

A SINGLE AMINO ACID SUBSTITUTION CONVERTS CYTOCHROME P450<sub>14DM</sub>  
TO AN INACTIVE FORM, CYTOCHROME P450<sub>SG1</sub>: COMPLETE  
PRIMARY STRUCTURES DEDUCED FROM CLONED DNAS

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Genes for lanosterol 14-demethylase, cytochrome P450<sub>14DM</sub>, and a mutated inactive cytochrome P450<sub>SG1</sub> were cloned from *S. cerevisiae* strains D587 and SG1, respectively. A single nucleotide change resulting in substitution of Asp for Gly-310 of cytochrome P450<sub>14DM</sub> was found to have occurred in cytochrome P450<sub>SG1</sub>. In this protein the 6th ligand to heme iron is a histidine residue instead of a water molecule, which may be the ligand for the active cytochrome P450<sub>14DM</sub>. Molecular models of the active sites of the cytochrome P450<sub>14DM</sub> and cytochrome P450<sub>SG1</sub> were built by computer modeling on the basis of the known structure of that of cytochrome P450<sub>CAM</sub> whose crystallographic data are available. The mechanisms which may cause a histidine residue to gain access to the heme iron are discussed. © 1988 Academic Press, Inc.

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A genetically altered cytochrome P450, P450<sub>SG1</sub> (1,2), which was derived from lanosterol 14-demethylase, P450<sub>14DM</sub> (3), has been isolated from a

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Abbreviations: P450<sub>14DM</sub>, lanosterol 14-demethylase from *S. cerevisiae*; P450<sub>SG1</sub>, cytochrome P450 isolated from lanosterol 14-demethylase deficient mutant *S. cerevisiae* SG1; P450<sub>CAM</sub>, camphor 5-exo-monooxygenase from *Pseudomonas putida*; P450<sub>SCC</sub>, cholesterol side-chain cleavage cytochrome P450 from bovine adrenal; P450<sub>b</sub> and P450<sub>c</sub>, rat liver microsomal cytochrome P450s; kbp, kilobase pairs.

lanosterol 14-demethylation deficient yeast mutant, *S. cerevisiae* SG1 (ATCC 46786) (4,5). P450<sub>SG1</sub> showed no lanosterol 14-demethylase activity. Spectroscopic properties of P450<sub>SG1</sub> (6) were critically different from those of normal cytochrome P450s including P450<sub>14DM</sub>, and indicated that the axial ligand trans to thiolate of P450<sub>SG1</sub> was a histidine residue (1,6), while that of the normal low-spin cytochrome P450 may be a water molecule (7,8,9,10). In spite of these differences, P450<sub>SG1</sub> and P450<sub>14DM</sub> were immunochemically identical and had identical peptide maps and 11 N-terminal amino acids (1). These observations suggested that the mutation giving rise to P450<sub>SG1</sub> caused local structural changes in the cytochrome which resulted in binding of a histidine residue to heme and loss of catalytic activity while retaining overall structural characteristics of P450<sub>14DM</sub>. Accordingly, it was expected that comparison of the primary structure of P450<sub>SG1</sub> with that of P450<sub>14DM</sub> would provide some important information on the structure of the heme environment of P450<sub>14DM</sub>.

The present communication describes primary structures of P450<sub>SG1</sub> and P450<sub>14DM</sub> deduced from the DNA sequences of their structural genes. The results indicated that only one amino acid substitution, i.e. Gly→Asp, occurred in P450<sub>SG1</sub>. A possible structural change caused by this amino acid substitution is discussed, based on computer aided molecular modeling.

#### MATERIALS AND METHODS

Cultivation of Yeast. The nystatin-resistant yeast, *S. cerevisiae* SG1 and its nystatin-sensitive parent, *S. cerevisiae* D587, were cultivated as described previously (2).

Construction and Screening of Yeast Genomic Libraries. Yeast genomic DNAs isolated as described (11) were digested with BamHI and HindIII, fractionated by agarose gel electrophoresis, hybridized with a 17-mer oligonucleotide (complementary to nucleotides 1396 to 1422, Fig.2) designed according to Kalb et al. (12) and <sup>32</sup>P-labeled using T<sub>4</sub> polynucleotide kinase. A single positive band was observed at about 5kbp. Accordingly, BamHI-HindIII fragments of the genomic DNA of about 5kbp were prepared and inserted into pUC18 to construct a genomic library. The library was screened with the <sup>32</sup>P-labeled 17-mer oligonucleotide. Positive colonies were isolated and plasmid DNAs were purified as described (13).

