A malleable catalyst dominates the metabolism of drugs

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In this issue of PNAS, Ekroos and Sjögren (1) present new structures of a cytochrome P450 (P450, or "CYP"), in one case bound with two ligands. The results are of considerable importance not only in regard to the practical issues in drug development but also because they have general significance in consideration of the flexibility of enzymes in recognizing substrates. The concept of how some enzymes accommodate a broad variety of ligands and catalyze multiple, regioselective reactions on a single substrate is a challenge to a classical lock-and-key model of catalysis. The current work has broad implications for a number of important enzyme systems and how we understand them.

Significance of P450-Based Drug Metabolism and P450 3A4

The P450 enzymes dominate the metabolism of drugs, accounting for ≈75% (2, 3). P450 3A4 is the most abundant P450 in the liver and small intestine and plays a role in the metabolism of one-half of the drugs on the market and in development (2–4). P450 3A4 was discovered two decades ago in early studies on the purification of P450s from human liver (5, 6) and was soon shown to have a wide repertoire of substrates, ranging in size from acetaminophen (M_r 151) to cyclosporin A (M_r 1201). The enzyme is highly inducible by barbiturates and numerous other compounds (7) and is also prone to both competitive and mechanism-based inhibition by drugs, leading to important drug–drug interactions. To date, attempts to understand the variability of P450 3A4 activity among humans at the level of allelic variants have been disappointing, and a pharmacogenetic approach to predicting interindividual patient variation in P450 3A4 and therapeutic effects has not been very successful (4).

How Does P450 3A4 Select Substrates?

Because of the significance of P450 3A4, a goal in pharmaceutical discovery and development is the prediction of modes of binding of new drugs. Another complicating factor is the cooperativity seen with some (but not all) ligands. Two types of cooperative behavior are recognized: (*i*) homotropic, in which sigmoidal patterns are seen for plots of the rate (of oxidation) versus concentration of a substrate, and (*ii*) heterotropic, in which one ligand can enhance the catalytic activity toward another (substrate) (4, 8). Some studies with P450 3A orthologues in experimental animals imply that such behavior may be clinically relevant (9). If this phenomenon does occur *in vivo* in humans, it is currently very unpredictable.

How Does P450 3A4 Work?

Numerous steady-state kinetic, homology modeling, and site-directed mutagenesis studies have been done with P450 3A4 to understand these phenomena, but biophysical studies have been limited. A number of amino acid residues have been implicated in the cooperativity, particularly in the work of Halpert's laboratory (10). A rather frequent suggestion has been that multiple ligands are bound within P450 3A4 and that the interaction of the ligands with each other, in the active site, forms the basis of the cooperativity (4, 8, 10). However, the physical evidence for this hypothesis has been limited. One relatively direct piece of evidence is the pyrene fluorescence stacking study of Dabrowski *et al.* (11). More recent studies by Fernando *et al.* (12) are consistent with the two-ligand stoichiometry for some (but not all) substrates (see also ref. 8). The first crystal structures of P450 3A4 were greeted with enthusiasm that the secrets of ligand binding would be revealed (13, 14). The structures are certainly valuable, although the ligand-binding issues were still not resolved. The crystals from Johnson's group (13) were grown in the presence of erythromycin, but the ligand was not found in the crystal. In the structure reported by Williams *et al.* (14), the substrate progesterone was bound but to a site clearly too distant from the iron atom for catalysis (of oxidation) to occur.

Defined "plastic" regions may be a common feature of the membrane-bound P450s.

In the new structures of Ekroos and Sjögren (1), several features are of particular interest. In contrast to the two original sets of P450 3A4 structures (13, 14), the protein has a dramatically altered conformation in the substrate-bound form, a characteristic of other P450s whose crystal structures are known both with and without ligands. Another feature is that the binding of a ligand can increase the size of the active site >80%. One of the structures (to 2.8 Å) has two molecules of ketoconazole (a relatively large substrate/inhibitor, M_r 531) bound in a stacked configuration. Although the authors concede that this could be a crystallization artifact, the results provide the most direct evidence that the long-postulated multiple component binding in P450 3A4 really can occur. Another crystal structure was solved with the substrate/inhibitor erythromycin, also to 2.8 Å. This structure differs from that of both unliganded P450 3A4 and the (bis) ketoconazole complex. Interestingly, the known site of *N*-demethylation is 17 Å from the heme iron, and this structure, like the progesterone-bound structure of Williams *et al.* (14), must be considered a nonproductive complex.

What are the implications regarding P450 3A4 biochemistry? First, the availability of multiple modes of loose binding (e.g., in the erythromycin structure) can explain some of the "loose" regioselectivity of P450 3A4. However, the view that the active site cavity is completely noninstructive must be rejected: the ligand ketoconazole shows interactions with amino acid residues in P450 3A4 (1); work from our own laboratory shows the striking stereoselectivity of β vs. α hydrogen abstraction in testosterone 6β-hydroxylation (15). Nevertheless, the availability of alternate and nonproductive binding modes can frustrate various biophysical approaches, in which stoichiometry measurements may or may not reflect functional interactions (8, 16). The results of the structural work of Ekroos and Sjögren (1) showing the binding of two ketoconazole molecules raise the question of the order of events leading to that structure. The (two) molecules are unlikely to dimerize before a single-event binding, and a more likely scenario involves some sequence of binding, protein conformational change, and binding of the second

