Drug-metabolizing enzymes: a group of promiscuous catalysts

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1 Introduction

Drug-metabolizing enzymes (DMEs) are a diverse group of enzymes responsible for the metabolism of a vast array of xenobiotics, that is, foreign chemicals humans encounter from the environment, including drugs, environmental carcinogens, and food toxicants.[1–4] DMEs also metabolize certain endobiotics produced endogenously, such as steroids, prostaglandins, and bile acids. DMEs are so named to reflect the prominent and historical role of the study of drug metabolism in understanding the enzymes. Since the first introduction of drug metabolism as a scientific discipline six decades ago,[5] the studies of DMEs have influenced many aspects of biology and medicine, including drug metabolism, enzymology, pharmacology, toxicology, cancer research, drug development, and risk assessment (***A Personal and Historical Perspective on Drug Metabolism***).

Many DME substrates, either exogenous or endogenous, are toxic to the body if left to accumulate to a certain level. Metabolism of the chemicals by DMEs in general leads to the formation of metabolites with increased water solubility and decreased activity to facilitate detoxification and elimination of the chemicals (***Functional Group Biotransformations***). As a result, the chemical homeostasis within the cell is maintained in the face of chemical challenges. In certain cases, drug metabolism by DMEs increases the biological activity (e.g., toxicity) of chemicals (***Metabolic Activation and Associated Drug Toxicity***). Metabolic activation is examplified by the metabolism of the polycyclic aromatic hydrocarbon (PAH) procarcinogen benzo[*a*]pyrene (B[*a*]P).[6,7] Oxygenation of B[*a*]P by cytochrome P4501A (CYP1A, P4501A) enzymes and hydration by epoxide hydrolase (EH) result in the formation of *trans*-7,8-diol-9,10-epoxide of B[*a*]P, an ultimate carcinogen capable of binding covalently to the guanine base of DNA (adduct formation) to cause gene mutations and cancerous transformation of the

Handbook of Metabolic Pathways of Xenobiotics, First Edition.
Edited by Philip W. Lee, Hiroyasu Aizawa, Lawrence L. Gan, Chandra Prakash, and Dafang Zhong.

cell. In aggregates, DMEs determine the efficacy, bioavailability, clearance, and toxicity of therapeutic drugs and environmental carcinogens and toxicants in the body by modulating the biotransformation and pharmacokinetic properties of chemicals.

Many DMEs are inducible by substrates.[8] Induction of DMEs generally occurs rapidly and subsides as the substrates are metabolized. In most cases, induction of DMEs accelerates the metabolism and reduces the toxicity and pharmacological activity of chemicals. In some cases, induction enhances chemical toxicity, as in the case of metabolic activation of PAH carcinogens. Induction may also cause undesired drug–drug interactions, if induction of a DME increases the metabolism of a co-administered drug. Induction of DMEs is coordinated by a group of ligand-activated receptor/transcription factors called xenobiotic-activated receptors (XARs).[9] This coupled chemical sensing and transcription by XARs allows fast induction and tight control of DMEs by the substrates. Among the XARs, the aryl hydrocarbon receptor (AhR) mediates the induction of CYP1A and 1B that metabolize therapeutic agents, such as caffeine, phenacetin, melatonin, theophylline, and lidocaine, in addition to PAH carcinogens.[10] A wide range of chemicals activate AhR, including environmental carcinogens B[*a*]P and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, the therapeutic agent omeprazole, and endogenous chemicals indole-3-carbinol, kynurenine, and tryptophan photoproduct 6-formylindolo[3,2-*b*]carbazole.[11,12] The antioxidant-activated nuclear factor erythroid 2-related factor 2 (Nrf2) mediates the induction of a number of DMEs including glutathione *S*-transferase (GST), glucuronosyltransferase (UGT), NQO1, and mEH through a common DNA-binding element called the antioxidant response element.[13,14] The pregnane X receptor (PXR, NR1I2) controls the induction of CYP3A4 by clinical drugs including rifampicin, dexamethasone, and St. John's Wort.[15] The constitutive androstane receptor (CAR, NR1I4) mediates the induction of CYP2B by phenobarbital and 5β-pregnanedione.[16] CYP3A4 is estimated to metabolize ~40–60% of clinical drugs. PXR and CAR together are believed to play a critical role in the regulation of the metabolism of clinical drugs in humans by controlling the induction of CYP3A, CYP2B, and several other DMEs.

2 Classification of drug-metabolizing enzymes

DMEs consist of diverse families of enzymes that are generally categorized by the reactions they catalyze. In the recent years, classification of DMEs has become a subject of considerable debate owing to the vast diversity and many exceptions to nearly every generalization of DME-catalyzed reactions.[17] Drug metabolism reactions are traditionally categorized into four classes: oxygenation, such as the microsomal CYP-catalyzed monooxygenation; reduction, such as the CYP and non-CYP-catalyzed nitro- and azo-reductions; hydrolysis, such as the hydrolysis of ester bonds by esterase; and conjugation (synthesis) reactions, such as the conjugations with glutathione, glucuronic acid, and sulfate by GST, UGT, and sulfotransferase, respectively.[5] From a mechanistic point of view, this classification does not adequately distinguish some DME reactions. For instance,

conjugation reactions with glucuronic acid and sulfate are S_N2 reactions where an electrophilic nucleoside-containing cofactor, such as UDP-glucuronic acid and 3′-phosphoadenosine-5′-phosphosulfate (PAPS), reacts with a nucleophilic functional group, such as –OH or $-NH_2$, of a xenobiotic. On the other hand, conjugation with glutathione occurs between the nucleophilic –SH group of glutathione and an electrophilic substrate. Furthermore, the classification does not include covalent binding of reactive xenobiotic electrophiles to macromolecules that forms protein or nucleic acid adducts, which is a key step in the metabolic activation and carcinogenesis of many drugs and carcinogens.

A popular classification of drug metabolism reactions was introduced during the early days of drug metabolism research.[18] The classification separates DME reactions into two phases: Phase I reactions consist of oxidation, reduction, and hydrolysis, whereas phase II are synthesis or conjugation reactions. This two-phase classification was intended to provide an "operational distinction" between "functionalization" reactions in which functional groups, such as –OH, are added to xenobiotics, and "conjugative" reactions in which chemicals with the functional groups become more water soluble through conjugation with small hydrophilic molecules. The classification also implies that phase I and phase II reactions are sequential in the metabolism of drugs. With respect to drug response, phase I reactions either decrease or increase drug activity, whereas phase II reactions are generally believed to result in detoxification. However, as more DMEs are discovered and more DME reactions characterized, many exceptions to these generalizations are found.[17] It has become clear that the two-phase classification is chemically incoherent and does not necessarily reflect the nature and complexity of drug metabolism reactions; nor does it provide prediction of the reaction sequence and metabolite activity for many drugs and toxicants. Perhaps, DMEs and their reactions are so diverse and complex that it is difficult for any classification to rationally and accurately comprehend.

