Cross-Linking Studies of Cytochrome P-450 and Reduced Nicotinamide Adenine Dinucleotide Phosphate–Cytochrome P-450 Reductase†

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ABSTRACT: Cross-linking studies were carried out to investigate the structural organization of monooxygenase enzymes. Liver microsomal cytochrome P-450 (P-450) and reduced nicotinamide adenine dinucleotide phosphate (NADPH)–cytochrome P-450 reductase were purified from phenobarbital-treated rats. When purified P-450 or reductase was reacted with 2 mM dimethylsuberimidate for 1 min, the predominant cross-linked species were the dimer and trimer of P-450 and the dimer of the reductase as analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Cross-linking P-450 with 2 mM dimethyl 3,3′-dithiobis(propionimidate) for 1 min gave a gel electrophoresis pattern of monomer through octamer of P-450. Reductase cross-linked under the same conditions gave a monomer through tetramer band pattern. Gel-filtration experiments indicated that the purified P-450 existed in protein micelles corresponding to a decamer. The results have been attributed to cross-linking within the protein micelle. When a mixture of P-450 and reductase was cross-linked with 2 mM dimethyl 3,3′-dithiobis(propionimidate) for 1 min, a mixed dimer of P-450 and reductase was the predominant cross-linked product in addition to the dimers of P-450 and reductase. Similar results were also obtained in cross-linking studies with dithiobis(succinimidyl propionate) and ethylene glycol bis(succinimidyl succinate). The addition of dilauroylphosphatidylcholine did not alter significantly the cross-linking pattern. The results provide evidence for the formation of a complex between P-450 and reductase under the experimental conditions.

Cytochrome P-450 (P-450)[1] and reduced nicotinamide adenine dinucleotide phosphate (NADPH)–cytochrome P-450 reductase (reductase) are essential components of the monooxygenase system. The enzymes are imbedded in the membrane and occur widely in various tissues, with the highest concentration being found in the endoplasmic reticulum of liver cells (Sato & Omura, 1978). The liver microsomal monooxygenase system is noted for its important roles in the biotransformation of steroids, fatty acids, drugs, carcinogens, and other xenobiotics (Conney, 1967; Gillette et al., 1972). In rat liver microsomes, the number of P-450 molecules is about 10–30 times that of reductase (Estabrook et al., 1976). In the absence of a known mobile electron carrier, it appears that one reductase molecule has to interact with a great number of P-450 molecules for efficient catalysis. The mechanism by which this is accomplished is not clearly understood. The mode of association and interaction between these two enzymes has been studied and reviewed by many investigators (Estabrook et al., 1976; DePierre & Ernster, 1977; Yang, 1977b). Whereas our previous studies suggested that these enzymes are nonrigidly organized and possess lateral mobility (Yang, 1975, 1977a; Yang & Strickhart, 1975; Yang et al., 1978), other models have also been proposed (Estabrook et al., 1976; Peterson et al., 1976). Recently, Miwa et al. (1979) have studied the association of P-450 and reductase in a reconstituted monooxygenase system and suggested the formation of a binary complex between the two enzymes during the catalytic process. French et al. (1978) have also reported the formation of a complex between these two proteins and isolated a large complex (M_r 800 000) composed of approximately equimolar quantities of rabbit liver P-450 and reductase.

Recently, we have investigated the structural orgnization of monooxygenase enzymes with a cross-linking approach. This is based on the rationale that proteins present in a molecular complex or aggregate will be more readily cross-linked by bifunctional reagents than proteins not associated together (Peters & Richards, 1977). This paper reports the results of cross-linking of monooxygenase enzymes separately and in a reconstituted system with the following amino-directed cross-linking reagents: dimethylsuberimidate (DMS), dimethyl 3,3′-dithiobis(propionimidate) (DTBP), dithiobis(succinimidyl propionate) (DSP), and ethylene glycol bis(succinimidyl succinate) (EGS).

