J Cancer Res Clin Oncol (1988) 114:335–340

Journal of Cancer Research and Clinical Oncology

Phospholipid bilayer membranes play decisive roles in the cytochrome P-450-dependent monooxygenase system * **

Hisaaki Taniguchi and Walter Pyerin

Institute of Experimental Pathology, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany

Summary. Hepatic microsomal monooxygenase was reconstituted by incorporating cytochrome P-450 and NADPH-cytochrome P-450 reductase, which had been purified from phenobarbital-pretreated rabbit liver microsomes, into phospholipid liposomal membranes. The NADPH-dependent monooxygenase activity of the reconstituted system was found to be dependent on the phospholipid-to-protein ratio, i.e., the two-dimensional concentration of the two proteins on the plane of the membranes. A similar concentration dependence was also observed in the cytochrome b_5 and NADH-cytochrome b_5 system, which had been incorporated into liposomal membranes. The diffusion process of the proteins in the membrane, therefore, plays an important role in the monooxygenase system. When the fluidity of the membrane was changed by utilizing a synthetic dimyristoylphosphatidylcholine, which shows a well-defined gel to liquid crystalline phase transition, the activation energy of the monooxygenase reaction was changed at around the phase transition temperature, suggesting a conformational change of cytochrome P-450 caused by the fluidity change of the membrane. The incorporation of P-450 into liposomes was also found to affect the binding of substrates to cytochrome P-450. The decrease in the apparent dissociation constant of substrates upon incorporation into membranes suggests that the lipid membrane acts as a pool for hydrophobic substrates, which are concentrated in the lipid phase, and that cytochrome P-450 takes substrates directly from the membrane phase. Phospholipid membranes, therefore, play very important roles in various phases of the reaction of cytochrome P-450-dependent monooxygenase.

Key words: Microsome – Monooxygenase – Cytochrome P-450 – Biomembrane – Chemical carcinogenesis

Introduction

The cytochrome P-450-dependent monooxygenase system is a key enzyme in chemical carcinogenesis. Although cytochrome P-450-dependent monooxygenation is the first step in the detoxification reaction of foreign compounds, i.e., xenobiotics, including various drugs as well as carcinogens, the monooxygenation of various chemical carcinogens such as arylhydrocarbons and nitrosamines sometimes leads to activation of the procarcinogens to ultimate carcinogens. The presence of various enzymes of the cytochrome P-450 multigene family, which has been firmly established in the last decade, accounts for the versatility of the enzyme system and for the concurrent activation and inactivation of carcinogens by cytochrome P-450 (Ortiz de Montellano 1986; Schenkman and Kupfer 1982).

In order to study the activation and inactivation mechanisms of chemical carcinogens by cytochrome P-450, it is essential to resolve the components of the monooxygenase system, which includes various cytochrome P-450 isoenzymes as well as other three protein components of the electron transfer system which supply reducing equivalents to cytochrome P-450 (Taniguchi et al. 1984b), to purify them, and then to reconstitute the monooxygenase system. The last step, however, turned out to be rather problematic. A simple mixing of the purified protein components failed to reconstitute the monooxygenase activity, and a heat-stable factor, which was later identified as phospholipid (Lu and Coon 1968; Lu et al. 1969; Strobel et al. 1970), was found to be necessary for the functional reconstitution. Although the addition of various detergents

* Dedicated to Professor H.W. Kunz on the occasion of his 65th birthday

** This work was supported in part by the Deutsche Forschungsgemeinschaft

Offprint requests to: H. Taniguchi

and phospholipids which have short fatty acids such as dilauroylphosphatidylcholine was then found to be able to reconstitute activity (Lu et l. 1969, 1974), this type of reconstituted system lacks the bilayer structure of the native microsomal membrane, and is inappropriate for studies which include various protein components (French et al. 1980; Morgan and Coon 1984).

Using purified cytochrome P-450 and NADPH-cytochrome P-450 reductase we successfully showed that the monooxygenase activity can be reconstituted by incorporating the two proteins into phospholipid membranes (Taniguchi et al. 1979, 1980, 1984b). This type of reconstituted system was found to be suitable for studying protein-protein interactions in membranes, and especially useful in elucidating the role of cytochrome b_5 in the microsomal monooxygenase system (Taniguchi et al. 1984b; Bösterling et al. 1982). The protein components incorporated into the liposomal membrane can diffuse easily on the plane of the membrane, and interact with each other as in microsomal membranes (Taniguchi et al. 1987).