Nucleotide Sequence Analysis. The HpaI-HindIII fragment or HpaI-DraI fragment (Fig. 1) of inserts were subcloned into M13mp18 or M13mp19. Sequencing was performed by the dideoxy chain terminator method (14,15) using chemically modified T<sub>7</sub> DNA polymerase (16) (Sequenase<sup>TM</sup>; United States Biochemical Corp.) Sequencing templates were prepared by deletion using T<sub>4</sub> DNA polymerase (17) (International Biotechnologies, Inc.). Sequence data were analyzed by using the DNASIS software (Hitachi) and the GCG software package (18).

#### RESULTS

##### Isolation of genomic DNA clones for P450<sub>SG1</sub> and P450<sub>14DM</sub>

Six and three clones out of about 1000 colonies from the genomic

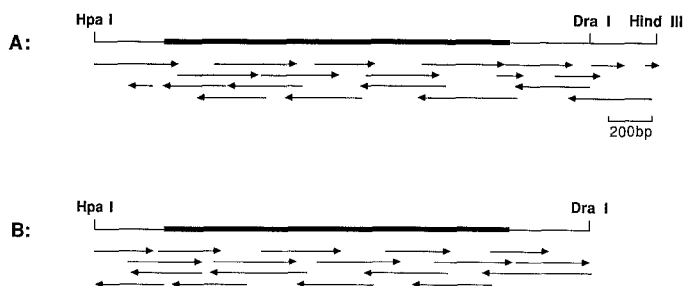


Fig.1 Sequencing strategy of P450<sub>SG1</sub> and P450<sub>14DM</sub> genes. Only those restriction enzymes relevant to the present work are shown. Thick and thin horizontal lines denote coding and noncoding regions, respectively. The direction and extent of sequence determination is shown by arrows. Sequencing was done as described in MATERIALS AND METHODS. A and B, P450<sub>SG1</sub> gene and P450<sub>14DM</sub> gene, respectively.

libraries of SG1 and D587, respectively, were found to be positive by colony hybridization analysis using the <sup>32</sup>P-labeled 17-mer oligonucleotide probe. Restriction map analysis of each of the clones showed that these clones were indistinguishable and we have chosen pgvSG1.1 and pgv14DM.8 as representing clones for P450<sub>SG1</sub> and P450<sub>14DM</sub>, respectively.

The HpaI-HindIII fragment and/or HpaI-DraI fragments of pgvSG1.1 and pgv14DM.8 were submitted to nucleotide sequence analysis as described in MATERIALS AND METHODS (Fig. 1).

#### Mutation in P450<sub>SG1</sub>

The DNA sequences and the deduced amino acid sequences of these genes are depicted in Fig. 2, where only residues different from those of P450<sub>SG1</sub> are shown for P450<sub>14DM</sub>. The initiating methionines were easily identified by referring to the N-terminal amino acid sequences of P450<sub>14DM</sub> and P450<sub>SG1</sub> which had been determined on the purified enzymes (1). This sequence was identical in the two proteins and has been confirmed by the present studies.

Both P450 genes code for a protein of 530 amino acid residues. In the P450<sub>SG1</sub> gene, a single substitution of A for G in P450<sub>14DM</sub> gene was observed at nucleotide residue 929. This substitution resulted in an amino acid change from Gly to Asp at amino acid residue 310 which was located 53 amino acids upstream from the analogous tridecapeptide (Fig. 2) reported in many P450s (19). No other difference was found in the overlapping regions (nucleotides -332 to 1972) between these two P450s including 3'- and 5'-flanking regions so far determined.