Materials and Methods

Materials. DMS, DTBP, and DSP were obtained from Pierce Chemical Co. EGS was a gift from Dr. Paul Smith of Pierce Chemical Co. Sodium dodecyl sulfate ($NaDodSO_4$) (DX2495) was obtained from Matheson Coleman and Bell. A gel electrophoresis molecular weight calibration kit was obtained from Pharmacia Inc. Cross-linked bovine serum albumin and other molecular weight standard proteins were obtained from Sigma Chemical Co. Dilauroylphosphatidylcholine (DLPC) was obtained from Serdary Research Labs.

Enzyme Preparation. P-450 was purified by the procedure of West et al. (1979) from liver microsomes of phenobarbital-induced rats and had properties similar to those described previously (Tsong & Yang, 1978). It moved as a single band with an estimated molecular weight of 52 000 in $NaDodSO_4$–polyacrylamide gel electrophoresis. Although minor lower molecular weight bands were detectable in certain gel systems, the minor bands did not seem to affect the result of the present study. Reductase was purified from microsomes by a previous procedure (Yang et al., 1978) except that the Emulgen 911 was omitted from the agarose–hexane–adenosine 2′,5′-diphosphate affinity chromatography step. The sample had properties similar to previous preparations (Yang et al., 1978).

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[1] Abbreviations used: P-450, cytochrome P-450; reductase, NADPH–cytochrome P-450 reductase; DMS, dimethylsuberimidate; DTBP, dimethyl 3,3′-dithiobis(propionimidate); DSP, dithiobis(succinimidyl propionate); EGS, ethylene glycol bis(succinimidyl succinate); $NaDodSO_4$, sodium dodecyl sulfate; P_1 (R_1), P_2 (R_2), P_3 (R_3), etc., monomer, dimer, trimer of P-450 (reductase), etc.; PR, mixed dimer of P-450 and reductase; DLPC, dilauroylphosphatidylcholine.

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Cross-Linking Reactions. Cross-linking with DMS and DTBP was generally carried out in 0.2 M triethanolamine–HCl buffer, pH 8.5 (Davies & Stark, 1970), at room temperature in a final volume of 100 μL. The protein was preincubated in 10–17 μL of 0.1 M sodium phosphate, pH 7.5, buffer at 30 °C for 10 min; then the triethanolamine–HCl buffer and cross-linking reagent were added. The reaction was quenched by 5 μmol of ammonium acetate (Wang & Richards, 1974). After incubation for 10 min at room temperature, 4 μmol of *N*-ethylmaleimide was added (in 5 μL of ethanol) to prevent disulfide–sulfhydryl exchange. Cross-linking with DSP (Lomant & Fairbanks, 1976) and EGS (Abdella et al., 1979) was carried out at pH 7.3 in a 50 mM sodium phosphate buffer. Other conditions were similar to those with the bisimidates. The cross-linking reactions were stopped by adding 5 μmol of glycine. *N*-Ethylmaleimide (4 μmol) was also added in the reactions with DSP. For electrophoresis, 100 μL of a 5% $NaDodSO_4$ tracking buffer (20 mM Tris-HCl, pH 6.8, 40% glycerol, and 0.003% bromphenol blue tracking dye) was added to the quenched reaction mixture. The mixture was heated for 5 min at 100 °C with 5% 2-mercaptoethanol for DMS samples and for 2 h at 37 °C for DTBP, DSP, and EGS samples. The samples were usually stored at −10 °C and warmed to 37 °C prior to gel electrophoresis.

One-Dimensional Cylindrical Gel Electrophoresis. Electrophoresis was performed on 3.5% polyacrylamide gels by the procedure of Davies & Stark (1970) except that the running buffer contained 50 mM instead of 100 mM sodium borate. Protein samples were subjected to electrophoresis at 24 °C at 8 mA/gel for 5–6 h or 2.5 mA/gel overnight. Cytochrome *c* which ran slightly ahead of the dye front was used as the reference front in the determination of molecular weights with cross-linked bovine serum albumin as a standard (Shapiro et al., 1967; Weber & Osborn, 1969). Gels were stained with Coomassie Blue R-250, destained by the gradient procedure of Fairbanks et al. (1971), and scanned spectrophotometrically at 550 nm on a Gilford Model 2520 spectrophotometer.

