The cytochromes P450 (CYPs)2 are a superfamily of monooxygenase enzymes that catalyze oxygen insertion into a vast array of different substrates such as lipids and steroids, as well as drug compounds and other xenobiotics (1). A prominent member of the P450 superfamily is CYP1A1, which metabolizes a diversity of substrates of various sizes including the fatty acid arachidonic acid, the fluoroquinolone antibiotic difloxacin, and the drug theophylline. CYP1A1 also acts on several procarcinogenic molecules, including aromatic and heterocyclic amines (2), as well as polycyclic aromatic hydrocarbons like benzo(a)pyrene (BaP). Indeed, CYP1A1-mediated epoxidation of BaP produces BaP-7,8-epoxide, which can be further oxidized to form the carcinogen BaP-7,8-dihydrodiol-9,10-epoxide (3). In light of CYP1A1’s roles in carcinogenesis, inhibition of this P450 could prove to be a good target for cancer prevention (4).

P450s can be promiscuous or highly substrate-specific, and understanding how the promiscuous P450s maintain their catalytic efficiency while accommodating so many substrates is of great intellectual and practical interest. Previous work has shown that many eukaryotic P450s are flexible enzymes that can adapt their structures to bind to different substrates. However, there are few structural data currently available for CYP1A1 or its related CYP1A2 and CYP1B1 enzymes in complex with substrates, limiting our insight into the workings of these family members. Structures of CYP1A1, CYP1A2, and CYP1B1 complexed with α-naphthoflavone (ANF) have been determined, in each case binding ANF in a small, planar active site (5, 6). These structures suggest that polycyclic aromatic hydrocarbons enter a narrow entrance cavity leading to an active site that has strong complementarity with that of the planar substrate. But, how do other molecules, with varying sizes and chemical properties, engage with CYP1A1?

In their exploration of this question (7), Bart and Scott noted that many substrates and inhibitors of human CYP1A1 did not conform to size constraints compatible with the binding mode observed for the human CYP1A1/ANF complex. This suggests that CYP1A1 has greater conformational flexibility than is evident from the structure of its ANF complex. The authors tested a range of known ligands with diverse chemical properties and structures for their ability to block the metabolism of a luminescent substrate, determining the IC₅₀ values in each case. Azole antifungal compounds (clotrimazole, miconazole, and tioconazole) were, unsurprisingly, among the most potent inhibitors, but the furanocoumarin bergamottin (found in grapefruit juice and bergamot orange oil) and erlotinib (a tyrosine kinase inhibitor that acts on the epidermal growth factor receptor) were also potent inhibitors, with IC₅₀ values of 140 and 180 nM, respectively. Their tight binding to CYP1A1 was confirmed using UV-visible spectroscopic titrations, producing Kᵋ values in the same range as that obtained for ANF. This result prompted the authors to co-crystallize these bulky ligands with CYP1A1 to identify their mode of binding and search for P450 structural reorganizations.

The authors were successful in the co-crystallization of both bergamottin and erlotinib (Fig. 1) using an engineered, soluble form of CYP1A1 from which the N-terminal transmembrane segment was deleted. In the bergamottin structure, its psoralen ring system is located distal to the heme and parallel to the P450 I-helix and forms π–π interactions with Phe-224 from the F-helix, while the carbonyl moiety on the psoralen ring also interacts with the side chain nitrogen of the Asn-222 residue. The bergamottin geranylxy chain is orientated toward the heme, with its 6′- and 7′-carbons approaching most closely to the heme iron. Importantly, CYP1A1 produced three metabolites, assigned as 6′,7′-dihydroxybergamottin and its singly hydroxylated 6′- and 7′-bergamottin precursors, consistent with the substrate binding mode observed in the crystal structure. For erlotinib, the quinazoline ring portion of the molecule is positioned close to the catalytically important I-helix, with π–π interactions again made by Phe-224 with the aromatic ring system. Two methoxetehyso side chains extend from the quinazoline core toward the top of the active site and make stabilizing interactions with the side chain nitrogen of Asn-255 in the G-helix and the Asp-320 carboxylate group. Further interactions are made between the secondary amine group of erlotinib...
and the Asp-320 carboxylate via an interstitial water molecule. Asn-222 also bonds to an oxygen atom of the methoxyethoxy group of one of the side chains. The phenyl-acetylene moiety of erlotinib is closest to the heme iron and, as in the case of bergamottin, this binding mode is consistent with the major products formed by CYP1A1—these being O-demethylation of the methoxyethoxy side chain, oxidation of the terminal alkyne, and hydroxylation at the para position on the phenyl-acetylene moiety (8). The fact that inactivation of CYP1A1 was not seen with erlotinib suggests that alkyne modification (which would produce locally reactive compounds) does not occur in this case and that instead para-hydroxylation ensues on the erlotinib anilino ring (9).

The new crystal structures of bergamottin and erlotinib complexed with CYP1A1 exhibit similarities to that of the CYP1A1/ANF complex through the positioning of the planar parts of these molecules in the same alignment as seen for the ANF complex (10). However, important lessons are also learned with respect to how CYP1A1 is able to bind those larger molecules. In particular, a 5-residue “break” in the F-helix region emerges as a common theme in each of the three structures. This reorganization likely enables the expansion of the active site, as seen most clearly in the case of erlotinib-bound CYP1A1. In this structure the repositioning of Asn-222 in the F-helix allows an “opening up” of the active site toward the surface of the P450 and a near-doubling of active site volume (from 408 Å³ in the ANF complex to 806 Å³ in the erlotinib complex). A similar strategy is likely used by other larger CYP1A1 substrates in order for them to fit in the CYP1A1 active site. This study thus reveals novel mechanisms by which CYP1A1 adapts to accommodate large substrate molecules, with potential ramifications for understanding substrate binding in other P450s from the CYP1A family and possibly beyond. These new structures should similarly drive computational analysis of CYP1A1 structure and ligand binding, enable docking studies to identify new ligands, and provide a clearer understanding of how carcino-}

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