SENSITIZER-LINKED SUBSTRATES AS PROBES OF HEME ENZYME STRUCTURE AND CATALYSIS

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Alexander R. Dunn

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ABSTRACT

Ruthenium-diimine sensitizers (Ru-wires) with the structure $[Ru(L_2)L']^{2+}$, where L' is a perfluorobiphenyl bridge connecting 4,4'-dimethylbipyridine to the substrate adamantane or the heme ligand imidazole, bind to cytochrome P450cam with micromolar dissociation constants. Ru-wires can be used to trigger redox reactions on timescales faster than those achievable using conventional stopped-flow techniques: photoinduced heme reduction with an imidazole-terminated Ru-wire occurs in 40 ns. The large variation in ET rates among the Ru-diimine:P450 conjugates strongly supports a through-bonds model of Ru:heme electronic coupling.

The Ru-wires also bind the murine inducible nitric oxide synthase (NOS) oxidase domain, both in the active site and to the hydrophobic surface patch that interacts with the NOS reductase domain. Rhenium-diimine probes with the structure [Re(4,7-dimethyl phenanthroline)$(CO)_3$L]$^+$, where L = imidazole-$C_{12}F_8$-imidazole (Re-im) or imidazole-$C_{12}F_9$ (Re-F_9bp), bind in the NOS active site. Re-im (K_d = 6 nM) ligates the heme iron. Re-F_9bp (K_d = 3.4 μM) produces a partial low- to high-spin conversion of the heme. Compounds with properties similar to the Ru- and Re-diimine probes may provide novel means of NOS inhibition.

Luminescent dansyl probes were designed to target cytochrome P450cam. D-4-Ad (dansyl-C_4-adamantane) luminescence is quenched by Förster energy transfer upon binding (K_d = 0.83 μM), but is restored when the probe is displaced from the active site by camphor. In contrast, D-8-Ad (K_d ~ 0.02 μM) is not displaced from the enzyme even in the presence of a large excess of camphor. Probes with properties similar to those of D-4-Ad potentially could be useful for screening P450 inhibitors.

Crystal structures of P450cam bound to ruthenium diimine and danysl probes reveal an open enzyme conformation that allows substrate access to the active center via a 22-Å deep channel. Interactions of the probes with the channel illustrate the importance of exploiting protein dynamics in inhibitor design. Movements of the F, G and B' helices couple to conformational changes in active site residues implicated in proton pumping and dioxygen activation. Common conformational states among P450cam and homologous enzymes indicate that the structural flexibility of the F/G helix region allows the 54 human P450s to oxidize diverse substrates.

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