Douzou, P. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6741-6744.
Douzou, P., & Hui Bon Hoa, G. (1987) in 2nd International Meeting on the Molecular and Cellular Regulation of Enzyme Activity, Berlin, April 1987, Pergamon, New York.
Douzou, P., Hui Bon Hoa, G., & Sligar, S. G. (1988) *Proc. Natl. Acad. Sci. U.S.A.* (submitted for publication).
Fisher, M. T., Scarlata, S. F., & Sligar, S. G. (1985) *Arch. Biochem. Biophys. 240*, 456-463.
Forster, T. (1959) *Discuss. Faraday Soc. No. 27*, 7-17.
Gunsalus, I. C., & Wagner, G. (1978) *Methods Enzymol. 52*, 116-188.
Hui Bon Hoa, G., & Marden, M. C. (1982) *Eur. J. Biochem. 124*, 311-315.
Hui Bon Hoa, G., Di Primo, C., Geze, M., Douzou, P., Kornblatt, J. A., & Sligar, S. G. (1988) *J. Am. Chem. Soc.* (submitted for publication).
Jung, C., Bendzko, P., Ristau, O., & Gunsalus, I. C. (1985) in Proceedings of the 5th International Conference of Cytochrome P-450. "Cytochrome P-450, Biochemistry, Biophysics and Induction (Akademiai Kiado, Budapest 1985) Aug 21-24, 1985, Hungary, pp 19-22.
Jung, C., Shyamsunder, E., Bowne, S. F., Ullah, A. H. J., Gunsalus, I. C., & Wagner, G. C. (1988) *J. Biol. Chem.* (submitted for publication).
Lange, R., Hui Bon Hoa, G., Debey, P., & Gunsalus, I. C. (1979) *Acta Biol. Med. Ger. 38*, 143-142.
Marden, M. C., & Hui Bon Hoa, G. (1982) *Eur. J. Biochem. 129*, 111-117.
Marden, M. C., & Hui Bon Hoa, G. (1986) *Biophys. J. 49*, 619-627.
Marden, M. C., & Hui Bon Hoa, G. (1987) *Arch. Biochem. Biophys. 253*, 100-107.
Newman, R. C., Kauzmann, W., & Zipp, A. (1973) *J. Phys. Chem. 77*, 2687-2691.
Ogunmola, G. B., Zipp, A., Chen, F., & Kauzmann, W. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1-4.
Poulos, T. L., Finzel, B. C., Gunsalus, I. C., Wagner, G. C., & Kraut, J. (1985) *J. Biol. Chem. 260*, 16122-16130.
Poulos, T. L., Finzel, B. C., & Howard, A. H. (1986) *Biochemistry 25*, 5314-5322.
Satake, H., Imai, Y., Hashimoto, C., Sato, R., Shimizu, P., Nozawa, Y., & Hatano, M. (1976) *Seikagaku 48*, 508-510.
Sato, R., & Omura, T. (1978) in *Cytochrome P-450* (Kodansha, Ed.) pp 50-62, Academic, New York.
Ullah, A. H. J., Bhattacharyya, P. K., Bhaktavatsalam, J., Wagner, G. C., & Gunsalus, I. C. (1983) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 42*, 1987.
Weber, G., & Drickamer, H. G. (1983) *Q. Rev. Biophys. 16*, 89-112.
Yu, C. A., & Gunsalus, I. C. (1974) *J. Biol. Chem. 249*, 102-106.

Secondary Structure Prediction of 52 Membrane-Bound Cytochromes P450 Shows a Strong Structural Similarity to $P450_{cam}$†

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ABSTRACT: The secondary structure of 52 aligned cytochrome P450 sequences, all of which are membrane bound, is predicted and collectively compared with the crystal structure of the soluble cytochrome $P450_{cam}$. Ten of 13 helical regions, 6 of 7 β-pair regions, and β-structure corresponding to a known β-bulge near the active site of $P450_{cam}$ are predicted to exist in the membrane-bound P450s. Three turns associated with β-structure in the soluble enzyme are also predicted for the membrane-bound forms. A strong structural similarity is evident between membrane P450s and the soluble $P450_{cam}$. Consequently, a multitransmembrane structure involving much of P450 seems highly unlikely. A structure with two N-terminal transmembrane segments is compatible with these observations.

