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AMINO ACID COMPOSITION OF CYTOCHROME P-450$_{scc}$ FROM BOVINE CORPUS LUTEUM

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SUMMARY: The amino acid composition of cytochrome P-450$_{scc}$, purified from bovine corpus luteal mitochondria was determined and expressed on the basis of the molecular weight 48,000, determined by SDS gel electrophoresis. The composition was similar to that reported for P-450$_{scc}$ from adrenocortical mitochondria, but different from P-450$_{11\beta}$ from bovine adrenal mitochondria, P-450$_{LM}$ from liver microsomes and P-450$_{cam}$ from Pseudomonas putida. The molecule is composed of about 400 amino acids, 49% of which are non-polar.

INTRODUCTION

Cytochrome P-450 proteins with different substrate specificities have been purified to homogeneity from liver microsomes (1-6), adrenocortical mitochondria (7-11), corpus luteum mitochondria (12) and Pseudomonas putida (13). At least four different forms of cytochrome P-450$_{LM}$ have been purified to specific content of 13-20 nmol per mg of protein from rat liver microsomes. They have distinct spectral, catalytic, immunological and electrophoretic properties (3-5). In adrenal mitochondria, P-450$_{scc}$ specific for cholesterol side chain cleavage and P-450$_{11\beta}$ specific for 11β-hydroxylation were estimated to be present in a ratio of approximately 2:1 in the sonicated mitochondria (9). P-450$_{scc}$ was purified to specific content of 12.3 nmol per mg protein (9, 10). These two P-450 proteins were immunochemically distinct (9,

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14). Cytochrome P-450$_{cam}$ purified to homogeneity from D-(+)- camphor-grown Pseudomonas putida strain P$_p$G786 has been crystallized (13) and shown to be composed of a single peptide chain of molecular weight, 45,000, and 1 mol of ferriprotoporphyrin IX.

Recently, the authors purified cytochrome P-450$_{scc}$ from bovine corpus luteum to homogeneity (12). This preparation was compared physicochemically and/or immunochemically with P-450$_{scc}$ from adrenocortical mitochondria and human placental mitochondria (12) and with P-450$_{11\beta}$ from bovine adrenocortical mitochondria (15).

In this paper, the amino acid composition of P-450$_{scc}$ from bovine corpus luteum is reported and compared with reported compositions of cytochrome P-450s from bovine adrenal mitochondria, Pseudomonas putida and with the multiple forms of rat liver microsomal P-450 induced by phenobarbital or methylcholanthrene.

METHODS

Cytochrome P-450$_{scc}$ from bovine corpus luteum mitochondria was purified to homogeneity and characterized as described previously (12). The purified P-450$_{scc}$ contained 16 nmole of heme per mg of protein, its enzymatically most active form had a molecular weight of about 200,000 and contained four identical peptide chains. The molecular weight of a single peptide chain was 48,000. The P-450$_{scc}$ preparation was dialyzed against distilled water to remove glycerol and salts.

Amino acid compositions were measured in a Beckman Spinco Model 121 MB Analyzer. Protein samples of 0.1 mg were hydrolyzed with 6 N HCL in sealed tubes at 108°C for 24 hours, 48 hours and 72 hours with 10 mM phenol present to protect tyrosine. Cystine plus cysteine were determined as cysteic acid on performic acid oxidized samples according to Hirs (16). Tryptophan was determined by the spectrophotometric method (17). Serine and threonine were normalized by extrapolation to zero time from the graph plotting the amounts after 24, 48, and 72 hours acid hydrolysis.

RESULTS

The amino acid compositions based on the molecular weight of 48,000 per single unit of cytochrome P-450$_{scc}$ are shown in Table I. The P-450$_{scc}$ contains about 400 amino acid residues and a relatively high content of leucine and glutamine-glutamate. The non-polar residue in the molecule was around 49%, which explains the hydrophobic characteristics of the protein. No unusual peaks were obtained on any of the chromatograms attributable to carbohydrate as were detected in hepatic microsomal P-450$_s$ (18) and P-450$_{cam}$ (19). In

Table I: Amino acid composition of cytochrome P-450$_{scc}$ from bovine corpus luteum mitochondria.

	24 hours	48 hours	72 hours
Aspartate+Asparagine(ASX)	34.4	34.1	34.8
Threonine	23.4	23.4	23.4
Serine	21.5	21.5	21.5
Glutamate+glutamine (GLX)	43.4	44.2	45.1
Proline	23.7	23.7	23.7
Glycine	22.0	22.9	23.8
Alanine	18.8	18.1	19.5
Valine	24.5	25.2	25.3
Methionine	11.0	10.7	11.2
Isoleucine	28.0	25.9	25.1
Leucine	43.4	40.8	41.0
Tyrosine	15.4	14.7	14.6
Phenylalanine	24.2	24.5	24.8
Tryptophan	2.5 *	2.5*	2.5*
Lysine	25.7	25.4	26.2
Histidine	13.3	16.7	11.5
Arginine	22.7	20.9	21.0
Half-cystine	2.8	2.8	2.8
Total residues	398.8	398.5	397.9
Molecular weight	48,000	48,000	48,000
Non-polar residues(%)	49.0	48.2	48.8

*Determined spectrophotometrically.