Two-Dimensional Gel Electrophoresis. After electrophoresis in the first dimension, the cylindrical gel was placed in the slot on top of a discontinuous slab gel system similar to that of Laemmli (1970). It consisted of a 3% polyacrylamide stacking gel on a 3–15% linear polyacrylamide gradient gel. Glycerol (20%) was added to the 15% solution to stabilize the gradient during casting (O'Farrell, 1975). Agarose (1%) containing 62.5 mM Tris-HCl (pH 6.8), 0.1% $NaDodSO_4$, 10% 2-mercaptoethanol, and 10% glycerol at 80 °C was added on top of the stacking gel to cover the cylindrical gel. The slab gel was run at 30 V overnight. Molecular weight standards were run in slots made in the agarose buffer. An electrode buffer consisting of 0.05 M Tris-HCl (pH 8.3), 0.38 M glycine, and 0.1% $NaDodSO_4$ was used. The gels were stained and destained as described above.

Results

Cross-Linking with DMS. Cross-linking with the bifunctional reagent DMS, which is 10.8 Å between reaction centers,[2] was investigated initially with purified P-450 and reductase preparations. When P-450 was treated with 2 mM DMS for 1 min, oligomers P_2, P_3, and P_4 were seen on cylindrical gels (Figure 1b). Under these mild conditions, the major cross-linked species was the dimer (P_2). Cross-linking of P-450 with 2 mM DMS for 45 min resulted in a pattern of seven bands

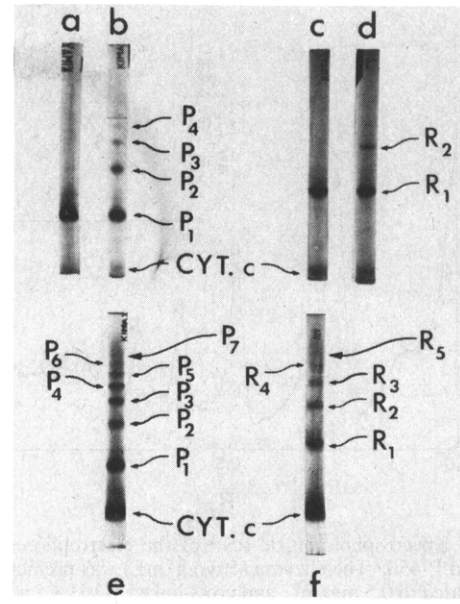

FIGURE 1: Gel electrophoresis of cross-linked monooxygenase enzymes. The following samples were analyzed by using 6 μg of protein per gel: (a) P-450; (b) P-450 (0.5 mg/mL) cross-linked with 2 mM DMS for 1 min in 0.1 M triethanolamine–HCl, pH 8.5, buffer; (c) reductase; (d) reductase (0.5 mg/mL) cross-linked with 2 mM DMS for 1 min in 0.2 M triethanolamine–HCl, pH 8.5, buffer. The following samples were analyzed by using 14 μg of protein per gel: (e) P-450 cross-linked with 10 mM DMS for 1 min; (f) reductase cross-linked with 10 mM DMS for 5 min.

(P_1–P_7), and the same pattern was observed when the cross-linking was carried out with protein concentrations ranging from 0.2 to 1.0 mg/mL. When reductase was treated with 2 mM DMS for 1 min, a dimer (R_2) was observed (Figure 1d). Over a protein concentration range of 0.1-0.5 mg/mL, reductase cross-linked with 2 mM DMS for 45 min gave a pattern of four main bands (R_1–R_4) and a faint fifth band (R_5). Again the cross-linking pattern was independent of the protein concentration. When P-450 or reductase was reacted with DMS under stronger reaction conditions, more bands were apparent on the gel. For instance, when P-450 was reacted with 10 mM DMS for 1 min, seven bands appeared on the gel (Figure 1e). The major cross-linked bands were the P_2, P_3, and P_4 bands which appeared to have about the same intensity. Reacting for 5 min resulted in a similar pattern except for the appearance of an eighth band. When reductase was reacted with 10 mM DMS for 10 min, five bands appeared on the gel (Figure 1f). Reactions up to 60 min did not alter the cross-linking band pattern of the reductase.