A broader, mechanism-based classification of DME reactions was proposed for teaching purposes recently (see Table 1).[17] Under this classification, oxidation reactions are similar to those defined traditionally, including microsomal CYP reactions, peroxidase-catalyzed reactions, alcohol dehydrogenase reactions, and so forth. Reduction reactions are limited to a rather small group of processes that stand alone, such as nitro- and azo-reductions, aldo-keto reduction, and quinone reduction. Conjugation reactions include only the reactions of electrophilic nucleoside-containing cofactors (UDP-glucuronic acid, PAPS, *S*-adenosyl methionine (SAM), acetyl CoA, etc.) with nucleophilic sites in xenobiotics (i.e., S_N2 reactions). Lastly, nucleophilic trapping processes encompass reactions of glutathione, water, or other cellular nucleophiles with electrophilic xenobiotics, including conjugation with glutathione and formation of protein and DNA adducts. Accordingly, DMEs are categorized into oxidation enzymes, reduction enzymes, conjugation enzymes, and enzymes involved in nucleophilic trapping reactions (see Table 2).

Nearly all DMEs consist of multiple isoforms. In addition to differences in substrates, reactions catalyzed, and regulation, DMEs and isoforms differ from each other considerably in tissue distribution, cellular localization, and cofactor requirement (see Table 2). On the other hand, as discussed in more detail in the following section, most DMEs are promiscuous proteins and share many common properties

Table 1 Drug metabolism reactions according to classification proposed by Josephy *et al.*[17]

Reaction	Definition	Examples of enzymes involved
Oxidation	Similar to traditionally defined oxidation reactions	Cytochrome P450 Flavin-containing monooxygenase Monoamine oxidase Alchohol dehydrogenase Aldehyde dehydrogenase Aldehyde oxidase Xanthine oxidase Prostaglandin H synthase
Reduction	A small group of processes for reduction that stand alone	Nitro-reductase Azo-reductase Aldo-keto-reductase Quinone reductase
Conjugation	Reactions of electrophilic nucleoside-containing cofactors (UDP, PAPS, SAM, acetyl CoA, ATP, etc.) with nucleophilic xenobiotics S_N2 reaction	UDP-glucuronosyltransferase Sulfotransferase Methyltransferase *N*-Acetyltransferase Amino acid conjugation enzyme
Nucleophilic trapping process	Reactions of cellular nucleophiles, such as glutathione and water, with electrophilic xenobiotics	Glutathione *S*-transferase Epoxide hydrolase Esterase Peptidase DNA and protein adduct formation

in enzymatic reactions.[19] Both the diversity at the system level and the promiscuity at the protein level are necessary traits of DMEs that enable the enzymes to metabolize the vast number of xenochemicals in tissue, cell type, development, substrate, and inducer-dependent manners.

3 Common properties of drug-metabolizing enzymes

In dealing with numerous and structurally diverse substrates, mammalian DMEs are evolved with remarkably similar properties that distinctly differ from those of classical (well-behaved) enzymes but are characteristic of promiscuous proteins.[19] Classical enzymes, such as those involved in intermediary metabolism, generally have the following properties: (a) high substrate specificity and affinity (i.e., having only a single or a few substrates); (b) high catalytic efficiency with high turnover number; (c) a single or limited types of reactions; (d) a single or very few metabolites; and (e) a Michaelis–Menten kinetics. On the contrary, DMEs generally show low specificity and affinity toward a large number of substrates; have low turnover numbers; catalyze multiple types of reactions; produce multiple metabolites from a single substrate; and very often exhibit atypical (non-Michaelis–Menten) enzyme kinetics (see Table 3). It is because of these promiscuous enzymatic properties that

Table 2 List of human DMEs: liver isoform, cellular localization, and cofactor requirement.

Enzyme	Example/major liver isoform[a]	Cellular localization	Cofactor requirement
Oxidation enzymes			
Cytochrome P450 (CYP, P450)	CYP3A4, 2D6, 2C, 1A2, 2E1	Microsomes	O_2, NADPH
Flavin-containing monooxygenase (FMO)	FMO3	Microsomes	O_2, NADPH
Monoamine oxidase (MAO)	MAO-A, MAO-B	Mitochondrial outer membrane	O_2, H_2O
Alcohol dehydrogenase	ADH1A, 1B, 1C	Cytosol	NAD^+
Aldehyde dehydrogenase	ALDH1, ALDH2	Mitochondria, cytosol	$NAD(P)^+$
Xanthine oxidase (XO)	XO	Cytosol	O_2, H_2O
Prostaglandin H synthase	PHS-1, PHS-2	Microsomes	O_2
Reduction enzymes			
Nitro- and azo-reductases	CYP, non-CYP enzymes	Microsomes, microflora, etc.	NADPH, etc.
Aldo-keto-reductase	AKR1A1, 1B1, 1C1-4, 1D1	Cytosol, microsomes	NADPH, NADH
Quinone reductase	NQO1, CYP reductase	Cytosol, microsomes	NAD(P)H, NADPH
Conjugating enzymes			
UDP-glucuronosyl-transferase (UGT)	UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B	Microsomes	UDPGA
Sulfotransferase (SULT)	SULT1A1, 1B1, 1E1, 2A1	Cytosol	PAPS
Methyltransferase	COMT, PNMT; TPMT, etc.	Cytosol, microsomes	SAM
N-Acetyltransferase	NAT1, NAT2	Cytosol, mitochondria	Acetyl CoA
Amino acid conjugating enzyme	Acyl-CoA synthetase, acyl-CoA: aminoacid N-acyltransferase, etc.	Cytosol, microsomes, mitochondria	ATP, acetyl CoA, amino acids
Enzymes involved in nucleophilic trapping reactions			
Glutathione *S*-transferase (GST)	GST A1-1, M1-1, P1-1	Cytoplasm	GSH
Epoxide hydrolase	EPHX1 (mEH), EPHX2 (sEH)	Microsomes, cytoplasm	
Esterase	hCE1, hCE2	Microsomes, cytosol, lysosomes	
Peptidase	Aminopeptidase, carboxypeptidase, endopeptidase	Lysosomes	

[a]Represent major isoforms in human liver or examples of enzymes if no isoform is discussed.

Table 3 Common properties of promiscuous DMEs.

Properties	DMEs
Substrate	Many substrates with low binding specificity and affinity
Turnover rate	Low turnover number
Type of reaction	Catalysis of many different types of reactions
Metabolite	Formation of multiple metabolites from single substrate in CYP-catalyzed reactions
	Formation of multiple metabolites from substrates with multiple functional groups, such as –OH, in conjugation reactions
Enzyme kinetics	Most reactions following apparently typical Michaelis–Menten kinetics
	Many showing atypical enzyme kinetics

DMEs become versatile and well suited to be the catalyst for the metabolism of innumerable xenobiotics.