Table II, the composition determined for this preparation of P-450 is compared with published compositions of cytochrome P-450s from several other sources (20, 21, 22). Similar high contents of hydrophobic amino acid residues within the range of 47.2 to 50.6% are found in all P-450s and are characteristic of membrane proteins. Notable differences are seen in the contents of half-cysteine, lysine, arginine, asparagine-asparatate, glutamine-glutamate, phenylalanine and alanine. The amino acid compositions of P-450$_{scc}$ from bovine adrenal and corpus luteum mitochondria are remarkably similar but distinct differences occur between the functionally different P-450$_{11\beta}$ and P-450$_{scc}$.

DISCUSSION

The cytochromes under the terminology of "P-450" have many similarities: spectral properties related to the heme prosthetic group, spectral changes associated with substrate and inhibitor ligands, electron transport mechanisms and reactions with oxygen and carbon monoxide. On the other hand, differences in the substrate site establish identity for each of the cytochrome P-450s and

Table II: Amino acid compositions of various cytochrome P-450s

Amino acid	Bovine adrenal Mitochondria		P. Putida	Rats liver microsome		
	$P\text{-}450_{scc}$	$P\text{-}450_{11\beta}$	$P\text{-}450_{cam}$	PB-treated P-450[1]	PB-treated P-450[2]	MC-treated P-450[3]
	(20)	(20)	(21)	(22)	(22)	(22)
Aspartate+asparagine(ASX)	36.5	28.4	36	42	41	43
Threonine	21.9	21.4	19	29	25	29
Serine	21.4	20.2	21	30	34	38
Glutamate+glutamine(GIX)	48.1	47.4	55	52	52	43
Proline	25.9	22.5	27	27	30	37
Glycine	21.2	21.1	26	33	54	32
Alanine	18.4	28.3	34	29	17	26
Valine	23.0	24.5	24	27	20	28
Methionine	11.5	11.2	9	11	15	10
Isoleucine	23.4	14.5	24	27	25	24
Leucine	39.1	45.8	40	59	40	54
Tyrosine	13.4	9.6	9	14	14	14
Phenylalanine	23.5	16.4	17	32	17	28
Tryptophan	4.5	6.4	1	2	1	6
Lysine	25.9	12.2	13	27	38	31
Histidine	12.4	10.8	12	13	10	13
Arginine	21.2	30.3	24	25	22	25
Half-cystine	3.3	3.4	6	7	7	6
Total	394.6	374.7	397	486	483	487
Molecular weight	48,000	45,000	45,000			
Non polar residue(%)	47.4	49.2	50.6	50.4	47.2	49.1

$(P\text{-}450)^1$, $(P\text{-}450)^2$- phenobarbital-induced rat microsomal P-450; the specific contents of P-450 were (^{1}) 18.0 nmol/mg protein); (^{2}) 14.1 nmol/mg protein.

$(P\text{-}450)^3$- 3-methylcholanthrene-induced rat P-450; the specific content was 17.1 nmol/mg protein.

differences in electron transport components is one of the factors which distinguish mitochondrial cytochromes P-450s from the microsomal P-450s.

These similarities and differences in function must be ascribable to differences in amino acid composition. The cytochrome P-450s are hydrophobic proteins composed of about 50% hydrophobic amino acids especially leucine and isoleucine. This hydrophobicity is to be expected since the enzymes are membrane-bound and, for the most part, have non-polar substrates.

The active site of the enzyme involves the heme prosthetic group, the substrate site and possibly an area where oxygen may be concentrated. The substrate site is clearly hydrophobic for the cholesterol side chain cleavage enzyme as the substrate has a single polar group for orientation of the molecule at the active site. If there is an affinity area for oxygen, it, too, must be hydrophobic in nature. Of particular interest is the coordination of the heme molecule with the apoprotein. The occurrence of only two or three half-cystines in $P\text{-}450_{scc}$ identifies its primary function as the coordination of the thiolate ion to the heme iron as proposed by Murakami and Mason (23) to explain the atypical Soret absorption of 450nm. In $P\text{-}450_{cam}$, all six half-cystines were found to be present as free sulfhydryls (13, 19) and some are considered to be involved in the binding of the heme and also the camphor substrate. Key to this assumption are the experiments in which small amino acid sequence fragments were split from purified $P\text{-}450_{cam}$ (24) and $P\text{-}450_{LM}$ (21) by selective chemical cleavage with BrCN. These fragments contained one cysteine residue, and one histidine residue per heme molecule and also retained many of the spectral characteristics of the substrate complex with the native P-450s. These data suggest axial thiolate coordination with the sixth heme ligand and histidyl coordination with the fifth. Recently, NMR (25) and MCD (26,27) techniques have confirmed the thiolate axial coordination.