Cross-Linking with DTBP. DTBP (Wang & Richards, 1974) is an 11.9-Å cross-linking reagent.[2] As such, it might be expected to be more reactive than the shorter reagent, DMS (Aizawa et al., 1977). The spectrophotometric scans in Figures 2 and 3 show that P-450 or reductase, when cross-linked with 2 mM DTBP for 1 min, formed oligomers from P_2 through P_8 or R_2 through R_4. The number of bands was more than that obtained with 2 mM DMS (Figure 1b,d) under similar conditions.

Cross-Linking with N-Hydroxysuccinimidyl Esters. Cross-linking was also carried out at pH 7.3 in a 0.1 M sodium phosphate buffer with DSP, an 11.9-Å cross-linker, and EGS, a 15.1-Å cross-linker.[2] Upon reaction of P-450 (0.25 mg/mL) with 4.3×10^{-5} M DSP or 3.3×10^{-5} M EGS for 30 min, P_1–P_8 were obtained. Cross-linking reductase under similar conditions resulted in a band pattern of R_1–R_4 (data not shown). These results resemble those obtained with the bisimidates, DMS and DTPB.

[2] The distance between reaction centers was calculated from the covalent radii for atoms listed in Pauling (1960).

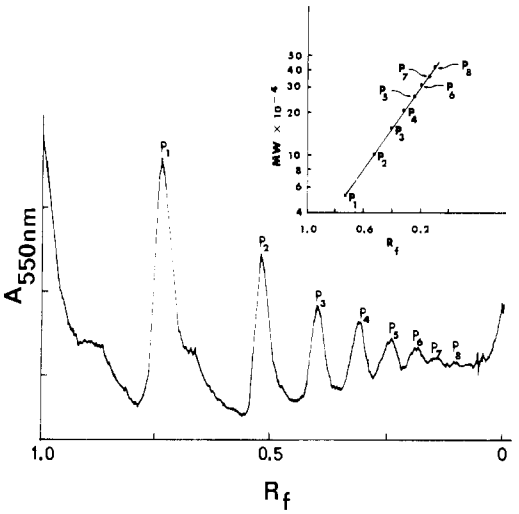

FIGURE 2: Spectrophotometric scan of the electrophoresis gel of cross-linked P-450. The enzyme (5.6 mg/mL) was preincubated at pH 7.5, diluted to 0.5 mg/mL, and cross-linked at pH 8.5 with 2 mM DTBP for 1 min. A sample containing 6 μg of P-450 was applied to a cylindrical gel and analyzed electrophoretically. The insert is a semilog plot of molecular weight (M_r) against electrophoretic mobility (R_f) relative to cytochrome c (R_f = 1.0).

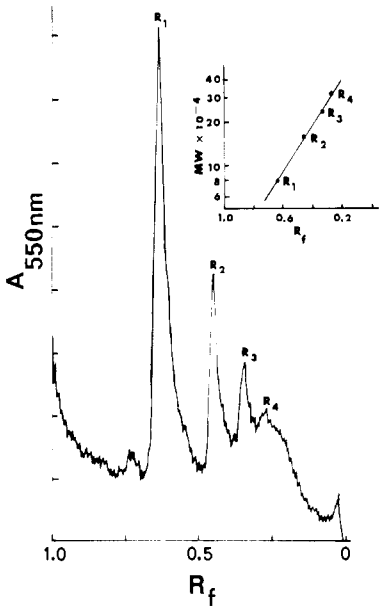

FIGURE 3: Spectrophotometric scan of the electrophoresis gel of cross-linked reductase. The enzyme (6 mg/mL) was preincubated, diluted to 0.5 mg/mL, and cross-linked with 2 mM DTBP for 1 min. The conditions were similar to those in Figure 2.