Cytochrome P450 designates a diverse class of *b*-type cytochromes that activate molecular oxygen. The activated oxygen then reacts in situ with many lipophilic substrates (Ortiz de Montellano, 1986; Black & Coon, 1987). There is considerable interest in the structure and function of these enzymes. Since 1982, over 100 P450 sequences have been published, representing at least 53 unique P450 proteins and many variants (Nebert et al., 1987, 1989). It is estimated that a single mammalian species may have from 50 to 200 cytochrome P450 genes (Nebert et al., 1987; Nelson & Strobel, 1987; Marx, 1985). These genes are organized in multigene familes on many different chromosomes (Nebert et al., 1987, 1989). Some P450 genes are polymorphic and are implicated in disease (Guengerich et al., 1987).

Sequence similarity around a 100% conserved cysteine suggests a common ancestral gene for all P450s. However, bacterial P450s are soluble, while eukaryotic P450s are membrane bound. Recently, the crystal structure of cytochrome $P450_{cam}$ from *Pseudomonas putida* was determined (Poulos et al., 1987). These authors proposed that mem-

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brane-bound P450s should resemble the bacterial enzyme on the basis of sequence similarities and structure/function constraints. In support of this suggestion, the secondary structure prediction of cytochrome P450*b* fits reasonably well with the structure of $P450_{cam}$ (Poulos et al., 1987; Gotoh et al., 1983). Though appealing, the proposed similarity in structure is difficult to reconcile with models of P450 that usually contain 6–10 transmembrane segments (Black & Coon, 1987; Tarr et al., 1983; Heinemann & Ozols, 1982; Ozols et al., 1985; Haniu et al., 1986). Recently, we have proposed a structure with only transmembrane segments at the extreme N-terminal (Nelson & Strobel, 1988). This model is compatible with bacterial and eukaryotic P450s sharing similar three-dimensional structures, except for the N-terminal 50–60 amino acids of the membrane-bound P450s. To offer further support for this model, we predicted the secondary structure of 52 aligned P450 sequences and compared the collective results with the structure of cytochrome $P450_{cam}$. There appears to be a marked similarity between the eukaryotic and prokaryotic enzymes. The method used should be applicable to other protein families.

Materials and Methods

Sequence Alignment. The alignment of cytochrome P450 sequences used here is an extension of our previously published alignment of 34 P450 sequences (Nelson & Strobel, 1987, 1988). Thirteen new sequences were added to the P450II family: pHP3 (Imai, 1987); 1–88 (Johnson et al., 1987; Zhao et al., 1987); b32–3 (Imai et al., 1988); 1 human (Kimura et al., 1987; Okino et al., 1987); mp (Umbenhauer et al., 1987; Yasumori et al., 1987; Kimura et al., 1987; Meehan et al., 1988); h (Yoshioka et al., 1987); IIB3 (Labbé et al., 1988); 15α mouse (Squires & Negishi, 1988); a (Nagata et al., 1987); 16α mouse (Wong et al., 1988); rat db1 and db2 (Gonzalez et al., 1987); and db1 human (Gonzalez et al., 1988). A porcine sequence was added to the C21 family [P450XXI] (Haniu et al., 1987). Two sequences were added to the PCN family [P450III]: PCN2 (Gonzalez et al., 1986); and rabbit 3c (Dalet et al., 1988). One sequence was added to the LAω family [P450IV]: p-2 (Matsubara et al., 1987). One sequence was added to the mitochondrial family [P450XI]: bovine 11β (Morohashi et al., 1987; Chua et al., 1987). Two new families were added: aromatase [P450XIX] (Simpson et al., 1987; Chen et al., 1988); and yeast lanosterol 14α-demethylase [P450LI] (Kalb et al., 1987). All sequences except aromatase, 11β, and 14DM were aligned visually because they had obvious similarity with other sequences. These three sequences were aligned by methods described previously (Nelson & Strobel, 1987). The alignment will be supplied with reprints of this paper or as supplementary material. All sequences were proofread against original sources. When sequences had been done by more than one group, amino acid sequence differences have been noted and care has been taken to choose the most appropriate amino acid. Some of these differences are given in an appendix to Nelson and Strobel (1987). A 16-page summary of 212 differences between closely related P450 sequences is available from the authors upon request.