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ketoconazole molecule. This would seem to be a minimum number of events (three?), and defining the sequence of these and the relevant rate constants (both forward and reverse) remains a challenge (8). Another point the authors (1) make is the extent of the differences between their ligand-bound structures, i.e., with erythromycin and ketoconazole. The implications are that understanding the details of the structure of P450 3A4 with one substrate bound (e.g., erythromycin) cannot necessarily predict the structure of the same protein with another ligand (e.g., ketoconazole, or vice versa). The authors make the point that "it is highly unlikely that the conformational space [possible] has been covered by the present structures alone." A corollary is that previous predictions made by modeling are suspect at best, and even modeling based on the current structures is equivocal, a point made by Ekroos and Sjögren. However, the authors do allude to other (unpublished) results of theirs showing that the P450 3A4-(bis) ketoconazole structure outperformed the ligand-free structure in predicting (known) sites of oxidation of P450 3A4 substrates (1).

Further Implications

In terms of the field of P450 research, Ekroos and Sjögren (1) propose that defined "plastic" (or malleable) regions may be a common feature of the membrane-bound P450s, or at least many of them. As an example, work with rabbit P450 2B4 (17) is consistent with the view that this enzyme also has considerable flexibility, which is considered to be at least a part of the basis for the broad substrate specificity of the catalyst. These results have implications in how P450 biochemistry proceeds to address issues in the field of structure–function relationships.

In a broader sense, P450s are probably not unique in this regard, and we can also consider implications of the work for many other enzymes with broad specificity, e.g., the drug transporter P-glycoprotein/Mdr1, for which evidence of multiple conformations has been shown indirectly (18). These general considerations are not new and go back to earlier biochemical discussions of allostery and induced fit (19). The original considerations were more directed toward how enzymes achieve high selectivity, but many features and issues are relevant here with issues of broad specificity. As Ekroos and Sjögren (1) discuss, their structural biology results do not necessarily distinguish between dynamic models in which (*i*) induced fit and substrate shaping are kinetically linked with substrate binding and (*ii*) a mosaic of protein conformations is already available and substrate interactions "catch" the most useful ones. There are general questions beyond P450s to address, but the P450 3A4 structural biology is an important step. As the authors point out (1), determination of more P450 3A4-ligand structures is clearly in order.

Finally, the relevance of these structures (1) should be emphasized in that ketoconazole and erythromycin both figure in numerous drug interactions and contraindications. Ketoconazole has a "black-box" warning for its use as a drug, i.e., a patient takes this at his or her own risk (20).

1. Ekroos M, Sjögren T (2006) *Proc Natl Acad Sci USA* 103:13682–13687.
2. Williams JA, Hyland R, Jones BC, Smith DA, Hurst S, Goosen TC, Peterkin V, Koup JR, Ball SE (2004) *Drug Metab Dispos* 32:1201–1208.
3. Wienkers LC, Heath TG (2005) *Nat Rev Drug Discov* 4:825–833.
4. Guengerich FP (2005) in *Cytochrome P450: Structure, Mechanism, and Biochemistry*, ed. Ortiz de Montellano, P. R. (Kluwer Academic/Plenum, New York), pp. 377–530.
5. Watkins PB, Wrighton SA, Maurel P, Schuetz EG, Mendez-Picon G, Parker GA, Guzelian PS (1985) *Proc Natl Acad Sci USA* 82:6310–6314.
6. Guengerich FP, Martin MV, Beaune PH, Kremers P, Wolff T, Waxman DJ (1986) *J Biol Chem* 261:5051–5060.
7. Lehmann JM, McKee DD, Watson MA, Wilson TM, Moore JT, Kliewer SA (1998) *J Clin Invest* 102:1016–1023.
8. Isin EM, Guengerich FP (2006) *J Biol Chem* 281:9127–9136.
9. Tang W, Stearns RA (2001) *Curr Drug Metab* 2:185–198.
10. Scott EE, Halpert JR (2005) *Trends Biochem Sci* 30:5–7.
11. Dabrowski MJ, Schrag ML, Wienkers LC, Atkins WM (2002) *J. Am. Chem. Soc.* 124:11866–11867.
12. Fernando H, Halpert JR, Davydov DR (2006) *Biochemistry* 45:4199–4209.
13. Yano JK, Wester MR, Schoch GA, Griffin KJ, Stout CD, Johnson EF (2004) *J Biol Chem* 279:38091–38094.
14. Williams PA, Cosme J, Vinkovic DM, Ward A, Angove HC, Day PJ, Vonrhein C, Tickle IJ, Jhoti H (2004) *Science* 305:683–686.
15. Krauser JA, Guengerich FP (2005) *J Biol Chem* 280:19496–19506.
16. Roberts AG, Campbell AP, Atkins WM (2005) *Biochemistry* 44:1353–1366.
17. Muralidhara BK, Negi S, Chin CC, Braun W, Halpert JR (2006) *J Biol Chem* 281:8051–8061.
18. Loo TW, Bartlett MC, Clarke DM (2003) *J Biol Chem* 278:13603–13606.
19. Koshland DE, Jr, Nemethy G, Filmer D (1966) *Biochemistry* 5:365–385.
20. Walgren JL, Mitchell MD, Thompson DC (2005) *Crit Rev Toxicol* 35:325–361.