#### Comparison between P450<sub>14DM</sub> genes in strains D587 and DBY939

When the sequence of the gene for P450<sub>14DM</sub> determined in the present study was compared to that determined by Kalb et al. (20) for the gene cloned from *S. cerevisiae* strain DBY939, several differences were noted (Fig. 2). In the overlapping sequences (-332 to 1972), two silent and one missense

	AACGACGTTTACGTACTCGCATGTATTCGAAAAGCCTCTAAAAATTGCCTCCATGCTGTAATCATTATATAGATAGCGTATTTCCCTTAGTCTTTCTTATCCCTCTTAATCTCT	-217
	CTTTCAAATGCTCTTTTTTTTTTTCCTTTTCCTTTTTCCTTTTCGTTATTTGGTAATATTTATATAAAAGATTGATAAGCAGTATCGTTACAGCGTGTGTTATTCGATAGGAAGTAATA	-99
1	ATTTTTCATTATATTAATTAATGATTTCCGAAAATAATTTTTTTCCTTCCAAATTGCAGCAGCGTTGAATAGAAACAGAACAAACGAGTAATACAAGG	15
	ATG TCT GCT ACC AAG Met Ser Ala Thr Lys	
6	TCA ATC GTT GGA GAG GCA TTG GAA TAC GTA AAC ATT GGT TTA AGT CAT TTC TTG GCT TTA CCA TTG GGC CAA AGA ATC TCT TTG ATC ATA	105
	Ser Ile Val Gly Glu Ala Leu Glu Tyr Val Asn Ile Gly Leu Ser His Phe Leu Ala Leu Pro Leu Ala Gln Arg Ile Ser Leu Ile Ile	
36	ATA ATT CCT TTC ATT TAC AAT ATT GTA TGG CAA TTA CTA TAT TCT TTG AGA AAG GAC CGT CCA CCT CTA GTG TTT TAC TGG ATT CCA TGG	195
	Ile Ile Pro Phe Ile Tyr Asn Ile Val Trp Gln Leu Leu Tyr Ser Leu Arg Lys Asp Arg Pro Pro Leu Val Phe Tyr Trp Ile Pro Trp	
66	GTC GGT AGT GCT GTT GTG TAC GGT ATG AAG CCA TAC GAG TTT TTC GAA AAG TGT CAA AAG AAA TAC GGT GAT ATT TTT TCA TTC GTT TTG	285
	Val Gly Ser Ala Val Val Tyr Gly Met Lys Pro Tyr Glu Phe Phe Glu Glu Cys Gln Lys Lys Tyr Gly Asp Ile Phe Ser Phe Val Leu	
96	TTA CGA AGA GTC ATG ACT GTG TAT TTA GGA CCA AAG GGT CAC GAA TTT GTC TTC AAC GCT AAG TTG CCA GAT GTT TCA GCA GAA GCT GCT	375
	Leu Gly Arg Val Met Thr Val Tyr Leu Gly Pro Lys Gly His Glu Phe Val Phe Asn Ala Lys Leu Ala Asp Val Ser Ala Glu Ala Ala	
126	TAC GCT CAT TTG ACT ACT CCA GTT TTC GAT AAC GGT GTT ATT TAC GAT TGT CCA AAT TCT AGA TTG ATG GAG CAA AAG AAG TTT GTT AAG	465
	Tyr Ala His Leu Thr Thr Pro Val Phe Gly Lys Gly Val Ile Tyr Asp Cys Pro Asn Ser Arg Leu Met Glu Gln Lys Lys Phe Val Lys	
156	GGT GCT CTA ACC AAA GAA GCC TTC AAG AGC TAC GTT CCA TTG ATT GCT GAA GAA GTG TAC AAG TAC TTC AGA GAC TCC AAA AAC TTC CGT	555
	Gly Ala Leu Thr Lys Glu Ala Gln Phe Lys Ser Tyr Val Pro Leu Ile Ala Glu Glu Val Tyr Lys Tyr Phe Arg Asp Ser Lys Asn Phe Arg	
186	TTG AAT GAA AGA ACT ACT GGT ACT TTC GAT CAC GTG ATG GTT ACT CAA CCT GAA ATG ACT ATT TTC ACC GCT TCA AGA TCA TTA TTG GGT AAG	645
	Leu Asn Glu Arg Thr Thr Gly Thr Ile Asp Val Met Val Thr Gln Pro Glu Met Thr Ile Phe Thr Ala Ser Arg Ser Leu Leu Gly Lys	
216	GAA ATG AGA GCA AAA TTG GAT ACC GAT TTT GCT TAC TTG TAC AGT GAT TTG GAT AAG GGT TTC ACT CCA ATC AAC TTC GTC TTC CCT AAC	735
	Glu Met Arg Ala Lys Leu Asp Thr Asp Phe Ala Tyr Leu Tyr Ser Asp Leu Asp Lys Gly Phe Thr Pro Ile Asn Phe Val Phe Pro Asn	
246	TTA CCA TTG GAA CAC TAT AGA AAG AGA GAT CAC GCT CAA AAG GCT ATC TCC GGT ACT TAC ATG TCT TTG ATT AAG GAA AGA AGA AAG AAC	825
