluc reaction. β-Gluc is the first enzyme of the hexuronide-hexuronate pathway (Ashwell 1962). *E. coli* β-gluc is a very stable enzyme, with a molecular weight of 290 kDa and is composed of four subunits (Kim *et al*. 1995). The enzyme displays a broad pH optimum in the neutral range with its activity being decreased by 50% at pH 4.3 and 8.5. *E. coli* β-gluc is resistant to thermal inactivation at 50°C (Ho and Ho 1985; Jefferson *et al*. 1986).

Bacterial β-gluc plays an important role in the enterohepatic circulation of drugs and endogenous compounds. This enterohepatic circulation is likely to be clinically significant because the alteration of circulation can considerably decrease or increase the clearance of some drugs like digitalis glycosides, morphine, steroids, indomethacin, amphetamine, several antibiotics, doxycycline and some benzodiazepines and nonsteroidal anti-inflammatory agents (Plaa 1975; Rollins and Klaassen 1979). Moreover, clearance of progesterone has been reported to be increased if the enterohepatic circulation is interrupted by *D*-saccharic acid 1,4-lactone which has been shown to be a potent inhibitor of β-gluc (Marselos *et al*. 1975). In addition, it has been reported that lorazepam is extensively conjugated resulting in the 3-*O*-phenolic glucuronide which undergoes enterohepatic circulation (Ruelius 1978). The clearance of the drug increased by about 25% when its circulation is blocked by neomycin, an antibiotic effective against β-gluc producing bacteria (Herman *et al*. 1989). Arylamine *N*-glucuronides were found to be susceptible to hydrolysis by *E. coli* β-gluc, suggesting the release of carcinogenic arylamines (Lilienblum and Bock 1984). Furthermore, it has been shown that glucuronides of 1-nitropyrene-metabolites secreted into the bile can be hydrolysed in the intestine by bacterial β-gluc to potent mutagenic aglycones (Morotomi *et al*. 1985). In fact, as many carcinogens are detoxified by glucuronidation, it has been proposed that inhibitors of β-gluc could protect against carcinogenesis by reducing the rate of deglucuronidation. For example, *D*-glucarate and *D*-saccharic acid 1,4-lactone have been shown to inhibit benzo(α)pyrene-induced mammary, colonic and pulmonary tumorigenesis (Kinoshita and Gelboin 1978; Walaszek *et al*. 1984; Walaszek *et al*. 1990). Intestinal toxicity of the anticancer agent irinotecan is due to biliary excretion of the glucuronic acid conjugate of its active metabolite SN-38 and subsequent deglucuronidation by bacterial β-gluc. Administration of the β-gluc inhibitor baicalin has been shown to protect against intestinal toxicity of irinotecan metabolites in rats (Takasuna *et al*. 1995, 1996). Other inhibitors of bacterial β-gluc are glycyrrhizin (Takasuna *et al*. 1995), as well as silymarin and silybin (Kim *et al*. 1994).

E. coli β-gluc expression is negatively controlled by the products of the two regulatory genes *uidR* and *uxuR* (Novel and Novel 1976) and inducible by methyl-β-

D-glucuronate and alkaline pH (Novel *et al*. 1974; Kim et al. 1992). Results from the studies of Caldini *et al*. (1999) have shown that starch metabolism is involved in *β*-gluc induction in *E. coli*.

OTHER GLUCURONIDASES

Heparanase is the dominant endoglucuronidase in mammalian tissues. The enzyme is capable of cleaving heparan sulphate glycosaminoglycans to short 5–6 kDa fragments (Gallagher *et al*. 1988). Heparanase was found to cleave the single *β-D*-glucuronidic linkage of a heparin octasaccharide with *O*-sulphate groups being essential for substrate recognition by heparanase (Pikas *et al*. 1998). Heparanase has been implicated in metastasis, because non-metastatic murine T lymphoma and melanoma cells transfected with the heparanase gene acquired a highly metastatic phenotype *in vivo* (Vlodavsky *et al*. 2000). A 4-fold increase in serum heparanase activity was found in patients with tissue metastases (Nakajima *et al*. 1988). In addition, these authors observed that serum heparanase levels in rats were 17-fold increased after injection of highly metastatic adenocarcinoma cells. Furthermore, elevated levels of heparanase were detected in tumour biopsies of cancer patients (Vlodavsky *et al*. 1999). Calcium spirulan, a sulphated polysaccharide chelating calcium from a blue-green alga, significantly inhibited degradation of heparan sulphate by heparanase and significantly reduced experimental lung metastasis (Mishima *et al*. 1998). Moreover, the anti-metastatic effect of non-anticoagulant species of heparin and of certain sulphated polysaccharides like laminarin and the phosphorothioate homopolymer of cytidine was attributed to their heparanase-inhibiting activity (Bitan *et al*. 1995; Miao *et al*. 1999). Furthermore, heparanase is involved in tumour angiogenesis both directly, by promoting invasion of endothelial cells and indirectly, by releasing heparan-bound basic fibroblast growth factor (Vlodavsky *et al*. 2000). Parish *et al*. (1999) found that sulphated oligosaccharides are potent inhibitors of heparanase activity and *in vitro* angiogenesis.