Molecular Size of the P-450 Micelle. Hydrophobic membrane proteins are known to form protein micelles in the absence of membrane or detergents (Simons et al., 1978). Gel-filtration analysis indicated that purified P-450 formed micelles or aggregates with a molecular weight of 520 000, corresponding to a decamer (Figure 4). A peak corresponding to the monomer of P-450 was not observed. This result is consistent with the value of 500 000 daltons for rat liver P-450 micelles as determined by sedimentation equilibrium (Guengerich & Holladay, 1979). The rabbit liver P-450, however, has been reported to exist as hexamers with molecular weights of about 300 000 (Coon et al., 1976).

Cross-Linking of a Mixture of Cytochrome P-450 and Reductase. P-450 and reductase were mixed and cross-linked with 2 mM DTBP for 1 min. The observed bands 1–6 (Figure 5) were assigned as species P_1, R_1, P_2, PR, R_2 (or P_3), and P_2R

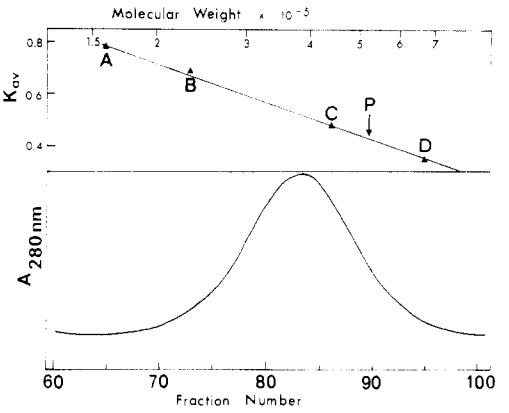

FIGURE 4: Gel filtration of cytochrome P-450 with Sepharose 6B. The column (1.5 × 74 cm) with a void volume of 42 mL was equilibrated and eluted with 50 mM potassium phosphate, pH 7.4, containing 20% glycerol and 0.1 mM EDTA. The upper panel shows the standard curve with (A) aldolase, M_r 158 000, (B) catalase, M_r 232 000, (C) ferritin, M_r 440 000, and (D) thyroglobulin, M_r 669 000. P-450 (P) was eluted at a position correponding to 520 000 ± 15 000 daltons. The lower panel shows the elution profile of 10 nmol of P-450.

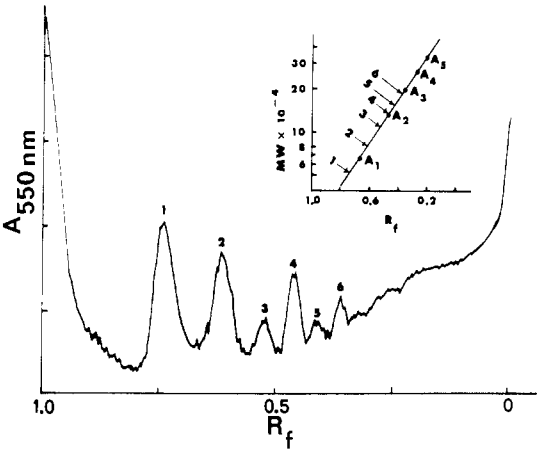

FIGURE 5: Cross-linking between P-450 and reductase. P-450 (50 μg) and reductase (50 μg) were preincubated at pH 7.4, diluted, and cross-linked with 2 mM DTBP for 1 min. A sample containing 6 μg each of P-450 and reductase was applied to a cylindrical gel. The insert is a semilog plot of molecular weight (M_r) against electrophoretic mobility (R_f) using cross-linked bovine serum albumin as a standard (A_1 = 66 000; A_2 = 132 000; A_3 = 198 000; A_4 = 264 000; A_5 = 330 000). Bands numbered 1–6 are described in Table I.