3.1 Mammalian microsomal cytochrome P450s

The mammalian microsomal CYPs are perhaps the most versatile catalysts found in nature that catalyze the oxygenation of most, if not all, xenobiotics in the body (***Role of Cytochrome P450 Enzymes in Biotransformation***). Moreover, their promiscuous behaviors are best studied among DMEs. Therefore, it is necessary to discuss mammalian microsomal CYPs first and separately from other DMEs.

Each microsomal CYP has a broad range of substrates that are generally lipophilic with diverse structures and sizes. For instance, the molecular mass of CYP substrates ranges from 28 Da (ethylene) to 1201 Da (cyclosporin A) (see Table 4). The CYP enzymes catalyze many types of reactions including aliphatic and aromatic hydroxylation, epoxidation, N- and S-oxygenation, N-, O-, and S-dealkylation, dehydrogenation, ester-bond cleavage, oxidative and reductive dehalogenation, oxidative deamination and desulfuration, and azo- and nitro-reduction (see Table 5). Formation of multiple metabolites from a single substrate by a CYP enzyme can be demonstrated experimentally using a single recombinant CYP or human liver microsomal preparations in vitro. As an example, indinavir, a protease inhibitor clinically used for the treatment of human

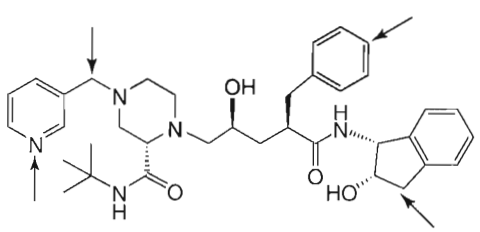
Table 4 DME substrate size variations.

DMEs	Substrate size (molecular mass, Da)
CYP	Ethylene (28), cyclosporin A (1201)
UGT	Ethanol (46), ziracin (1631)
SULT	Ethanol (46), ziracin (1631)
FMO	Trimethylamine (59), 8-PTZ (422)
EH	Ethylene epoxide (44), benzo(*a*)pyrene 7,8-oxide (268)
GST	Benzene oxide (94), ecteinascidin 743 (762)

Table 5 Multiple reactions catalyzed by DMEs.

Enzymes	Reactions
Cytochrome P450	Aliphatic and aromatic hydroxylation
	Epoxidation
	N- and S-oxygenation
	N-, O-, and S-dealkylation
	Dehydrogenation
	Ester-bond cleavage
	Oxidative and reductive dehalogenation
	Oxidative deamination and desulfuration
	Azo- and nitro-reduction
	Others
UGT	O-, N-, S-, and C-glucuronidation
SULT	Sulfation of alcohol, amine, hyroxylamine, hydroxyamide, catechol, and *N*-oxide
GST	Conjugation, isomerization, peroxidation
FMO	Oxygenation of amine, hydrazine, sulfide, thiol, and phosphine
EH	Hydration of alkene epoxide and arene oxide

immunodeficiency virus (HIV) infection, is metabolized to several major metabolites (primary and secondary products), including *N*-oxide, *N*-oxide and indane-OH, desmethylpyridine, desmethylpyridine and indane-OH, and minor metabolites such as *p*-OH-phenyl, *trans*-dihydroxyindanyl, and *cis*-dihydroxyindanyl (see Reaction 1).[20] Antibody inhibition experiment with anti-CYP3A4 antibodies reveals a total inhibition of indinavir metabolism. In addition, recombinant human CYP3A4 exhibits high activities in catalyzing the formation of all indinavir metabolites found in human samples. Therefore, CYP3A4 is likely responsible for the formation of all oxidative metabolites of indinavir in humans.



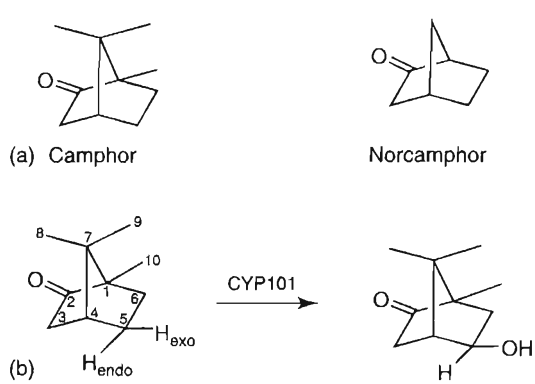
Reaction 1 Structure and sites of oxidation of indinavir. Arrows indicate the major primary sites for CYP3A4 action. Sequential metabolism at these sites results in secondary metabolites. Several minor metabolites (structures unknown) can also be detected, suggesting metabolic attack by CYP3A4 can occur at other undetermined sites of indinavir.

The metabolism of *d*-camphor by mammalian liver microsomal CYP and microbial $P450_{CAM}$ (CYP101, from *Pseudomonas putida*) illustrates the sharp contrast between promiscuous microsomal CYPs and well-behaved enzymes with respect to substrate–enzyme interaction and catalysis (see Table 6). In addition to being

Table 6 Promiscuous mammalian liver CYP: comparison with microbial CYP101.

Parameter	Liver CYP/drug	CYP101/camphor	CYP101/norcamphor
Substrate specificity	Nonspecific	Highly selective	–
Substrate binding	Lack of complementarity	Absolute complementarity	Lack of complementarity
Metabolites formed	Multiple	One	Multiple
Stereo- and regio-specific metabolite	Generally not specific	5-Exo	5-Exo, 6-exo, and 3-exo
Metabolic switching with deuterated substrate	Yes, in many cases	No	Yes
Elecron transport	Leaky	Tightly coupled	Leaky

a soluble protein and using reduced form of nicotinamide adenine dinucleotide (NADH) instead of reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor, CYP101 is very different from mammalian liver microsomal CYPs but behaves similarly to a classical enzyme. CYP101 uses only *d*-camphor and a few structural analogs as efficient substrates (see Reaction 2a). Metabolism of *d*-camphor by CYP101 produces a single and regio-specific metabolite at the 5-exo position (5-exohydroxycamphor, 100%) (see Reaction 2b).[21,22] The reaction is highly efficient with a very high turnover number.[21] Substitution of camphor with deuterated camphor does not cause metabolic switching,[23] indicating that binding of *d*-camphor produces an absolute complementation between camphor and the substrate-binding cavity (SBC) of CYP101.[24,25] Finally, electron transfer of the reaction is tightly coupled to the hydroxylation of the substrate[26] with a low level of H_2O_2 production. In contrast, the liver microsomal CYP enzyme 2B4 (CYP2B4), which also uses *d*-camphor as a substrate, metabolizes many chemicals



Reaction 2 Hydroxylation of camphor by CYP101. (a) Structures of camphor and norcamphor. (b) Oxidation of camphor by CYP101 at the 5-exo position. Only 5-exo-hydroxycamphor is produced from camphor, whereas oxidation of norcamphor by CYP101 generates three isomers at the 5-exo (45%), 6-exo (47%), and 3-exo (8%) positions (not shown).

in addition to *d*-camphor. CYP2B4 converts *d*-camphor into three isomeric metabolites, namely, 3-endo- (16%), 5-exo- (14%), and 5-endo-hydroxycamphor (63%).[21] Catalysis is rather inefficient with a turnover number of only 1.3. The distribution of the products from CYP2B4 nearly reflects the rank order of the chemical reactivity of various hydroxylable positions, suggesting a lack of complementation between camphor and CYP2B4 SBC but considerable mobility of camphor within the SBC.[21] Substitution with deuterated substrates causes metabolic switching in many CYP2B4-catalyzed reactions, further supporting a high degree of mobility of bound substrates within the SBC of CYP2B4. Electron transport in 2B4 reaction is rather leaky, resulting in an overall low efficiency of coupling to substrate hydroxylation and production of a relatively high amount of H_2O_2.