Human heparanase is a 50 kDa enzyme (Toyoshima and Nakajima 1999) and its amino acid sequence is highly homologous to mouse and rat heparanase (Hulett *et al*. 1999; Dong *et al*. 2000). The intracellular heparanase from mouse melanoma cells is similar to the human platelet enzyme in terms of pH optimum and pI value, but appears to be bigger in size (Graham and Underwood 1996). Kussie *et al*. (1999) have shown high expression of heparanase in placenta and spleen. Furthermore, heparanase activity has been described in a number of tissues and cell types including rat liver (Gallagher *et al*. 1988), human placenta (Klein and von Figura 1979), human platelets (Hoogewerf *et al*. 1995), cultured human skin fibroblasts (Klein and von Figura 1976), human neutrophils (Matzner *et al*. 1985), rat T-lymphocytes (Naparstek *et al*. 1984), murine B-lymphocytes (Laskov *et al*. 1991) and human monocytes (Bartlett *et al*. 1995).

Bilirubin monoglucuronide transglucuronidase (bilirubin-glucuronoside glucuronosyltransferase, EC 2.4.1.95) catalyses a two- step degradation reaction of bilirubin mono- and di-glucuronides: (1) hexosyl group transfer and (2) transglucuronidation. The enzyme (16 kDa) with a pH optimum of 6.6 and a K_m value of 32–34 μM was

found in rat liver and is localised in plasma membrane and microsomes (Jansen *et al*. 1977; Chowdhury *et al*. 1979; Chowdhury and Arias 1981).

α-Glucuronidase (EC 3.2.1.139) is a xylanolytic enzyme produced by a wide variety of fungi, such as *Trichoderma reesei* (Siika-aho *et al*. 1994), *Thermoascus aurantiacus* (Khandke *et al*. 1989), *Agaricus bisporus* (Puls *et al*. 1987) and *Aspergillus niger* (Uchida *et al*. 1992). Furthermore, α-glucuronidase activity has been identified in bacteria, e.g. *Streptomyces* (MacKenzie *et al*. 1987), *Fibrobacter* (Smith and Forsberg 1991), *Clostridium* (Trudeau *et al*. 1992; Bronnenmeier *et al*. 1995) and *Thermoanaerobacterium* strains (Bronnenmeier *et al*. 1995).

Hyaluronidase (EC 3.2.1.36) catalyses *O*-glycosyl bond hydrolysis of 1,3-linkages between β-*D*-glucuronate and *N*-acetyl-*D*-glucosamine residues in hyaluronic acid and tetrasaccharides. The activity of this enzyme is inhibited by butane-2,3-dione and phenylglyoxal. The enzyme is localised in the salivary glands of *Hirudo medicinalis* (Linker *et al*. 1960; Hipkin *et al*. 1989).

Glycyrrhizinate β-glucuronidase (EC 3.2.1.128) from *Aspergillus niger* and *Eubacterium sp*. is specific for the hydrolysis of *O*-glycosyl compounds, such as the triterpenoid glycoside glycyrrhizinate from roots of *Glycyrrhiza sp*. The enzyme with a pH optimum of 4.1 to 4.5 and a temperature optimum of 45°C has a molecular weight of 15 kDa and is localised in the cytosol (Muro *et al*. 1986; Akao *et al*. 1987; Sasaki *et al*. 1988).

Glucuronosyl-disulphoglucosamine glucuronidase (EC 3.2.1.56) with a pH optimum of 6.5 and a temperature optimum of 30°C was isolated from cells of *Flavobacterium heparinum* and catalysed the hydrolysis of *O*-glycosyl bonds from desulphated disaccharides and heparin (Dietrich 1969; Dietrich *et al*. 1973; Hovingh and Linker 1977).

Conclusions

Sulphatases are widely distributed in various animal species and tissues. Whereas less information is available on their role in metabolising drugs and other xenobiotics, the main function appears to be metabolism of endogenous compounds. These include glycosaminoglycans, glycolipids, steroid as well as thyroid hormones and catecholamines. Activation of steroid hormones by steroid sulphatase plays an important role in the pathogenesis of several tumours like breast or prostate cancer. Therefore inhibitors of steroid sulphatase are of great interest for prevention and therapy of these tumours. Bacterial sulphatase activity modulates bioavailability of substances that undergo enterohepatic circulation, especially thyroid and steroid hormones used in replacement therapy. In addition, transformation of substances into carcinogens via desulphation has been reported.

β-Glucuronidase is the most important enzyme among the glucuronidases known. Localisation of the enzyme differs among species. Whereas human β-glucuronidase is located mainly in lysosomes, remarkable amounts of rodent β-glucuronidase have been detected in the endoplasmic reticulum. Thus the extent of net glucuronidation of xenobiotics in rodents depends on the level of hepatic microsomal β-glucuronidase. After glucuronidation, several drugs and endogenous compounds are known to be eliminated via the bile. Bacterial expression of β-glucuronidase in the intestine results

in deglucuronidation and reabsorption of these compounds. Interference with this enterohepatic circulation sets the stage for a wealth of drug interactions. Finally, drugs have been developed which are selectively bioactivated by β-glucuronidase for the targeted treatment of cancer, thereby reducing adverse side effects.

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