Secondary Structure Prediction. Secondary structure was predicted by the method of Garnier et al. (1978). Decision constants were selected by assuming the helix content was between 20 and 50% and β-structure was greater than 20% on the basis of the results of Chiang and Coon (1979). Each amino acid in the alignment is replaced by a code for its predicted secondary structure. The number of amino acids in a given conformation is summed for each position in the alignment, and a histogram is made for each type of structure. The location of gaps in the alignment are also plotted. The results are shown in Figure 1. The Garnier et al. algorithm is based on the crystal structures of soluble proteins. Its use is justified for membrane-bound P450s by our earlier work, which requires a cytosolic location for all but the extreme N-terminal of these proteins (Nelson & Strobel, 198).

Results and Discussion

The crystal structure of cytochrome $P450_{cam}$ has 13 helices with alignment positions indicated by the shaded areas in Figure 1A. With a 77% cutoff imposed (40 or more of the 52 sequences), helical structure is predicted at 10 locations in the alignment. Nine peaks occur within the shaded areas corresponding to helices B, C, D, E, G, I, J, K, and L. The 10th peak straddles one edge of the helix H region.

Examination of the alignment in the helix H region shows this area is very poorly conserved. In our alignment, there is a gap of seven residues in the $P450_{cam}$ sequence between positions 364 and 372 [339 and 347 in Nelson and Strobel (1987, 1988)]. Due to poor conservation in this region, the exact location of this gap is arbitrary. If the $P450_{cam}$ segment from positions 355 to 364 [amino acids 219–228 or positions 330–339 in Nelson and Strobel (1987, 1988)] were shifted to the right seven positions, then the helix H region in Figure 1A would be shifted to include the peak that presently lies just to its right. Such a revision of the alignment may be indicated by these results.

The helix A region of $P450_{cam}$ is predicted to be helical in one-third to half of the aligned sequences. This helix is in the helix-poor part of the $P450_{cam}$ structure, and it may not be helical in all P450s. Helix B′ is a short helix not detected in the 2.6-Å structure of $P450_{cam}$ but seen in the 1.63-Å refined structure (Poulos et al., 1987). This helix is near the active site with one residue contributing a hydrogen bond to the substrate. This region's structure will probably vary depending on each enzyme's substrate specificity.

The Garnier et al. algorithm failed to predict helix F. This helix lies above the middle of helix I in the helix-rich portion of $P450_{cam}$. This region has been suggested to be structurally conserved in all P450s (Poulos et al., 1987). The failure to predict helix F is unexpected. When the alignment is examined, two conserved prolines are seen at positions 303 (40/53 residues) and 310 (46/52 residues). These prolines correspond to alignment positions 278 and 286 in Nelson and Strobel (1987, 1988). Proline is considered to disrupt helices, which may explain the failure of the algorithm to predict a helix in this region. The bias in this algorithm against prolines occurring in the helices may be overweighted, because prolines are seen in helices. The E helix in $P450_{cam}$ has two internal prolines. The C, F, G, and K helices also contain internal prolines. We suspect that the F region of membrane-bound P450s really is helical, but this is not predicted due to an algorithm bias.

