Substrate-specific cytochromes P450 play major roles in steroid and eicosanoid biosynthesis and thus constitute important drug design targets. In contrast, P450 isozymes expressed in the liver take part in the metabolism of nearly all drugs. Adverse drug reactions, for instance to Prozac, result from individual variations in hepatic P450s. It is thus important to predict which P450s interact with a potential drug candidate and to understand the nature of these interactions.

We have developed fluorescent probe molecules for P450cam (the prototypical P450) that consist of an \(\alpha,\omega\)-diaminoalkane chain connecting a dansyl fluorophore to the P450cam substrate adamantane:

A shift in Soret absorption (Figure 1) and greatly diminished dansyl luminescence attributable to Förster energy transfer to the heme (Figure 2) accompany probe binding. When D-4-Ad is displaced from the active site by camphor, fluorescence is restored (Figure 2a). Because a bright signal stands out against a dark background, substrate or inhibitor binding is readily detected. This assay, which is both simple and sensitive, can be employed to screen combinatorial chemical libraries.

Both fluorescence and absorption spectra show that D-4-Ad binding to P450cam is competitive with camphor. The Soret shift (416–414 nm) induced by D-4-Ad indicates that it binds in the active site. With a \(K_d\) of 0.83 \(\mu\)M, D-4-Ad binds twice as strongly as the natural substrate. D-8-Ad also induces a shift in the Soret maximum from 416 to 414 nm: from the integrated D-8-Ad fluorescence in the presence and in the absence of P450cam, we estimate an upper limit \(K_d\) of 0.02 \(\mu\)M for this probe.

The crystal structure of the P450cam:D-8-Ad complex shows that the probe binds in the same channel as Ru(bpy)\(_3\)−linker−Ad (bpy = 2,2′-bipyridine) analogues (Figure 3). The eight-carbon chain is nearly fully extended, allowing the dansyl moiety to bind at the surface of the protein. The good fit is attributable to conformational flexibility; that is, the F and G helices open just enough to allow the probe to enter and bind. The observed conformation is midway between the “closed” (camphor) and “open” (Ru-linker-Ad) structures.

The structure reveals a hydrogen bond between the amide carbonyl of the probe and Tyr96 in P450cam:D-8-Ad, mimicking the hydrogen bond between camphor and Tyr96 in the P450cam:substrate complex. In addition, there are a great many hydrophobic interactions between the probe molecule and the enzyme; analysis of these contacts shows that much of the solvent-accessible surface area is buried. The estimated \(K_d\) of 0.02 \(\mu\)M corresponds to a binding energy of \(\sim 11\) kcal/mol, or \(\sim 11\) cal/(mol Å\(^2\)) (Figure 4).

The Ru(bpy)\(_3\)\(^2+\) analogues (Ru-9-Ad and Ru-F\(_8\)bpy-Ad) do not bind as tightly to P450cam, but the free energy changes per buried...
the mobility of the B′, F, and G helices. Both solution and crystallographic studies of other P450s suggest that this feature is common to the P450 superfamily.

The two probes described herein illustrate the usefulness of our methodology. D-4-Ad can be employed to screen potential P450 inhibitors, as it is easily displaced by other molecules with comparable or lower dissociation constants. In contrast, D-8-Ad binds extremely tightly: the conformational flexibility of the P450 fold allows the enzyme to close around the probe, thereby making a great many productive hydrophobic contacts. The insight gained from the D-8-Ad:P450cam structure could potentially lead to a more rational design strategy for P450 inhibitors.

Acknowledgment. This work was supported by the Fannie and John Hertz Foundation (A.R.D.), the National Science Foundation, and the National Institutes of Health (Metalloprotein Program Project Grant P01 GM48495; NRSA fellowship GM20703 to A.-M.A.H.).

Supporting Information Available: Syntheses of compounds; spectroscopic data; and crystallographic experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) California Institute of Technology. (b) The Scripps Research Institute.
(7) The fluorescence of D-8-Ad (λmax = 480 nm) is blue shifted from that of D-4-Ad (550 nm), indicating that the D-8-Ad environment is less polar.
(10) As with D-4-Ad, titration of a 1:1 mixture of P450cam and D-8-Ad results in conversion to high-spin ferric P450cam (Figure 1b).

JA0271678