	Leu Pro Leu Glu His Tyr Arg Lys Arg Asp His Ala Gln Lys Ala Ile Ser Gly Thr Tyr Met Ser Leu Lys Glu Arg Leu Lys Lys	
276	AAC GAC ATT CAA GAC AGA GAT TTG ATC GAT TCC TTG ATG AAG AAC TCT ACC TAC AAG GAT GGT GTG AAG ATG ACT GAT CAA GAA ATC GCT	915
	Asn Asp Ile Gln Asp Arg Asp Leu Ile Asp Ser Leu Met Lys Asn Ser Thr Tyr Lys Asp Gly Val Lys Met Thr Asp Gln Glu Ile Ala	
306	AAC TTG TTA ATT GAT GTC TTA ATG GGT GGT CCA CAT ACT TCT GCT GCC ACT TCT GCT TGG ATT TTG TTG CAC TTG GCT GAA AGA CCA GAT	1005
	Asn Leu Leu Ile <u>Asp</u> Val Leu Arg Met His Lys Gln His Thr Ser Ala Thr Ser Ala Trp Ile Leu Leu His Ala Glu Arg Leu Lys Asp	
336	GTC CAA CAA GAA TTG TAC GAA GAA CAA ATG CGT GTT TTG GAT GGT GGT AAG AAG GAA TTG ACC TAC GAT TTA TTA CAA GAA ATG CCA TTG	1095
	Val Gln Gln Glu Leu Tyr Glu Glu Gln Met Arg Val Leu Asp Gly Gly Lys Lys Glu Leu Thr Tyr Asp Leu Leu Gln Glu <u>Met Pro Leu</u>	
366	TTG AAC CAA ACT ATT AAG GAA ACT CTA AGA ATG CAC CAT CCA TTG CAC TCT TTG TTC CGT AAG GTT ATG AAA GAT ATG CAC GTT CCA AAC	1185
	<u>Leu Asn Gln Thr Thr Lys Glu Thr Leu Arg</u> Met His His Pro Leu His Ser Leu Phe Arg Lys Val Met Ser Leu Lys Glu Arg Leu Lys Asn	
396	ACT TCT TAT GTC ATC CCA GCA GGT TAT CAC GTT TTG GTT TCT CCA GGT TAC ACC CAT TTA AGA GAC GAA TAC TTC CCT AAT GCT CAC CAA	1275
	Thr Ser Tyr Val Ile Pro Ala Gly Tyr His Val Leu Val Ser Pro Gly Tyr Thr His Leu Arg Asp Glu Tyr Phe Pro Asn Ala His Gln	
426	TTC AAC ATT CAC CGT TGG AAC AAC GAT TCT GCC TCC TCT TAT TCC GTC GGT GAA GAA GTC GAT TAC GGT TTC GGT GCC ATT TCT AAG GGT	1365
	Phe Asn Ile His Arg Trp Asn <u>Asn</u> Asp Ser Ala Ser Ser Tyr Ser Val Gly Glu Glu Val Asp Tyr Gly Phe His Leu Ala Ile Ser Lys Gly	
456	GTC AGC TCT CCA TAC TTA CCT TTC GGT GGT GGT AGA CAC AGA TGT ATC GGT GAA CAC TTT GCT TAC TGT CAG CTA GGT GTT CTA ATG TCC	1455
	Val Ser Ser Pro Tyr Leu Pro <u>Phe Gly Gly Gly Arg His Arg Cys Ile Gly Glu His Phe Ala Tyr Cys Gln Leu Gly Val Leu</u> Met Ser	
486	ATC TTT ATC AGA ACA TTA AAA TGG CAT TAC CCA GAG GGT AAG ACC GTT CCA CCT CCT GAC TTT ACA TCT ATG GTT ACT CTT CCA ACC GGT	1545
	Ile Phe Ile His Arg Trp Lys Trp His Tyr Pro Glu Gly Lys Thr Val Pro Pro Pro Asp Phe Thr Ser Met Val Thr Leu Pro Thr Gly	
516	CCA GCC AAG ATC ATC TGG GAA AAG AGA AAT CCA GAA CAA AAG ATC TAAGGTTTGTAACTGAGAAAAAAGAGAAAAAGAGAGAGTTGTACATTCA	1649
	Pro Ala Lys Ile Ile Trp Glu Lys Arg Asn Pro Glu Gln Lys Ile ***	
	CGTGGCCCTTATGCCCACTCATCCATCCCAATGATTTATTTTATTTGCCAGTACGTTTGATATGACATCGAGTTATGTTCTTTAGTTACTTTCAAACITGGCAACCACTCCCGCAT	1768
	TATTTATGAGTGGTATGTTTCACTTCTTTATTTATAAATATTTATACACGAAATATATATAGATTACATTTCTAAAAACAACATCAAAATTAAGGTTTATTTATGCAACATGCCATC	1887
	TGGATTAGAAGAGAAACACACAGCTCACAGGCCAAGCGGAAATAAAGCAGTCTTTTCGCAAACTGTGAATCTTTTTCGAATTTAAATTAATCAAGAGAGGCCAGGAGGAGGAG	2006
	TTTGGGAGGGGCTACTGAGGAGCTCTCTTAATATGGATTCACTGAATAAATGTATATAAGATTCATTACTGCTTTTATAGTGATAAATCCAAATGATGAGTGAATGAACCAT	2125
	CCTTAGTATCCATTGTTTATAAAGCACCGCTA <sup>T</sup> AAAAATAATATATGCT <sup>T</sup> AAAAATGATATAATAATATATTACGTAAATACCTGAAACGCCATAAAAAATATACCTGAATTAAC	2244
	CTTAAAAATCTATCCATAAGCTT	2268