Table I: Cross-Linked Oligomers of P-450 and Reductase[a]

samples	R_f (band no.)	$M_r \times 10^{-3}$	assignment (calcd $M_r \times 10^{-3}$)
P-450	0.74	52	P
	0.53	107	P_2 (104)
	0.41	165	P_3 (156)
reductase	0.62	80	R
	0.42	160	R_2 (160)
P-450 plus reductase	0.74 (1)	52	P
	0.62 (2)	80	R
	0.53 (3)	107	P_2 (104)
	0.47 (4)	133	PR (132)
	0.43 (5)	155	R_2 (160)
	0.38 (6)	185	P_2R (184)
	0.33 (7)	225	R_3 (240)

[a] The data were from Figures 2, 3, 5, and 6.

based on their measured molecular weights of 52 000, 80 000, 107 000, 133 000, 155 000, and 185 000, respectively (Table I). Band 7 with a molecular weight of 225 000 was not assigned. The predominant cross-linked species was the mixed

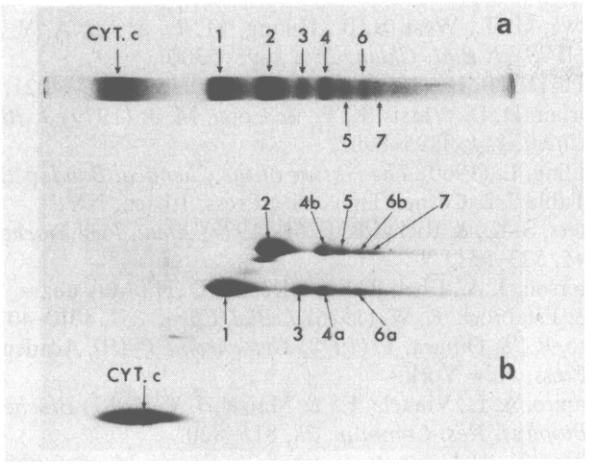

FIGURE 6: Two-dimensional gel electrophoresis of cross-linked monooxygenase enzymes. P-450 (50 μg) and reductase (50 μg) were preincubated, diluted, and cross-linked with 2 mM DTBP for 1 min. (a) First dimension: a cross-linked sample containing 11 μg of each protein was electrophoresed on a cylindrical gel and stained. On a duplicate gel a sample of 43.5 μg of each protein was electrophoresed and stored unstained at −90 °C. (b) Second dimension: the unstained cylindrical gel was placed over a slab gel and electrophoresed overnight. The bands numbered 1–7 are described in Table I. Bands 4 and 6 are shown to contain both P-450 and reductase.

dimer PR (band 4). Both the P-450 dimer (band 3) and band 5 were less prevalent. The P_2R species (band 6) was more intense than band 5.

Two-dimensional NaDodSO$_4$–polyacrylamide gel electrophoresis experiments were conducted to confirm the assignments of the cross-linked species (Figure 6a,b). The DTBP cross-linked proteins were cleaved by 2-mercaptoethanol in the second dimension to regenerate P_1 and R_1. The results led to the assignment of bands 3, 4, 5, 6, and 7 as P_2, PR, R_2, P_2R, and R_3, respectively. Up to 100 μg of protein was run in the first dimension and then in the second dimension to enhance the visualization of the higher order cross-linked species. This procedure resulted in an overloading of the unreacted monomers but clearly confirmed the presence of the complexes formed between P-450 and reductase. Cross-linking reactions were also carried out in a mixture containing P-450 and reductase (each at 0.25 mg/mL) and DSP (4.3×10^{-5} M) or EGS (3.3×10^{-5} M) at pH 7.3. The results also confirmed the formation of a mixed dimer between P-450 and reductase (data not shown).