In the present study, we have investigated the roles of bilayer membranes in the microsomal monooxygenase system utilizing the advantage of the liposomal reconstituted system. In this system the composition can be varied easily, and well-defined lipids can be used instead of complex mixtures of various lipids. The results obtained clearly show the importance of the microsomal membrane in the various phases of cytochrome P-450-catalyzed monooxygenase reactions. The microsomal membrane can modify the overall monooxygenase reaction through a change in membrane fluidity, and the conformation of cytochrome P-450 is affected by the membrane. The microsomal membrane also serves as a pool for hydrophobic substrates, and cytochrome P-450 binds substrates not from the cytosol but directly from the microsomal membrane.

Materials and methods

Materials. Cytochrome P-450, NADPH-cytochrome P-450 reductase, cytochrome b_5, and NADH-cytochrome b_5 reductase were purified simultaneously from phenobarbital-pretreated rabbit liver microsomes as described elsewhere (Taniguchi et al. 1984b). Egg yolk phosphatidylcholine was purchased from Lipid Products, South Nutfield, England, while dimyristoylphosphatidylcholine was obtained from Sigma, München, FRG, and cholate from Merck, Darmstadt, FRG. Other chemicals and biochemicals were of the highest grade commercially available.

Reconstitution of monooxygenase. Cytochrome P-450, NADPH-cytochrome P-450 reductase, cytochrome b_5, and NADH-cytochrome b_5 reductase were incorporated into liposomes of egg yolk phosphatidylcholine or of dimyristoylphosphatidylcholine as described previously (Taniguchi et al. 1979, 1980, 1984b, 1987). Briefly, phospholipids and the proteins were solubilized in 1% cholate, and the mixture was dialyzed against cholate-free buffer. Phospholipids spontaneously form liposomes in which the proteins are incorporated (Taniguchi et al. 1979, 1984b).

Analytical methods. NADPH-dependent benzphetamine and *N*-methylaniline *N*-demethylase activities were determined by measuring the formation of formaldehyde by the method of Nash (Nash 1953), as described previously (Taniguchi et al. 1979). The reaction mixture contained 0.1 *M* potassium phosphate buffer (pH 7.25), 1 m*M* benzphetamine or 8 m*M* *N*-methylaniline, 1 m*M* NADPH, and the reconstituted system (about 0.7 μ*M* in terms of cytochrome P-450) in a final volume of 1 ml. The reaction was started by adding NADPH, and stopped with 0.2 ml of 40% trichloroacetic acid. The supernatant after centrifugation was mixed with an equal volume of Nash reagent, and incubated at 60 °C for 20 min. The binding constant of benzphetamine was determined from the substrate-induced spectral change as described previously (Taniguchi et al. 1984a). The spectral data were fitted to the usual Michaelis-Menten equation using a nonlinear least square method (Taniguchi et al. 1984a), and the constants obtained showed less than 10% SD in five separate experiments. Cytochrome P-450 was determined from the absorbance difference between 418 and 500 nm, assuming a millimolar extinction coefficient of 101 (Hashimoto-Yutsudo et al. 1980). The NADPH-cytochrome *c* reductase was determined from the intensity of flavin absorption at 455 nm, using a millimolar extinction coefficient of 10.7 per bound flavin (Iyanagi and Mason 1973). Cytochrome b_5 was determined from the absorbance at 413 nm, assuming a millimolar extinction coefficient of 117 (Strittmatter and Velick 1956). The NADH-cytochrome b_5 reductase was determined from the flavin absorption at 461 nm, using a millimolar coefficient of 10.6 (Mihara and Sato 1976). The NADH-dependent cytochrome *c* reductase activity catalyzed by cytochrome b_5 and NADH-cytochrome b_5 reductase in liposomes was measured in 1 ml of 0.1 *M* potassium phosphate buffer (pH 7.3) containing 50 μ*M* cytochrome *c* and 1 m*M* NADH.