Fig.2 Sequence of P450<sub>SG1</sub> and P450<sub>14DM</sub> genes and their deduced amino acid sequences. Nucleotide residues are numbered in 5' to 3' direction, beginning with the first residue of the initiating methionine codon for P450<sub>SG1</sub>. Amino acid residues deduced are expressed in three letter notation under nucleotide sequences and numbered beginning with the initiating methionine of SG1. The number of nucleotide and amino acid residues is given at the right and the left ends of each line, respectively. The sequence of the P450<sub>14DM</sub> gene of strain D587 was determined from -335 to 1972 corresponding to that of P450<sub>SG1</sub> gene. Nucleotides and the amino acids different from those of SG1 are shown in bold face letters. Residues in DBY939 gene (20) different from those of D587 and/or SG1 are shown above and below the underlined nucleotides or amino acids, respectively. Arrows indicate that the nucleotides are missing in DBY939 gene. Asterisks show the termination codon. The analogous tridecapeptides (19) and the HR2 region (23) are shown by long underlines.

substitutions were seen in the coding region and in 3'-flanking sequence, an extra A residue was observed for that of D587. As for the sequence

downstream of the nucleotide 1972, only comparison between those of SG1 and DBY939 was possible. However, if we take the comparison of the overlapping sequences of SG1, D587 and DBY939 into account, one insertion and two substitutions are likely to reflect also the difference between the sequences of strains D587 and DBY939.

Apparently, substitution of Asn-433 for Lys-433 in DBY939 has no serious effect on the enzyme activity of P450<sub>14DM</sub> (20).