Effect of Phospholipid. DLPC or other phospholipids are required for the maximal catalytic activity of the reconstituted monooxygenase system (Strobel et al., 1970). The addition of DLPC, however, did not increase the amount of the DTPB cross-linked mixed dimer, PR, nor did it alter appreciably the amounts of other cross-linked species. This was true when 10 μg of DLPC was preincubated with P-450 and reductase (50 μg of each) in 100 μL of a pH 7.4 buffer for 10 min at 30 °C, or when 25 μg of DLPC was preincubated similarly with 12.5 μg each of P-450 and reductase, before the cross-linking reaction. When the cross-linking reaction was carried out with lower concentrations of enzymes (e.g., 4 μg of each protein), the results were complicated by the artifacts introduced by concentrating the sample before electrophoresis. In a study with DMS, DLPC was also found not to increase the extent of cross-linking between P-450 and reductase (data not shown).

Discussion

Cross-linking offers an experimental approach for studying the molecular organization of P-450 and reductase in solution and in the membrane. In solution, purified P-450 exists in micelles with a molecular weight corresponding to a decamer (Figure 4). Reductase is also known to exist in micelles or aggregates (French et al., 1978). A major problem in interpreting the results of cross-linking studies, however, is to distinguish the cross-linking of proteins within a protein complex or micelle from that caused by the random collision of proteins. Davies & Stark (1970) proposed using low protein concentrations to decrease cross-linking due to random collisions. The present results, showing that the formation of cross-linked oligomers is independent of the protein concentration, are consistent with the concept that the cross-linked oligomers originated from proteins existing in the same protein micelle or aggregate.

Theoretically, cross-linking of proteins within the P-450 decamer micelles would yield oligomers of P_2–P_{10}. The relative amount of each oligomer should be dependent on the concentration of the reagent and reaction time. This prediction was observed partially in our study; i.e., the quantity of oligomers decreased sequentially from P_2 to P_8, and the amounts of the larger oligomers increased with stronger reaction conditions (Figure 2). Under even stronger conditions, higher molecular weight species appeared, but P_6–P_{10} were not clearly resolved in the gel. The existence of cross-linked P-450 species with molecular weights greater than 520 000 indicated that intermicelle cross-linking of P-450 also took place. We chose, however, to carry out most of our study under mild reaction conditions and short reaction times, conditions under which intermicelle cross-linking appears not to take place.

In this study, we have used four cross-linking reagents. The experiments were conducted in two buffer systems at pH 8.5 and 7.3. Since similar cross-linking results were observed with all four reagents, we believe that the observed cross-linking pattern reflected the molecular organization of the monooxygenase enzymes, rather than artifacts from using a specific reagent, buffer system, or pH. A significant finding in this study is that P-450 and reductase are readily cross-linked under our experimental conditions. The result suggests that these two monooxygenase enzymes form a complex. The fact that the PR mixed dimer is the major cross-linked species appears to reflect the high affinity (or frequency) for PR association as compared to PP and RR associations. The spatial arrangement of the amino groups, nevertheless, may also be a factor in this consideration. It is possible that the two enzymes form mixed micelles, and we have cross-linked the most abundant nearest neighbors, P-450 and reductase. A similar result was also obtained in the presence of DLPC. The result is consistent with the concept proposed by French et al. (1978), Oprian et al. (1979), and Miwa et al. (1979) that P-450 and reductase form a complex in the reconstituted system. The lack of a lipid effect is somewhat surprising since phospholipids are known to increase the rate of P-450 reduction (Oprian et al., 1979) and the catalytic activities of the reconstituted system (Strobel et al., 1970). With our preparations, a sixfold enhancement in benzphetamine demethylase activity was observed upon the addition of DLPC (unpublished experiments). The relationship between the presently cross-linked complex and the catalytic active complex is not clearly understood. Although complex formation between monooxygenase enzymes was observed in reconstituted nonmembrane systems, it is not known whether a stable complex between monooxygenase systems is formed in the microsomal membranes. The latter system is being investigated. The present study, however, has revealed some structural features of monooxygenase enzymes in solution and provided a background for investigating the structural organization of these enzymes in the membrane.

Acknowledgments

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References

Abdella, P. M., Smith, P. K., & Royer, G. P. (1979) *Biochem. Biophys. Res. Commun. 87*, 734–742.