Microsomal CYP reactions exhibit varying degrees of substrate–substrate cooperativity resulting in non-Michaelis–Menten kinetics. The CYP3A4-based substrate–substrate interactions are clinically relevant in many cases due to the prominent role of CYP3A4 in the metabolism of clinical drugs and the fact that the enzyme is highly allosteric toward many therapeutic agents. CYP3A4 shows positive homotropic cooperativities toward a number of substrates including testosterone, aflatoxin B1, progesterone, 17β-estradiol, and diazepam. In the plot of reaction rate versus substrate concentration (v vs S), the reactions with these substrates exhibit sigmoidal curves instead of the hyperbolic curves typical of Michaelis–Menten reactions. In CYP3A4-catalyzed testosterone 6β-hydroxylation, the reaction rate increases as the substrate concentration rises, but much more so in the middle range of substrate concentrations than at low and high concentrations.[27,28] Hydroxylation of midazolam by CYP3A4 follows negative homotropic cooperative kinetics in which the reaction rate is reduced at high concentrations of midazolam.[29] CYP-catalyzed reactions may show heterotropic cooperativities between substrates in which one substrate (or effector) affects the binding and/or catalysis of a separate substrate. In CYP3A4-catalyzed hydroxylation of aflatoxin B1, the reaction exhibits a positive homotropic cooperativity in the absence of a second substrate; however, co-incubation with α-naphthoflavone (ANF) stimulates the 8,9-epoxidation (positive heterotropic cooperativity) but inhibits the 3α hydroxylation (negative heterotropic cooperativity) of aflatoxin B1.[28]

In many cases, heterotropic cooperativities of CYP reactions vary with substrates, giving rise to substrate-dependent drug–drug interactions. In one example, four commonly used CYP3A4 substrates (testosterone, terfenadine, midazolam, and nifedipine) were analyzed in pairs for substrate–substrate interactions.[30] Some pairs show mutual inhibition, but testosterone and nifedipine exhibit differential effects toward each other in that nifedipine inhibits testosterone 6β-hydroxylation, whereas testosterone does not affect nifedipine oxidation. On the other hand, erythromycin, midazolam, and terfenadine inhibit nifedipine oxidation by 20, 60, and 80%, respectively (see Table 7).[30] Testosterone and 7,8-benzoflavone show regioselective but opposite effects on midazolam hydroxylation: testosterone stimulates the 4-hydroxylation but inhibits the 1′-hydroxylation of midazolam, whereas 7,8-benzoflavone inhibits the 4-hydroxylation but stimulates the 1′-hydroxylation reaction.[30]

Table 7 Substrate-dependent drug–drug interactions.

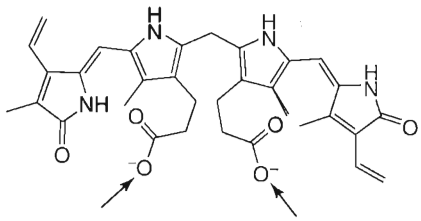
Human enzymes	Substrate used in assay	Alternate substrate	Maximal effect of alternate substrate on enzyme activity
CYP3A4[a]	Nifedipine oxidation	Testosterone	Little or no effect
		Erythromycin	20% decrease
		Midazolam	60% decrease
		Terfenadine	80% decrease
UGT1A4[b]	Dihydrotestosterone (DHT) glucuronidation	Tamoxifen	40% decrease at low DHT concentrations 120% increase at high DHT concentrations
		Lamotrigine	60% decrease
Epoxide hydrolase[c]	Styrene 7,8-oxide	Cyclohexene oxide	70% increase
	Benzo(*a*)pyrene 11,12-oxide	Cyclohexene oxide	95% decrease

[a]Reference 30.
[b]Reference 31.
[c]Reference 32.

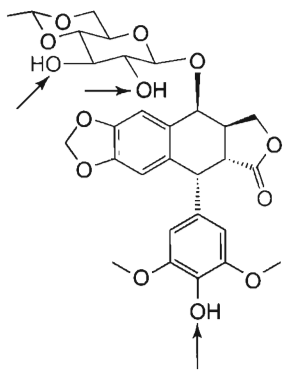
3.2 *UGT, SULT, EH, and other DMEs*

Similar to microsomal CYPs, most other DMEs behave as promiscuous proteins (see Table 3). Each of these DMEs has multiple substrates with diverse structures and sizes (see Table 4) and is capable of catalyzing multiple types of reactions (see Table 5). UGTs are responsible for catalyzing glucuronidation at the oxygen, nitrogen, sulfur, or carbon atoms of substrates; sulfotransferases (SULTs) for the sulfation of alcohol, amine, hydroxylamine, hydroxyamide, catechol, and *N*-oxide; flavin-containing monooxygenases (FMOs) for the oxygenation of amine, hydrazine, sulfide, thiol, and phosphine; and EHs for the hydration of alkene epoxide and arene oxide. GSTs catalyze glutathione conjugation as well as isomerization and peroxidation reactions.

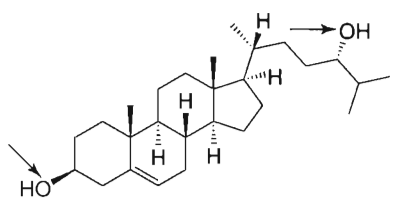
DME conjugation reactions produce multiple products from a single substrate if more than one functional group, such as –OH, is present. For example, glucuronidation of bilirubin by UGT1A1 occurs at one or two of the carboxyl positions to form mono and diglucuronide metabolites (see Reaction 3).[33] In a separate example, the anticancer drug etoposide undergoes glucuronidation in vitro and in vivo, forming one phenolic (EPG, major product) and two alcoholic glucuronides (EAG1 and EAG2) (see Reaction 4).[34] In vitro experiments with recombinant UGTs show that UGT1A1 is principally responsible for the formation of all three etoposide glucuronides with K_m values of 285, 230, and 753 μM, respectively. 24(*S*)-Hydroxycholesterol (24-OH-chol) is a major metabolite of cholesterol in human brain. 24-OH-chol is secreted across the blood–brain barrier and further metabolized in the liver to bile acids by CYP7A, or conjugates with sulfate or glucuronic acid by SULT or UGT. Conjugation with sulfate is catalyzed by three cytosolic SULT isoforms. SULT2A1 and 1E1 catalyze the conjugation at the 3- and



Reaction 3 Structure and glucuronidation of bilirubin. UGT1A1 catalyzes the formation of mono- and diglucuronide metabolites of bilirubin at one or two sites indicated by arrows.