$P450_{cam}$ helices C, E, I, and K occupy regions in the alignment that are wider than the peaks that appear in Figure 1A. Helices C, E, and K have gaps in the alignment of $P450_{cam}$ with the eukaryotic sequences. These gaps account for the wide shaded areas of regions C, E, and K. Helix I is a special case. This is a 34 amino acid helix with a distortion at the center to allow for oxygen binding (Poulos et al., 1987). In this central area there is a conserved cluster of Ala, Gly, Thr, and Ser residues that will thwart prediction of helical structure. The algorithm predicted the central residues would have a coil conformation, while the adjacent residues would have a β-structure. The outermost residues on either side were predicted to be helical. A similar prediction was made for the

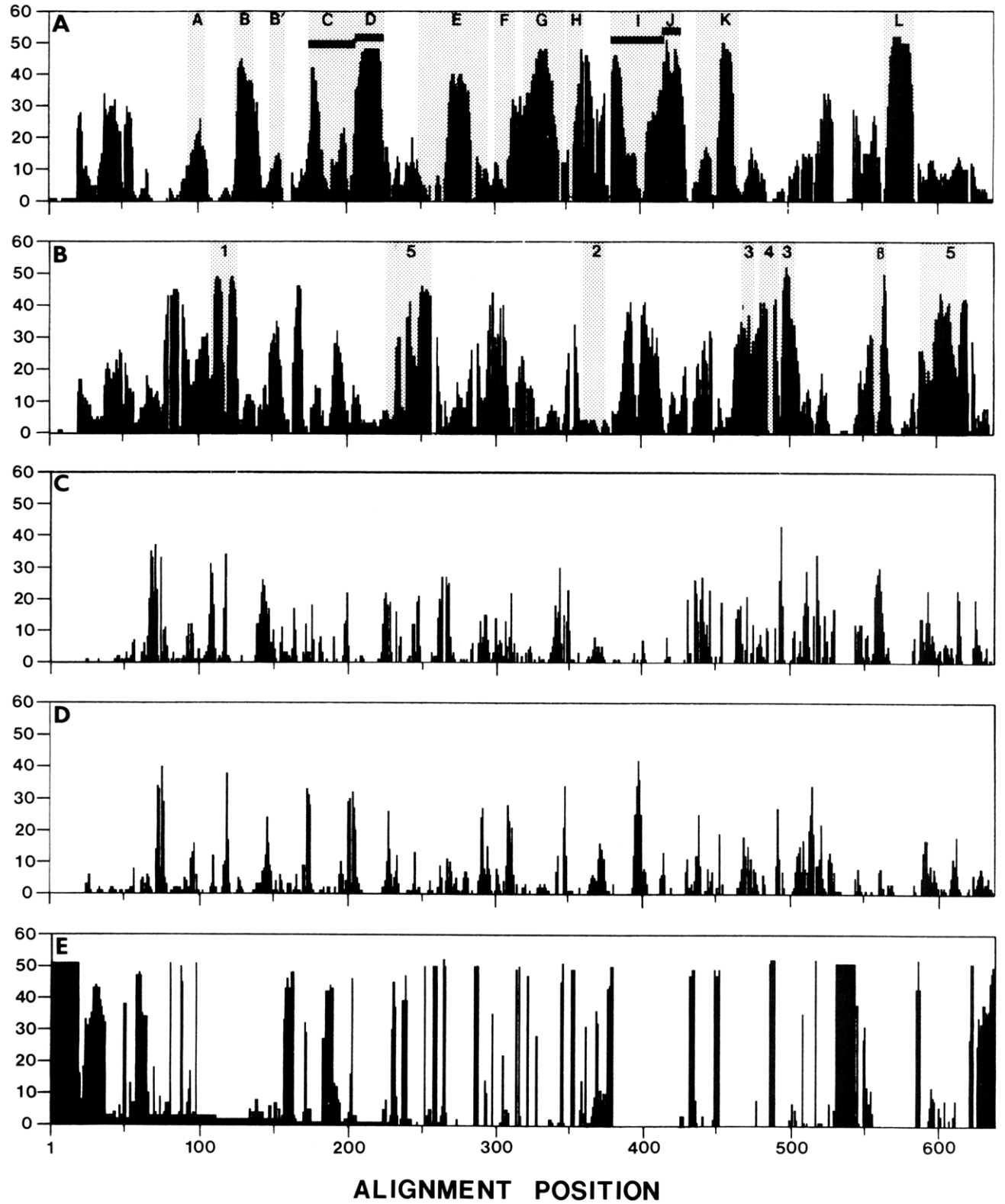

FIGURE 1: Collective secondary structure prediction of 52 aligned cytochrome P450 protein sequences. These histograms represent the number of amino acids predicted to be in (A) helical, (B) β-sheet, (C) turn and (D) coil structure at each position in the sequence alignment. (E) shows the location of gaps in the alignment. Shaded regions in (A) and (B) correspond to the location of helices A–L and β-pair segments $\beta 1$–$\beta 5$, respectively, in the aligned $P450_{cam}$ sequence. Note that $\beta 3$ and $\beta 5$ occur in two regions separated from each other in the sequence. A β-bulge in $P450_{cam}$ is labeled as β in panel B.

$P450_{cam}$ sequence alone. The algorithm's failure to predict this helix in $P450_{cam}$ and its similar prediction for the eukaryotic enzymes suggest that the structures are equivalent in the prokaryotic and eukaryotic proteins.