Aizawa, S., Kurimoto, F., & Yokono, O. (1977) *Biochem. Biophys. Res. Commun. 75*, 870–878.

Conney, A. H. (1967) *Pharmacol. Rev. 19*, 317–366.

Coon, M. J., Haugen, D. A., Guengerich, F. P., Vermilion, J. L., & Dean, W. L. (1976) in *The Structural Basis of Membrane Function* (Hatefi, Y., & Djavadi-Ohaniance, L., Eds.) pp 409–427, Academic Press, New York.

Davies, G. E., & Stark, G. R. (1970) *Proc. Natl. Acad. Sci. U.S.A. 66*, 651–656.

DePierre, J. W., & Ernster, L. (1977) *Annu. Rev. Biochem. 46*, 201–262.

Estabrook, R. W., Werringloer, J., Masters, B. S. S., Jonen, H., Matsubara, T., Ebel, R., O'Keeffe, D., & Peterson, J. A. (1976) in *The Structural Basis of Membrane Function* (Hatefi, Y., & Djavadi-Ohaniance, L., Eds.) pp 429–445, Academic Press, New York.

Fairbanks, G., Steck, T. L., & Wallach, D. F. H. (1971) *Biochemistry 10*, 2606–2617.

French, J. S., Guengerich, F. P., & Coon, M. J. (1978) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 37*, 1568 (Abstract 1640).

Gillette, J. R., Davis, D. C., & Sasame, H. A. (1972) *Annu. Rev. Pharmacol. 12*, 57–84.

Guengerich, F. P., & Holladay, L. A. (1979) *Biochemistry 18*, 5442–5449.

Laemmli, V. K. (1970) *Nature (London) 227*, 680–685.

Lomant, A. J., & Fairbanks, G. (1976) *J. Mol. Biol. 104*, 243–261.

Miwa, G. T., West, S. B., Huang, M. T., & Lu, A. Y. H. (1979) *J. Biol. Chem. 254*, 5695–5700.

O'Farrell, P. H. (1975) *J. Biol. Chem. 250*, 4007–4021.

Oprian, D. D., Vatsis, K. P., & Coon, M. J. (1979) *J. Biol. Chem. 254*, 8895–8902.

Pauling, L. (1960) *The Nature of the Chemical Bond*, p 224, Table 7-2, Cornell University Press, Ithaca, NY.

Peters, S. K., & Richards, F. M. (1977) *Annu. Rev. Biochem. 46*, 523–551.

Peterson, J. A., Ebel, R. E., O'Keeffe, D. H., Matsubara, T., & Estabrook, R. W. (1976) *J. Biol. Chem. 251*, 4010–4016.

Sato, R., & Omura, T. (1978) *Cytochrome P-450*, Academic Press, New York.

Shapiro, A. L., Vinuela, E., & Maizel, J. V. (1967) *Biochem. Biophys. Res. Commun. 28*, 815–820.

Simons, K., Helenius, A., Leonard, K., Sarvas, M., & Gething, M. J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5306–5310.

Strobel, H. W., Lu, A. Y. H., Heidema, J., & Coon, M. J. (1970) *J. Biol. Chem. 245*, 4851–4854.

Tsong, T. Y., & Yang, C. S. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5955–5959.

Wang, K., & Richards, F. M. (1974) *J. Biol. Chem. 249*, 8005–8018.

Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406–4412.

West, S. B., Huang, M. T., Miwa, G. T., & Lu, A. Y. H. (1979) *Arch. Biochem. Biophys. 193*, 42–50.

Yang, C. S. (1975) *FEBS Lett. 54*, 61–64.

Yang, C. S. (1977a) *J. Biol. Chem. 252*, 293–298.

Yang, C. S. (1977b) *Life Sci. 21*, 1047–1058.

Yang, C. S., & Strickhart, F. S. (1975) *J. Biol. Chem. 250*, 7968–7972.

Yang, C. S., Strickhart, F. S., & Kicha, L. P. (1978) *Biochim. Biophys. Acta 509*, 326–337.