#### DISCUSSION

P450<sub>SG1</sub> (1,2,6), from a lanosterol 14-demethylase deficient mutant, *S. cerevisiae* SG1, is an altered and inactive form of P450<sub>14DM</sub>, lanosterol 14-demethylase (3,21). P450<sub>SG1</sub> is, however, immunochemically indistinguishable from P450<sub>14DM</sub>; in addition, their peptide maps are identical (1). Hence P450<sub>SG1</sub> may be considered to be formed by a minor amino acid substitution in P450<sub>14DM</sub> which would not change overall conformation of the protein significantly. Actually, only one amino acid substitution was found between P450<sub>SG1</sub> and P450<sub>14DM</sub>: Gly-310 of P450<sub>14DM</sub> was changed to Asp-310 in P450<sub>SG1</sub> (Fig. 2). As described previously, the most striking feature of P450<sub>SG1</sub> is the coordination of a histidine as the 6th ligand of the heme (1). Since P450<sub>SG1</sub> did not have newly introduced histidine, one of the pre-existing histidine residues must have gained access to the heme iron as the 6th ligand owing to substitution of Asp-310 for Gly-310. This observation suggested that the single amino acid substitution caused a significant local conformational change in the cytochrome, resulting in loss of activity. In contrast is the substitution in P450<sub>DM</sub> of yeast strain DBY939 of Lys-433 (20) for Asn-433, which was also residue 433 in P450<sub>DM</sub> of strain D587. Since DBY939 was not lanosterol 14-demethylase deficient (20), substitution of Asn-433 for Lys-433 or vice versa appears to show no essential effect on the activity of P450<sub>14DM</sub>. In order to elucidate the structural basis for the shift of a histidine residue to the sixth ligand position of the heme resulting from an amino acid substitution, we have constructed a molecular model to compare the structure of the active site and its vicinity in P450<sub>14DM</sub> and P450<sub>SG1</sub>.

By comparing with the sequences of distal helices in various P450s compiled by Poulos et al. (22), we assigned residues 309-325 of P450<sub>14DM</sub> as the distal helix sequence and aligned it with other sequences (Table 1). It is clear that the sequence has features common to the distal helices (22), e.g. residues 314-315 are Gly-Gly which are always Gly-Gly or Ala-Gly for the distal helices, residue 318 is Thr which is conserved in all P450s, and residues 311-316 form the hydrophobic stretch running over the distal heme

Table 1. Alignment of distal helices in various P450s

P450 <sub>CAM</sub>	G L244 L L V G G L D251 T V V N F L S F
P450 <sub>SCC</sub>	T E321 M L A G G V N328 T T S M T L Q W
P450b	L S294 L F F A G T E301 T T S S T T L R
P450c	F D317 L F G A G F D324 T I T T A I S W
P450 <sub>14DM</sub>	I G310 V L M G G Q H317 T S A A T S A W
P450 <sub>SG1</sub>	I D310 V L M G G Q H317 T S A A T S A W

surface (Table 1). Interestingly, the residues 309-325 contain the residue 310 which is the only residue different between P450<sub>14DM</sub> and P450<sub>SG1</sub>, and a histidine residue at 317 (Table 1). A model of a part of the active site of P450<sub>14DM</sub> including the heme and the distal helix was built by using crystallographic data of P450<sub>CAM</sub> (22) registered in the Protein Data Bank (Fig. 3), and was used to analyze the probable structural changes caused by substitution of the residue 310.

In P450<sub>14DM</sub>, His-317 is not located over the heme center and its imidazole does not interact with the heme iron (Fig. 3, thin line). The mutation of Gly-310 (P450<sub>14DM</sub>) to hydrophilic Asp in a rather hydrophobic environment would cause a shift of Asp-310 (P450<sub>SG1</sub>) toward the protein surface where it would interact with water and/or basic amino acid residues such as Lys-176 and Arg-179 (not shown in Fig. 3). Translational and rotational shifts of Asp-310 along the helix would result in an effective interaction of His-317 residue with the heme iron (Fig. 3, thick line). Local deformation (not shown in Fig. 3) of the following region, Thr-318 to Ala-324, may be afforded by the presence of highly populous threonines and serines (24), and may facilitate the shift without significantly affecting

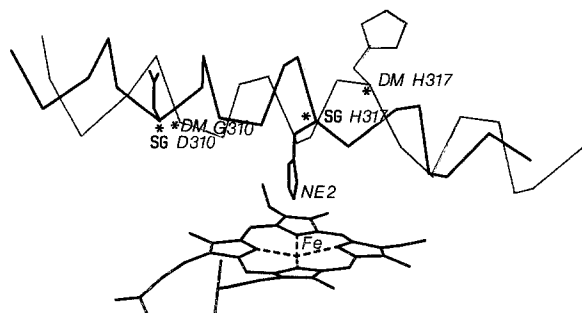


Fig.3 Structural model showing correlation between distal helices and heme molecule of P450<sub>14DM</sub> and P450<sub>SG1</sub>. Residues 310 and 317 of P450<sub>14DM</sub> (DM) and P450<sub>SG1</sub> (SG) are labeled, \*, on  $\alpha$ -carbon backbone helices (thin line for P450<sub>14DM</sub> and thick line for P450<sub>SG1</sub>) with their side chains. Shift of helix would result in the coordination of nitrogen atom (NE2) of histidine residue to the heme iron (Fe).