Figure 1B shows the prediction of β-structure. The shaded regions indicate β-pair segments seen in $P450_{cam}$. Six of the seven shaded regions coincide with significant prediction of β-structure. $\beta 2$, a short segment in an external loop of $P450_{cam}$, is not predicted. There may be no conservation of this feature in eukaryotic P450s. Significant β-structure is predicted in the F and I helix regions. We feel these regions are incorrectly predicted by the algorithm due to the presence of Pro, Gly, Thr, and Ser residues. The prominent β-peak near helix L (β in Figure 1B) corresponds to the β-bulge noted in the crystal

Table I: Location of Helices and β-Structure in $P450_{cam}$ and Figure 1[a]

helix	cam numbering	alignment position	β-structure	cam numbering	alignment position
A	37–46	94–104 (1)	β1	52–66	110–126 (2)
B	67–77	127–137 (0)	β5	146–150	227–257 (26)
B′	89–96	150–157 (0)	β2	226–233	362–376 (7)
C	106–126	175–206 (11)	β3	295–301	469–476 (1)
D	127–145	207–226 (1)	β4	305–312	481–492 (4)
E	149–169	249–296 (27)	β3	315–323	495–503 (0)
			β-bulge	350–357	557–564 (0)
F	173–185	301–314 (1)	β5	382–405	589–619 (7)
G	192–214	321–347 (4)			
H	218–225	351–361 (3)			
I	234–267	381–415 (1)			
J	267–276	415–427 (3)			
K	280–292	438–466 (16)			
L	359–378	566–585 (0)			

[a] Helices and β-structure positions are taken from Poulos et al. (1987). Numbers in parentheses after alignment position indicate the number of gap positions in the $P450_{cam}$ sequence in this region of the alignment.

structure of $P450_{cam}$ (Poulos et al., 1987). This peak is maximal at Cys 564 [357 in $P450_{cam}$, position 522 in Nelson and Strobel (1987, 1988)]. There is a hairpin turn associated with this β-bulge (Poulos et al., 1987) that is initiated by a 100% conserved Gly [560 in our alignment, 518 in Nelson and Strobel (1987, 1988), 353 in $P450_{cam}$]. The predicted turn structure in Figure 1C is maximal at this position. The two β-peaks near helix B′ actually represent one region of β-structure split by a gap in the alignment (see Figure 1E). This peak may represent β-structure present in eukaryotic P450s but not in $P450_{cam}$.