Reaction 4 Structure and glucuronidation of etoposide. UGT1A1 is the principal enzyme for the glucuronization of etoposide in human liver to give rise to one phenolic and two alcoholic glucuronides at sites indicated by arrows.



Reaction 5 Structure and sulfation of 24(*S*)-hydroxycholesterol. Two sites of sulfation are shown by arrows. SULT2A1 and SULT1E1 catalyze the sulfation at both the 3- and 24-hydroxyl positions to form the 24-OH-chol-3,24-disulfate, whereas SULT2B1b forms only 24-OH-chol-3-sulfate.

24-hydroxyl positions to form 24-OH-chol-3,24-disulfate, whereas SULT2B1b catalyzes the formation of 24-OH-chol-3-monosulfate (see Reaction 5).[35]

Atypical kinetics and substrate–substrate interactions are observed in UGT and mEH-catalyzed reactions. UGT1A4 catalyzes the glucuronidation of dihydrotestosterone (DHT), tamoxifen, and lamotrigine. When tested alone, DHT and lamotrigine exhibit hyperbolic kinetics, whereas tamoxifen displays substrate inhibition kinetics (negative homotropic cooperativity).[31] Neither tamoxifen (high-affinity substrate,

K_m = 0.9 μM) nor lamotrigine (low-affinity substrate, K_m of 1564 μM) affects the glucuronidation of DHT (K_m = 19.6 μM) through competitive inhibition. Instead, tamoxifen causes concentration-dependent activation/inhibition of the reaction, and lamotrigine inhibits the reaction by 60% through noncompetitive inhibition (see Table 7). The results are consistent with enzyme kinetics resulting from multiple binding sites within the UGT1A4 SBC.

Large effects of substrate-dependent drug–drug interactions are observed in mEH-catalyzed hydration of alkene epoxide and arene oxide. In one example, cyclohexene oxide causes a 70% increase in the hydration of styrene 7,8-oxide, but a 95% decrease in the hydration of benzo(*a*)pyrene 11,12-oxide by mEH (see Table 7).[32] Metyrapone and cyclohexene oxide exhibit opposite effects on mEH for certain substrates. Metyrapone increases the rate of hydration of benzo[*a*]anthracene 5,6-oxide and octane 1,2-oxide, but has no effect on that of benzo[*a*]pyrene 4,5-oxide.[36] Cyclohexene oxide does not affect the hydration of benzo[*a*]anthracene 5,6-oxide and octane 1,2-oxide, but inhibits the hydration of benzo[*a*]pyrene 4,5-oxide. On the other hand, metyrapone inhibits the hydration of benzo[*a*]pyrene 11,12-oxide and dibenzo[*a*, *h*]-anthracene 5,6-oxide, similarly to cyclohexene oxide.[36]

4 Mechanistic aspects of DME promiscuity

Mechanistic studies have revealed significant insights into the molecular bases that govern the substrate–enzyme interaction, catalysis, and kinetics of DMEs in recent years. In particular, three-dimensional structures of many DMEs with substrates bound to the enzymes have been investigated by X-ray crystallography at atomic resolutions. The availability of the crystal structures, together with function–structural analyses by site-directed mutagenesis and biophysical measurements, has made it possible to understand the mechanism responsible for the promiscuous behaviors of DMEs at molecular levels.

4.1 Large substrate binding cavity

The first noticeable common feature from the crystal structures of DMEs is that most DMEs contain a large SBC.[19] Conceptually, a large SBC contributes to DME's promiscuous substrate–enzyme interaction in several ways. First, a large SBC permits the binding of substrates with different sizes, shapes, and chemical properties to result in a broad substrate specificity. Second, it allows the binding of multiple substrates in the same SBC at the same time, which potentially causes substrate–substrate interactions and atypical enzymatic kinetics. Third, it increases substrate mobility within the SBC due to loose binding or lack of complementation between the substrate and SBC. Increased mobility causes substrate rotation and exposure of different reactive groups of a substrate to the site of catalysis resulting in multiple products from a single substrate. Increased mobility also causes binding of a substrate in productive and nonproductive configurations to reduce catalytic

efficiency and cause negative cooperative kinetics. As discussed below, evidence supporting each of the above scenarios has been provided.

The structures of microsomal CYPs are best studied among DMEs. Mammalian CYPs exhibit conserved secondary structures and folding patterns to form an overall similar shape and functional domain structure.[37] The core structure is largely invariable and contains spatially conserved helices E, I, J, K, and L, as well as portions of β-sheet 1. The core provides the binding site for the heme and the cysteine residues that form the axial ligands to heme iron. Molecular oxygen binds to heme iron and is reduced to form the hypervalent heme iron-oxo intermediate that oxidizes a substrate. The heme forms the base of the active site cavity above which a substrate binds and is held in place such that the correct atom is exposed to the iron-oxo intermediate for regio- and stereo-selective oxygenation. The strong oxidant nature of the high-valence iron-oxo complex of CYPs provides the chemical basis for the extreme diversity of CYP reactions. The outer surfaces of the active site cavity are formed by portions of β-sheets 1 and 4, helices F–G, and the B–C loop. The outer surfaces are generally divergent and plastic to provide structural discrimination in substrate binding.

CYP3A4 is the most versatile CYP with substrates as large as cyclosporin A (M_r 1203 Da) and as small as acetaminophen (M_r 151 Da). The SBC of CYP3A4 is about 1386 Å^3 in volume; moreover, the cavity is large near the heme iron, further reducing constraints on the motion of substrates near the site of catalysis.[38] In a substrate-bound crystal structure of CYP3A4, two molecules of ketoconazole (M_r 534 Da) – an inhibitor of 3A4 – were found to bind at the SBC in an antiparallel orientation above the heme base.[39] In a separate crystal structure, erythromycin was found to bind in the SBC in a nonproductive configuration.[39] These findings clearly demonstrate that CYP3A4 not only has a very large SBC but also accommodates multisubstrates in the SBC at the same time, permits rotation of substrates at the active site, and binds substrates in multiple orientations or conformations, all of which contribute to the versatility and promiscuity of the enzyme.