the conformation of the region that follows the helix (not shown in Fig. 3). Thus, we are fairly confident in the prediction that the 6th ligand to the heme iron of the inactive P450<sub>SG1</sub> is the imidazole group of His-317 residue. Further structural investigation of P450<sub>14DM</sub> and P450<sub>SG1</sub> using site directed mutagenesis and molecular modeling of the helix is in progress.

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## REFERENCES

1. Aoyama, Y., Yoshida, Y., Nishino, T., Katsuki, H., Maitra, U. S., Mohan, V. P. and Sprinson, D. B. (1987) *J. Biol. Chem.* 262, 14260-14264
2. Aoyama, Y., Yoshida, Y., Hata, S., Nishino, T., Katsuki, H., Maitra, U. S., Mohan, V. P. and Sprinson, D. B. (1983) *J. Biol. Chem.* 258, 9040-9042
3. Yoshida, Y. and Aoyama, Y. (1984) *J. Biol. Chem.* 259, 1655-1660
4. Trocha, P. J., Jasne, S. J. and Sprinson, D. B. (1974) *Biochem. Biophys. Res. Commun.* 59, 666-671.
5. Trocha, P. J., Jasne, S. J. and Sprinson, D. B. (1977) *Biochemistry* 16, 4721-4726
6. Yoshida, Y., Aoyama, Y., Nishino, T., Katsuki, H., Maitra, U.S., Mohan, V.P. and Sprinson, D.B. (1985) *Biochem. Biophys. Res. Commun.* 127, 623-628
7. Yoshida, Y., Imai, Y. and Hashimoto-Yutsudo, C. (1982) *J. Biochem.* 91, 1651-1659
8. White, R.E. and Coon, M.J. (1982) *J. Biol. Chem.* 257, 3073-3080
9. Dawson, J.H., Andersson, L.A. and Sono, M. (1982) *J. Biol. Chem.* 257, 13637-13645
10. Poulos, T.L., Finzel, B.C. and Howard, A.J. (1986) *Biochemistry* 25, 5314-5322
11. Cryer, D. R., Eccleshall, R. and Marmur, J. (1975) *Methods Cell Biol.* 12, 39-44
12. Kalb, V. F., Loper, J. C., Day, C. R., Woods, C. W. and Sutter, T. R. (1986) *Gene* 45, 237-245
13. Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) *Molecular Cloning, A Laboratory Manual*. Cold Spring Harbor Laboratory, New York.
14. Sanger, F., Nicklen, S. and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463-5467
15. Messing, J., Crea, R. and Seeburg, P. H. (1981) *Nucl. Acids Res.* 9, 309-321
16. Tabor, S. and Richardson, C. C. (1987) *Proc. Natl. Acad. Sci. USA* 84, 4767-4771
17. Dale, R. M. K., McClure, B. a. and Houchins, J. P. (1985) *Plasmid* 13, 31-40
18. Devereux, Haeberli and Smithies, (1984), *Nucl. Acids Res.* 12 387-395
19. Ozols, J. and Heinemann, F. S. (1981) *J. Biol. Chem.* 256, 11405-11408
20. Kalb, V. F., Woods, C. W., Turi, T. G., Dey, C. R., Sutter, T. R. and Loper, J. C. (1987) *DNA* 6, 529-537
21. Aoyama, Y., Yoshida, Y. and Sato, R. (1984) *J. Biol. Chem.* 259, 1661-1666
22. Poulos, T. L., Finzel, B. C., Gunsalus, I. C., Wagner, G. C. and Kraut, J. (1985) *J. Biol. Chem.* 260, 16122-16130
23. Gotoh, O., Tagashira, Y., Iizuka, T. and Fujii-Kuriyama, Y. (1983) *J. Biochem.* 93, 807-817
24. Poulos, T.L. and Howard, A.J. (1987) *Biochemistry* 26, 8165-8174