Membrane-bound P450s also have a membrane anchor that $P450_{cam}$ is missing. We have proposed that only the N-terminal of eukaryotic P450s is involved in transmembrane binding (Nelson & Strobel, 1988). Two transmembrane helical segments are proposed to occur in the same positions predicted by others (Black & Coon, 1987; Ozols et al., 1985). These segments correspond to the first predicted helical region in Figure 1A [alignment positions 42–57, positions 19–38 in Nelson and Strobel (1987, 1988)] and the first predicted β-structure in Figure 1B [alignment positions 78–92, positions 59–71 in Nelson and Strobel (1987, 1988)]. Again, the prediction of β-structure rather than helix is probably influenced by a proline-rich region in this second transmembrane segment. This region has been predicted by others to be a polyproline helical segment (Black & Coon,1987).

Our model requires a turn between the first and second transmembrane segments. Figure 1C shows a very strong turn prediction in just this area. The strongest turn prediction (43 of 52 sequences) occurs at position 494 [466 in Nelson and Strobel (1987, 1988)], a highly conserved Lys residue. In $P450_{cam}$, this amino acid lies between β3 and β4 and has been implicated in electron transfer between rabbit cytochrome P450 LM2 [P450IIB4] and NADPH cytochrome P450 reductase (Bernhardt et al., 1984). Two other locations have turns predicted in 34 sequences. One is at position 118 [96 in Nelson and Strobel (1987, 1988)], a highly conserved Gly between the two strands of β1. The other is at position 518 [489 in Nelson and Strobel (1987, 1988)]. The function of a turn at this location is not obvious. The turns at positions 118, 494, and 560 [96, 466, and 518 in Nelson and Strobel, (1987, 1988)] occur where turns are expected according to the crystal structure of $P450_{cam}$ and the alignment.

Figure 2 shows the secondary structure prediction of $P450_{cam}$ compared with the known structure as given in Table 2 of Poulos et al. (1987). Of the known helical segments in $P450_{cam}$, 60% of the amino acids in these segments were predicted to be in a helical conformation. Only 25% of the remaining amino acids were predicted to be helical, and 10

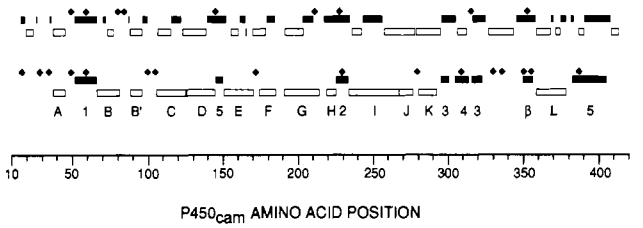

FIGURE 2: Secondary structure prediction of $P450_{cam}$ (upper line) compared to the known secondary structure from Table 2 of Poulos et al. (1987) (lower line): (□) helix; (■) β-structure; (◆) turns. The first nine amino acids are not included, since they were not visible in the crystal structure. Labels are the same as Figure 1.

of 50 were extensions of known helices. Prediction of β-structure was slightly better, yielding 64% correct prediction for known β-pair segments. Only 28% of the non-β-structure was incorrectly predicted to be in a β-conformation. These values are close to the reported accuracy of the Garnier et al. algorithm of about 60–63%.

Qualitatively, every helix in $P450_{cam}$ with the exception of helix H was at least partially predicted. Helices A, B′, J, and K were the most accurately predicted with 40 of 41 residues being correctly assigned. Four helical segments of less than 8 amino acids and one of 17 amino acids were predicted where no helices existed in $P450_{cam}$. Two of these occurred in β4 and β5. Most of the β-structure was predicted except for β4 and the N-terminal region of β5, where short helices were predicted instead. Other β-structure was predicted in helical areas including small parts of helices B, C, D, E, F, G, and L and all of helix H. A large central portion of helix I was predicted to be β-structure. This also occurred in the membrane-bound P450s as discussed above. Six segments of less than five amino acids were predicted as β, where no helix or β-structure was seen in $P450_{cam}$.

