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Supporting Information

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for

Engineering Cytochrome P450 Enzymes for Improved Activity towards Biomimetic 1,4-NADH Cofactors

Jessica D. Ryan, Richard H. Fish,* and Douglas S. Clark*

Site Directed Mutagenesis of PdR and P450 BM-3: Site directed mutagenesis was accomplished using the Stratagene Quickchange Kit according to the manufacturer's protocols, employing the following primers to effect the mutations in PdR:

E300L forward: 5'-GACCGCTGGGTGCGTATCCT**TTC**GGTGC-3'

E300L reverse: 5'-GCATTGGGCACCGA**AAG**GATACGCACCC-3'

E300A forward: 5'-GGTGCGTATCG**CAT**CGGTGCCCAATGC-3'

E300A reverse: 5'-GCATTGGGCACCGA**TGC**GATACGCACC-3'

The primers for P450 BM-3 mutations (W1064S/R966D) were identical to those used by Maurer et al.[1]

Expression and Purification of P450cam: Three plasmids, each containing one of the P450cam proteins, were obtained from the laboratory of Jay D. Keasling (U. C. Berkeley). The expression and purification protocols were as described by Grayson et al.[2], except that α-levulinic acid was added to P450cam cultures (0.5 mM final concentration) at the time of induction. Concentrations of each protein were calculated using the following extinction coefficients: 102.0 cm^{-1} mM^{-1} for P450cam, l = 391 nm; 11.1 cm^{-1} mM^{-1} for PdX, l = 415 nm; 10.0 cm^{-1} mM^{-1} for PdR, l = 454 nm.

Expression and Purification of P450 BM-3: The pc-wori plasmid containing the gene for P450 was a generous gift of Francis H. Arnold (California Institute of Technology, USA). Proteins were expressed and purified according to previously published methods[3]. The concentration of P450 BM-3 was determined by CO difference spectra using an extincion coefficient of 91.1 mM^{-1} cm^{-1}, as reported elsewhere[4].

Reduction of cytochrome *c* by P450 BM-3: Assays were in carried out 0.1 M Tris buffer (0.1 M), pH 8.0. The reaction (100 µL) contained cytochrome C (60 µM) and P450 (11 or 33 nM). P450 BM-3 was incubated with the cytochrome C for 1 min prior to initiation of the reaction by the addition of a cofactor stock solution (10 µL). The reduction of cytochrome c was monitored at 550 nm, and the concentration of reduced cytochrome c was calculated using an extinction coefficient of 21.1 mM^{-1} cm^{-1}[5].

Gas chromatography analysis of P450cam reactions: The concentrations of camphor, hydroxycamphor, and 2,5-diketocamphane were determined by gas chromatography using a 25-m, 250-mm (ID) Varian Factor Four VF-5ms capillary column installed in a Varian 3900 gas chromatograph equipped with an FID detector (Varian Instruments, Walnut Creek, CA). The injector and detector temperatures were 250 and 300°C, respectively. The samples were injected at a split ratio of 20 with helium as the carrier gas at a flow of 1.0 ml/min. The column temperature was held at 60°C for 2 min, and the temperature was then ramped up to 190°C at a rate of 20°C/min. Decane (internal standard), camphor, and hydroxycamphor eluted at 4.94, 6.34, and 7.97 min, respectively.

References

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