The sizes of the SBCs of other microsomal CYPs are also consistent with the predictions from their substrates. Human CYP2C8 plays a central role in the metabolism of a number of therapeutic drugs including the anticancer drug taxol (M_r 852 Da), the antidiabetic drug troglitazone (M_r 441 Da), the calcium channel blocker verapamil (M_r 455 Da), and the antimalarial drug amodiaquine (M_r 356 Da). In addition, 2C8 metabolizes certain endogenous substrates, such as retinoic acid (M_r 300 Da) and arachidonic acid (M_r 305 Da). The SBC of CYP2C8 is 1438 Å^3, which is large enough to allow the enzyme to metabolize these relatively large-size substrates.[40] The SBC of CYP2C9 is much larger than its common substrates such as warfarin (M_r 308 Da), phenytoin (M_r 252 Da), and flurbiprofen (M_r 305 Da), leaving room for binding of more than one substrate at the same time.[41] The SBC of CYP2D6 is 540 Å^3 and takes the shape of a "right foot"; both features facilitate the binding of its typical substrates that generally contain a basic nitrogen atom and a planar aromatic ring.[42] Polymorphisms of CYP2D6, which cause the poor metabolism phenotypes of debrisoquine, affect a significant portion of the Caucasian population.

The SBCs of CYP1A2 and 2A6 are smaller than those of the above CYPs and are more adapted to substrates with certain traits. The CYP1A2 SBC is about

375 Å^3 and is well suited for the positioning and oxidation of relatively large and planar aromatic substrates, such as PAH and halogenated aromatic hydrocarbons.[43] The SBC of 2A6 is about 260 Å^3, which is in an agreement with its substrate spectrum for smaller substrates, such as coumarin (M_r 146 Da), methoxalen (M_r 216 Da), and nicotine (M_r 162 Da).[44]

Large SBCs are also demonstrated in other DMEs. SULT1A1 is a major human SULT that catalyzes the sulfation of a wide range of xenobiotics and endobiotics, including large L-shaped aromatics (iodothyronine, M_r 525 Da), extended planar aromatic ring systems (β-estradiol, E_2, M_r 272 Da; troglitazone M_r 441 Da), and small planar aromatics (*p*-nitrophenol (*p*NP), M_r 139 Da). The active site binds a molecule of PAPS that it provides the sulfate group. Crystal structures of substrate-bound SULT1A1 reveal several unique features of its SBC.[45,46] First, the SBC is large and takes a "L" shape, which readily explains the binding of large L-shaped aromatics, such as iodothyronine. Second, the large SBC can accommodate more than one small aromatic molecule at the same time. Indeed, two molecules of *p*NP are found to bind in the SBC in a crystal structure. Binding of two *p*NP molecules appears to account for the two-phase kinetics observed in the sulfation of the compound. Third, the SBC undergoes substantial conformational change to accommodate rigid, fused steroid ring substrates, such as E_2. Finally, E_2 is found to bind the SBC in a nonproductive configuration in an E_2-bound crystal structure of SULT1A1; the finding is in agreement with partial substrate inhibition kinetics observed at high concentrations of E_2.

In contrast to the large SBCs and loose substrate binding in DMEs, well-behaved enzymes in general exhibit tight and complementary substrate binding which is often strengthened by various interactions between substrate and SBC residues. As discussed in Section 3.1, the metabolism of *d*-camphor by microbial CYP101 and mammalian microsomal CYP2B4 appears to be dictated by its complementary binding to the SBCs (see Table 6). A rational question that follows is whether well-behaved CYP101 can be converted into a promiscuous, CYP2B4-like enzyme by manipulating the substrate-binding characteristics. Experimental data show that this is indeed achievable by altering the SBC of CYP101 or the chemical properties of substrates. Changing Tyr96 to Phe in the SBC of CYP101, which eliminates the hydrogen bonding between Tyr96 and the carbonyl oxygen of *d*-camphor, weakens the binding and causes rotation of *d*-camphor in the SBC, resulting in the formation of multiple hydroxylation metabolites as opposed to a single product by wild-type CYP101.[22] Substitution of *d*-camphor with norcamphor that lacks the three methyl groups of *d*-camphor (replaced by hydrogen) also produces promiscuous phenotypes (see Table 6). First, the reaction with norcamphor produces three isomeric products, 5-exo- (45%), 6-exo- (47%), and 3-exo-hydroxy (8%) metabolites instead of one product (5-exo-) from *d*-camphor.[22,23] Second, hydroxylation efficiency with norcamphor is reduced to 12% as opposed to 100% with camphor.[26] Third, substitution with deuterated norcamphor causes metabolic switching.[23] Fourth, hydrogen peroxide production with norcamphor is increased more than fourfold in comparison with that of camphor.[23] These phenotypic changes likely reflect reduced hydrophobic interactions between norcamphor and CYP101 SBC, which increase the mobility and rotation of norcamphor within the SBC.

4.2 Multiple conformations of DMEs

Both structural and enzymatic studies have revealed multiple conformations of DMEs that exhibit different properties of substrate–enzyme interaction, catalysis, and kinetics. Several mechanisms may account for the multiplicity of DME conformation. Comparison of crystal structures of DMEs with or without substrates reveals that, in addition to their large sizes, the SBCs of many DMEs are highly flexible. In the case of microsomal CYPs, the plastic region can undergo substantial conformational changes that drastically reshape the active site to allow the binding and catalysis of substrates with very different sizes, shapes, and chemical properties, even though the overall CYP folds appear to be maintained.[37] Mechanistically, this extraordinary malleability of the SBCs of microsomal CYPs (and other DMEs) is similar to "induced fit" typically observed in classical substrate–enzyme interactions except that it is utilized to achieve substrate diversity in DME reactions. Evidence also supports allosteric regulation of DME conformations in which a substrate (or effector) binds to an allosteric site and alters the conformation of the SBC, resulting in either positive or negative cooperativity with a separate substrate.[47,48] In addition, some DMEs exist in multiple, persistent conformations or "mosaic" states prior to the exposure to substrates.[49] The equilibrium of the mosaic states in the "persistent heterogeneous pools" of a DME can be modulated by substrates. One such example is substrate-induced oligomerization of CYPs, which likely produces substrate-dependent cooperative kinetics.[50] The effect of allosteric binding and oligomerization on the kinetic properties of DMEs is discussed in more detail in Section 4.3.

Substrate-induced conformational changes modulate substrate binding and catalysis of DMEs in a number of ways.