The mechanism of electron transport to the mitochondrial or microsomal P-450's from the iron sulfur protein (ISP) or ISP reductase respectively has not been elucidated. Obviously, the electron transport protein binding site of the microsomal P-450s must differ from the analogous binding site of the mito-

chondrial P-450s. Because the electron carriers are less hydrophobic than the cytochromes, one might speculate that more non-polar amino acids are located in this site.

In summary, reconstruction of the P-450 molecule leads to the speculation that there is a hydrophobic external area which is membrane-oriented, a hydrophobic substrate site and a more electrophilic site for the electron transport protein. Other hydrophilic amino acids, i.e., histidine and cysteine, are necessary for heme linkage but it is likely that the majority of the hydrophilic amino acids lie within the insulated core of the protein.

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REFERENCES

1. Imai, Y. and Sato, R. (1974) Biochem. Biophys. Rec. Commun. 60, 8-14.
2. van der Hoever, T.A., Haugen, D.A. and Coon, M.J. (1974) Biochem. Biophys. Res. Commun. 60, 596-575.
3. Haugen, D.A. and Coon, M.J. (1976) J. Biol. Chem. 251, 7929-7939.
4. Hashimoto, C., Imai, Y. (1976) Biochem. Biophys. Res. Commun. 68, 821-827.
5. Ryan, D.E., Thomas, P.E., Kotzeniowski, D. and Levin, W. (1979) J. Biol. Chem. 254, 1365-1374.
6. Takemori, S., Sato, H., Gomi, T., Suhara, K. and Katagiri, M. (1975) Biochem. Biophys. Res. Commun. 67, 1151-1157.
7. Takemori, S., Suhara, K., Hashimoto, S., Hashimoto, M., Sato, H., Gomi, T. and Katagiri, M. (1975) Biochem. Biophys. Res. Commun. 64, 588-593.
8. Katagiri, M., Takemori, S., Itagaki, E., Suhara, K., Gomi, T. and Sato, H., (1976) Adv. Exp. Med. Biol. 74, 281-289.
9. Suhara, K., Gomi, T., Sato, H., Itagaki, E., Takemori, S. and Katagiri, M. (1978) Arch. Biochem. Biophys. 190, 290-299.
10. Sato, H., Ashida, M., Suhara, K., Itagaki, E., Takemori, S. and Katagiri, M. (1978) Arch. Biochem. Biophys. 190, 307-314.
11. Takikawa, O., Gomi, T., Suhara, K., Itagaki, E., Takemori, S. and Katagiri, M. (1978) Arch. Biochem. Biophys. 190, 300-306.
12. Kashiwagi, K., Dafeldecker, W.P. and Salhanick, H.A. (1980) J. Biol. Chem. 255, 2606-2611.
13. Yu, C.-A., Gunsalus, I.C., Katagiri, M., Suhara, K., and Takemori, S. (1974) J. Biol. Chem. 249, 94-101.
14. Watanuki, M., Granger, G.A. and Hall, P.F. (1978) J. Biol. Chem. 253, 2927-2931.
15. Kashiwagi, K., MacDonald, A.B. and Salhanick, H.A. (submitted for publication).
16. Hirs, C.H.W. (1956) J. Biol. Chem. 219, 611-621.
17. Goodwin, T.W. and Morton, R.A. (1946) Biochem. J., 40, 628-631.
18. Haugen, D.A. and Coon, M.J. (1976) J. Biol. Chem. 251, 7929-7939.
19. Dus, K., Katagiri, M., Yu, C-A., Erbes, D.L. and Gunsalus, I.C. (1970) Biochem. Biophys. Res. Commun. 40, 1423-1430.
20. Katagiri, M., Takemori, S., Itagaki, E., Suhara, K., Gomi, T. and Sato, H. (1976) in Iron and Copper Proteins (Yasunobu, K.T., Mower, H.F. and Hayaishi, O., eds.), pp. 281-289. Plenum Press, New York.

21. Dus, K., Litchfield, W.J., Miguel, A.G., van der Hoever, T.A., Dean, W.L. and Coon, M.J. (1974) Biochem. Biophys. Res. Commun. 60, 15-21.
22. Guengerich, F.P. (1978) J. Biol. Chem. 253, 7931-7939.
23. Murakami, K. and Mason, H.S. (1967) J. Biol. Chem. 242, 1102-1110.
24. Dus, K., Miguel, A.G., Smith, P.C., Litchfield, W.J. and Harrison, J.R. (1973) 9th International Congress of Biochemistry, Stockholm, Abstracts, p. 341.
25. Morishima, I., Izuka, T. and Ishimura, Y. (1974) Seikagaku. 46, 535.
26. Shimizu, T., Nozawa, T., Hatano, M., Imai, Y. and Sato, R. (1975) Biochemistry 14, 4172-4178.
27. Hashimoto, C. and Imai, Y. (1976) Biochem. Biophys. Res. Commun. 68, 821-827.