The similarity in secondary structure between eukaryotic and prokaryotic P450 proteins is compelling. Ten of 13 helical segments, 6 of 7 β-segments, a β-bulge, and 3 significant turns centered at positions 118, 494, and 560 are correctly predicted. A fourth predicted turn supports the proposal of two N-terminal transmembrane segments in the molecule. We are aware of one other structure prediction based on aligned sequences as we describe here. Crawford et al. (1987) predicted an 8-fold α/β-barrel for the tertiary structure of the α-subunit of tryptophan synthase based on 10 aligned sequences. Their model was subsequently confirmed by X-ray crystallography and lauded as possibly the best effort yet at predicting a protein's three-dimensional structure from its sequence (Chothia, 1988). We hope a membrane cytochrome P450 can be crystallized and its structure determined. Until that hap-

pens, the present analysis strongly suggests that $P450_{cam}$ can be used as a model structure for membrane-bound cytochrome P450.

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SUPPLEMENTARY MATERIAL AVAILABLE

Alignment of 53 P450s used for this analysis and a table listing the location of α-helices and β-structure in $P450_{cam}$ and their corresponding alignment positions in the 53 sequence alignment (3 pages). Ordering information is given on any current masthead page.

Registry No. P450, 9035-51-2.

REFERENCES

Bernhardt, R., Makower, A., Jänig, G.-R., & Ruckpaul, K. (1984) *Biochim. Biophys. Acta 785*, 186.

Black, S. D., & Coon, M. J. (1987) *Adv. Enzymol. Relat. Areas Mol. Biol. 60*, 35.

Chen, S., Besman, M. J., Sparkes, R. S., Zollman, S., Klisak, I., Mohandas, T., Hall, P. F., & Shively, J. E. (1988) *DNA 7*, 27.

Chiang, Y.-L., & Coon, M. J. (1979) *Arch. Biochem. Biophys. 195*, 178.

Chothia, C. (1988) *Nature (London) 333*, 598.

Chua, S. C., Szabo, P., Vitek, A., Grzeschik, K.-H., John, M., & White, P. C. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7193.

Crawford, I. P., Niermann, T., & Kirschner, K. (1987) *Proteins: Struct., Funct., Genet. 2*, 118.

Dalet, C., Clair, P., Daujat, M., Fort, P., Blanchard, J.-M., & Maurel, P. (1988) *DNA 7*, 39.

Garnier, J., Osguthorpe, D. J., & Robson, B. (1978) *J. Mol. Biol. 120*, 97.

Gonzalez, F. J., Song, B.-J., & Hardwick, J. P. (1986) *Mol.Cell. Biol. 6*, 2969.

Gonzalez, F. J., Matsunaga, T., Nagata, K., Meyer, U. A., Nebert, D. W., Pastewka, J., Kozak, C. A., Gillette, J., Gelboin, H. V., & Hardwick, J. P. (1987) *DNA 6*, 149.

Gonzalez, F. J., Skoda, R. C., Kimura, S., Umeno, M., Zanger, U. M., Nebert, D. W., Gelboin, H. V., Hardwick, J. P., & Meyer, U. A. (1988) *Nature (London) 331*, 442.

Gotoh, O., Tagashira, Y., Iizuka, T., & Fujii-Kuriyama, Y. (1983) *J. Biochem. (Tokyo) 93*, 807.

Guengerich, F. P., Umbenhauer, D. R., Churchill, P. F., Beaune, P. H., Bishocker, R., Knodell, R. G., Martin, M. V., & Lloyd, R. S. (1987) *Xenobiotica 17*, 311.

Haniu, M., Ryan, D. E., Levin, W., & Shively, J. E. (1986) *Arch. Biochem. Biophys. 244*, 323.

Haniu, M., Yanagibashi, K., Hall, P. F., & Shively, J. E. (1987) *Arch. Biochem. Biophys. 254*, 380.

Heinemann, F. S., & Ozols, J. (1982) *J. Biol. Chem. 257*, 14988.

Imai, Y. (1987) *J. Biochem. (Tokyo) 101*, 1129.

Imai, Y., Komori, M., & Sato, R. (1988) *Biochemistry 27*, 80.

Johnson, E. F., Barnes, H. J., Griffin, K. J., Okino, S., & Tukey, R. J. (1987) *J. Biol. Chem. 262*, 5918.