First, the sizes of DME SBCs are highly malleable upon binding with substrates. In one crystal structure, the SBC volume of CYP3A4 is increased by 80% upon binding with ketoconazole to allow the binding of two molecules of the inhibitor within the SBC at the same time.[39] For smaller size substrates, such as metyrapone and progesterone, the CYP3A4 SBC is reduced in volume to achieve closer contact with the substrate. In one crystal structure, metyrapone occupies a volume of $\sim$230 Å^3 with a good shape complementation within the SBC of CYP3A4.[51]

Second, multiple conformations of DME SBCs are observed in crystal structures of DMEs bound with different substrates. The ligand-free CYP2B4 SBC takes a wide-open U-shaped conformation.[52] Upon binding with inhibitor 4-(4-chlorophenyl)imidazole (4-CPI), the SBC changes its shape to create a small and closed pocket for the inhibitor.[53] Binding with bifonazole, a substrate larger than 4-CPI, changes the SBC to an O-shaped cleft that is wide in the middle but narrow at the top.[54] As discussed in Section 4.1, the L-shaped SBC of human SULT 1A1 readily accommodates large L-shaped aromatics, such as diiodothyronine, or two small molecules, such as *p*NP, but does not easily accept rigid, fused ring substrates, such as E_2.[45] To bind E_2, the SBC conformation takes substantial changes. The two loop regions (residues 146–154) that close tightly in the SULT1A1·*p*NP complex are opened to increase the space for rigid, fused ring steroid substrates. Additionally, the Phe-247 residue in the loop connecting α12

and α13 adopts a different conformation from that in the SULT1A1·*p*NP complex to further increase the space for E_2 binding.[46]

Third, crystal structural studies revealed unexpected binding sites and orientations of many substrates in DME SBCs. In the SULT1A1·E_2 complex, E_2 is bound in a nonproductive configuration in the SBC.[46] The nonproductive binding may represent an intermediate state toward productive binding for catalysis, or a true secondary binding site within the SBC responsible for the negative homotropic cooperative kinetics observed at high concentrations of E_2. In either scenario, additional conformational changes not yet observed in crystal structures are necessary for a productive binding of E_2 in the SULT1A1 SBC.

A number of substrates are found to bind CYP3A4 SBC in multiple configurations. In crystal structures with small compounds, binding of metyrapone appears productive in that the alkyl pyridine nitrogen is directly positioned to the heme iron and the binding exhibits a good shape complementation within the active site.[51] On the other hand, binding of progesterone is non-productive in a peripheral site away from heme iron (>17 Å) for catalysis.[51] In crystal structures with large substrates, binding of erythromycin to the SBC is in a nonproductive configuration; the D-desosamine group is 17 Å away from heme iron, which is too distant for demethylation to occur.[39] In a separate structure, two molecules of ketoconazole bind to the SBC by stacking above the heme base in antiparallel orientations, neither of which would be productive for catalysis.[39]

The CYP2C5 substrate 4-methyl-*N*-methyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl) benzenesulfon-amide (DMZ) can bind to CYP2C5 SBC in two antiparallel orientations along the long axis of DMZ. One orientation exposes the principal site of hydroxylation, the 4-methyl group, 4.4 Å from the heme iron, whereas the alternate orientation positions the second, infrequent hydroxylation site >5.9 Å from the catalytic site, which may account for the alternate hydroxylation product formation.[55]

4.3 Models of cooperativity

Significant interests in the atypical kinetic behaviors of DMEs have arisen in recent years from the realization that atypical kinetics of DMEs could complicate the prediction and interpretation of in vitro drug–drug interaction data to in vivo clinical outcomes. Structural and kinetic studies have revealed new and interesting aspects of protein–substrate (effector) and protein–protein interactions of DMEs that likely form the basis of the cooperative behaviors of the enzymes. Three models have been developed based on these findings: multiple substrate binding, allosteric conformation transition, and modulation of persistent heterogeneity. These models are not totally independent, but are interconnected with each other to account for the atypical kinetic behaviors of DMEs, even though one model may dominate the kinetics of a DME with a particular substrate(s).

The initial model that explains the cooperativity of DMEs involves the binding of multiple substrates in the same SBC of an enzyme.[19] The concept stems from the notion that a loose binding of substrate in a spacious SBC, which is characteristic of many DME substrate bindings, requires the binding of another or more substrates in order to be catalytically more effective (i.e., positive cooperativity). An opposite scenario arises when the binding of multiple substrates

in the SBC results in an inhibition of catalysis via competitive or noncompetitive mechanisms (i.e., negative cooperativity). It is well established that DMEs, owing to their large SBCs and broad substrate specificity, do bind more than one substrate of the same or different chemicals in their SBCs. Therefore, multiple substrate binding accounts for at least some of the substrate–substrate interactions in DMEs. In the example of SULT1A1, binding of two molecules *p*NP produces a two-phase kinetics of *p*NP sulfation: at low concentrations, the reaction slightly deviates from a hyperbolic curve to give a small positive cooperativity; but increasing the concentration of *p*NP to >2 μM results in substrate inhibition.[45] Consistent with the *p*NP-bound crystal structure, the kinetics can be explained by a two-substrate-binding-site model in the SBC: Binding of *p*NP at site 1 forms an ES_1 complex that does not prevent subsequent binding of *p*NP at site 2, which gives an ES_1S_2 complex; on the other hand, binding of *p*NP at binding site 2 produces an ES_2 complex that blocks *p*NP from binding to site 1. Both ES_1 and ES_1S_2 are catalytically competent, whereas ES_2 is nonproductive. Increased formation of ES_2 at high concentrations of *p*NP results in the inhibitory kinetics.[45]

An important advancement in understanding DMEs is the uncovering of many conformational changes of DMEs associated with substrate/effector binding, leading to the concept that DMEs behave as allosteric enzymes in many of the substrate–enzyme interactions as opposed to a static space-filling model. In this allosteric model, binding of a substrate (or effector) at an allosteric site, such as an alternate but nonproductive site within the SBC or a peripheral site outside the SBC, alters the conformation of the SBC and thereby, affects the binding and/or catalysis of another substrate at the catalytic site.[48] As discussed above, both an alternate binding site in SBC and a peripheral binding site outside SBC have been demonstrated in crystal structures of substrate/effector-bound DMEs, such as the binding of progesterone or erythromycin at nonproductive sites in CYP3A4 SBC,[39,51] *p*NP in the second substrate-binding site of SULT1A1 SBC,[45] and two molecules of palmitic acid in the middle region of the symmetric homodimer of CYP2C8 outside its SBC.[40]

CYP3A4-containing nanodiscs mimic the membranous environment of native CYPs and prevent their aggregation to give a functionally homogeneous 1:1 complex of CYP3A4 and cytochrome P450 reductase. Experiments with the nanodiscs reveal that the formation of the high-spin complex of CYP3A4 with testosterone (productive conformation) requires the binding of three molecules of testosterone to the enzyme, each with different contributions to the turnover of the CYP3A4 intermediate.[56] Binding of the first testosterone accelerates NADPH consumption with no product formation; binding of the second produces the maximum rate of product formation; and binding of the third significantly improves coupling efficiency with no further increase in the rate of product formation. Furthermore, the extra binding site(s) of testosterone is located peripheral to the heme and outside the SBC.[57] Together, these findings support an allosteric mechanism in the homotropic cooperative kinetics of testosterone hydroxylation by CYP3A4. Pressure–perturbation studies of substrate-induced CYP spin shift also support allosteric conformational dynamics of CYP3A4 in substrate binding. In this scenario, binding of testosterone or 1-pyrenebutabol (1-PB), both of which are allosteric substrates, but not bromocriptine (BCT), which is a nonallosteric

substrate, stabilizes the high-spin state of the heme protein at high hydrostatic pressures.[58] These findings suggest a decrease in the accessibility and hydration of the active sites due to allosteric conformational changes in the SBC induced by allosteric substrates.