Kalb, V. F., Woods, C. W., Turi, T. G., Dey, C. R., Sutter, T. R., & Loper, J. C. (1987) *DNA 6*, 529.

Kimura, S., Pastewka, J., Gelboin, H. V., & Gonzales, F. J. (1987) *Nucleic Acids Res. 15*, 10053.

Labbé, D., Jean, A., & Anderson, A. (1988) *DNA 7*, 253.

Marx, J. L. (1985) *Science (Washington, D.C.) 228*, 975.

Matsubara, S., Yamamoto, S., Sogawa, K., Yokotani, N., Fujii-Kuriyama, Y., Haniu, M., Shively, J. E., Gotoh, O., Kusunose, E., & Kusunose, M. (1987) *J. Biol.Chem. 262*, 13366.

Meehan, R. R., Gosden, J. R., Rout, D., Hastie, N. D., Friedberg, T., Adesnik, M., Buckland, R., Heyningen, V. van, Fletcher, J., Spurr, N. K., Sweeney, J., & Wolf, C. R. (1988) *Am. J. Hum. Genet. 24*, 26.

Morohashi, K., Yoshioka, H., Gotoh, O., Okada, Y., Yamamoto, K., Miyata, T., Sogawa, K., Fujii-Kuriyama, Y., & Omura, T. (1987) *J. Biochem. (Tokyo) 102*, 559.

Nagata, K., Matsunaga, T., Gillette, J., Gelboin, H. V., & Gonzalez, F. J. (1987) *J. Biol. Chem. 262*, 2787.

Nebert, D. W., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W., Phillips, I. R., Sato, R., & Waterman, M. R. (1987) *DNA 6*, 1.

Nebert, D. W., Nelson, D. R., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W., Phillips, I. R., Sato, R., & Waterman, M. R. (1989) *DNA* (in press).

Nelson, D. R., & Strobel, H. W. (1987) *Mol. Biol. Evol. 4*, 572.

Nelson, D. R., & Strobel, H. W. (1988) *J. Biol. Chem. 263*, 6038.

Okino, S. T., Quattrochi, L. C., Pendurthi, U. R., McBride, O. W., & Tukey, R. H. (1987) *J. Biol. Chem. 262*, 16072.

Ortiz de Montellano, P. R. (1986) in *Cytochrome P450 Structure, Mechanism and Biochemistry* (Ortiz de Montellano, P. R., Ed.) p 217, Plenum, New York.

Ozols, J., Heinemann, F. S., & Johnson, R. F. (1985) *J. Biol. Chem. 260*, 5427.

Poulos, T. L., Finzel, B. C., & Howard, A. J. (1987) *J. Mol. Biol. 195*, 687.

Simpson, E. R., Evans, C. T., Corbin, C. J., Powell, F. E., Ledesma, D. B., & Mendelson, C. R. (1987) *Mol. Cell. Endocrinol. 52*, 267.

Squires, E. J., & Negishi, M. (1988) *J. Biol. Chem. 263*, 4166.

Tarr, G. E., Black, S. D., Fujita, V. S., & Coon, M. J. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 6552.

Umbenhauer, D. R., Martin, M. V., Lloyd, R. S., & Guengerich, F. P. (1987) *Biochemistry 26*, 1094.

Wong, G., Kawjiri, K., & Negishi, M. (1987) *Biochemistry 26*, 8683.

Yasumori, T., Kawano, S., Nagata, K., Shimada, M., Yamazoe, Y., & Kato, R. (1987) *J. Biochem. (Tokyo) 102*, 1075.

Yoshioka, H., Morohashi, K.-I., Sogawa, K., Miyatat, T., Kawajiri, K., Hirose, T., Inayama, S., Fujii-Kuriyama, Y., & Omura, T. (1987) *J. Biol. Chem. 262*, 1706.

Zhao, J., Leighton, J. K., & Kemper, B. (1987) *Biochem. Biophys. Res. Commun. 146*, 224.