Allosteric regulation of substrate cooperativity may be explained by a sequential, multistep binding mechanism, which, in principle, is similar to the three-step binding model initially proposed for the binding of BCT to CYP3A4.[59,60] The mechanism involves the formation of at least three complexes: An initial (encounter) complex of the enzyme with an effector at an allosteric site promotes a conformational change and binding of a substrate in the SBC; this induces the subsequent conformational transition that is necessary for the binding of a substrate at the catalytic site and for a full-scale substrate-induced spin shift to occur. Although the steps and complexes may vary with effectors, substrates, and enzymes, this mechanistic model combines the multisubstrate binding and allosteric regulation and can be used to better explain many of the substrate–substrate and effector–substrate interactions of DMEs.

The discovery and characterization of persistent heterogeneity of DMEs and modulation of the equilibration of conformational pools by substrates and effectors lead to yet another model of cooperativity. In this model, binding of an effector modulates the equilibrium of persistent heterogeneous forms of a DME, for instance by inducing the oligomerization of the enzyme, which results in a change in the conformation of SBC and the characteristics of binding and catalysis of a substrate.[60] The presence of persistent functional heterogeneity of CYPs and its role in heterotropic cooperativity were first shown in the examination of the effect of substrates on the kinetics of CO rebinding after flash photolysis.[49] CYP3A4 was found to exist in two pools with distinct substrate specificity and catalysis. ANF stimulates the hydroxylation of B[*a*]P by CYP3A4, and the activating effect correlates with a redistribution of CYP3A4 between the two functional pools induced by ANF.[49]

Because CYPs and other DMEs exist in different oligomer configurations in both membranous and aqueous environments, it is reasonable to believe that oligomerization of CYPs contributes to the functional heterogeneity of the enzymes and, consequently, affects their kinetic behaviors. A supporting evidence of CYP oligomerization as a mechanism of cooperative behavior came from a study with the CYP3A4 mutant L211F/D214E/F304W.[50] It is known that CYP3A4 exhibits cooperativity in the interaction with 1-PB or in progesterone and testosterone 6β-hydroxylation. However, the cooperativity is lost with the mutant enzyme preparation. On the other hand, removal of apoproteins (CYP proteins without heme) known to be present in the mutant preparation restores the cooperativity and increases the proportion of the mutant enzyme of high-spin state in the CYP pool. The data suggest that interactions between holo- and apo-CYP oligomers result in conformational changes that eliminate the cooperativity and reduce the proportion of the high-spin state in the heterogeneous pools of the enzyme.[50]

5 Conclusion

The realization that mammalian DMEs function essentially as a group of promiscuous proteins has profoundly changed the understanding of chemical–host interaction

creating a new paradigm wherein the DMEs constitute the most complex and versatile enzymatic system found in nature well suited for the metabolism and disposal of all xenobiotics (and certain endobiotics) in the mammalian species. Not only is this paradigm understood in the context of evolution but, importantly, it reveals the molecular basis of the promiscuous behaviors of the enzymes that has significant implications in biology and medicine from both mechanistic and practical points of view.

The molecular secrecy of promiscuity of DMEs, as revealed by atomic-resolution crystal structures of DMEs along with molecular and biophysical analyses, is complex and, in many cases, phenomenal with regard to chemical–enzyme interactions. In essence, the DMEs have utilized all means of chemical–protein interactions, such as "induced fit" and "mosaic conformations", which are also seen in other biological systems, to bind and catalyze the conversion of chemicals. However, the interactions in DMEs are pushed to the extremes in many cases to bring about spacious and highly malleable SBCs, binding of multiple substrates, binding of substrates in multiple orientation/configurations, high mobility and rotation of substrates in the SBC, allosteric conformation transition, and oligomerization in homo- and hetero-configurations, all of which contribute to the enzymatic versatility of the DMEs for substrate recognition and catalysis, which, very often, is at the expense of substrate specificity and catalytic efficiency.

The practical interests in DME promiscuous behaviors stem from their potential applications in the evaluation of clinical drug–drug interactions and adverse drug effects; the prediction of the metabolism, pharmacokinetics, and potential toxicity of drug candidates in drug discovery and drug development; and the risk assessment of exposure to environmental carcinogens and toxic chemicals. Many undesirable clinical drug–drug interactions have been attributed to promiscuous metabolism and kinetics of the therapeutic agents. Prediction of the metabolic and pharmacokinetic properties of candidate drugs has proven to be very challenging, largely due to the extreme complexity and promiscuous behaviors of DMEs and other proteins involved in drug metabolism and disposition, such as drug transporters and xenobiotic-activated receptors. The same challenge is confronted in risk assessment of environmental chemicals that impose health threats to the public. Therefore, the molecular understanding of the promiscuous chemical–DME interactions provides a new framework in which the aforementioned issues can be analyzed with new approaches and models.

One potentially important but unaddressed question of DME promiscuity is what role, if any, it plays in the metabolism of chemicals under physiological conditions. In one example, reduced glutathione (GSH) at physiological concentrations (1–4 mM) is found to induce conformational changes of CYP3A4 that alter the cooperativity of the enzyme with a number of substrates/effectors including 1-PB, 7-benzyloxy-4-(trifluoromethyl)coumarin, and 7-benzyloxy-quinoline.[61] These findings suggest a potential regulatory mechanism by which the CYP3A4 activity is coupled to the cellular oxidative status via GSH. From this prospective, further studies are needed to substantiate physiological connections among DME promiscuity, cellular function, and, potentially, disease pathogenesis in the future.

Acknowledgments

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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Handbook of Metabolic Pathways of Xenobiotics

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WILEY

This edition first published 2014

Registered office

John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom

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Library of Congress Cataloging-in-Publication Data

Handbook of metabolic pathways of xenobiotics / editor-in-chief, Dr. Philip W. Lee.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-0-470-74910-4 (cloth)
I. Lee, Philip W., 1948- editor of compilation.
[DNLM: 1. Xenobiotics–metabolism. QU 120]
RM267
615.3'29–dc23

2013024701

A catalogue record for this book is available from the British Library.

ISBN-13: 978-0-470-74910-4

Set in 10/12pt Times by Laserwords Private Limited, Chennai, India
Printed and bound in Singapore by Markono Print Media Pte Ltd