Results

Effect of lipid-to-protein ratio on monooxygenase activity

When cytochrome P-450 and NADPH-cytochrome P-450 reductase were incorporated into the liposomal membrane of egg yolk phosphatidylcholine, the two proteins catalyzed NADPH-dependent benzphetamine *N*-demethylation at reaction rates comparable to that of microsomes. In order to elucidate the role of the membrane in the monooxygenase system, equal amounts of cytochrome P-450 and the reductase were incorporated into liposomes at various protein-to-lipid ratios. As shown in Fig. 1, the monooxygenase activity increased markedly when the protein-to-lipid ratio was increased. In these experiments the concentrations of cytochrome P-450 and the reductase in the reaction mixture, i.e., the three-dimensional concentrations, were kept constant, and the results were expressed in terms of turnover number per nanomole cytochrome P-450. Only the lipid-to-protein ratios, i.e., the two-dimensional concentrations of the two proteins on the plane of the liposomal membrane, were varied. When the two-dimensional concentrations of proteins were increased, the monooxygenase activity

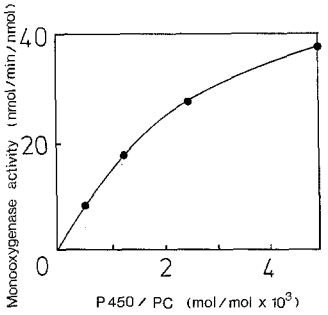

Fig. 1. Effect of the protein-to-lipid ratio on NADPH-dependent benzphetamine *N*-demethylase activity. Cytochrome P-450 and NADPH-cytochrome P-450 reductase were incorporated into liposomal membranes of egg yolk phosphatidylcholine at various lipid-to-protein ratios as indicated. The ratio between the cytochrome and the reductase was kept constant at 1:1

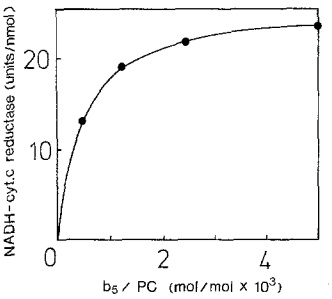

Fig. 2. Effect of the protein-to-lipid ratio on NADH-dependent cytochrome *c* reductase activity. Cytochrome b_5 and NADH-cytochrome b_5 reductase were incorporated into liposomal membranes of egg yolk phosphatidylcholine at various lipid-to-protein ratios as indicated. The ratio between cytochrome b_5 and the reductase was kept constant at 7:1

increased. The increase was linear at low concentrations, and showed saturation behavior at higher concentrations. The diameter of the reconstituted liposomes was around 100 nm, which corresponds to roughly 100,000 lipid molecules per liposome (Taniguchi et al. 1979, 1984b). Since the liposomes were homogeneous both in size and in density, the protein components were evenly distributed in each liposome, and there were enough protein components in each liposome. The intervesicular interactions were found to be negligible (Taniguchi et al. 1979). These results led to the following conclusions. Firstly, the protein-to-lipid ratio plays a decisive role in the monooxygenase reaction. In other words, the monooxygenase activity can be modulated by changing the amounts of lipids. Secondly, the monooxygenase reaction is a diffusion-limited reaction. The monooxygenase activity increases linearly when the two-dimensional concentration of the two protein components is increased.

Effect of protein-to-lipid ratio on the interaction between cytochrome b_5 and NADH-cytochrome b_5 reductase

Similar experiments were then carried out with the cytochrome b_5 and NADH-cytochrome b_5 reductase system. The two proteins, which had been purified from phenobarbital-pretreated rabbit liver microsomes simultaneously with cytochrome P-450 and NADPH-cytochrome P-450 reductase, were incorporated into liposomes of egg yolk phosphatidylcholine by the cholate dialysis method. The interaction between the two proteins was determined by measuring NADH-dependent cytochrome *c* reductase activity.

While reduced cytochrome b_5 can reduce cytochrome *c* directly, NADH-cytochrome b_5 reductase can not. The NADH-cytochrome *c* reductase activity, therefore, was a measure of the electron transfer between the two proteins. The activity increased in a similar way to the cytochrome P-450-dependent monooxygenase activity when the protein-to-lipid ratio, i.e., the two-dimensional concentration of proteins in the membrane was increased (Fig. 2). The relatively earlier saturation observed was probably due to the change in the rate limiting step in the reaction from the interaction between the two proteins to the flavin reduction. The turnover rate of NADH-cytochrome b_5 reductase itself measured in the presence of ferricyanide was approximately 25 μmol/min per nmol reductase, which was very close to the saturation level observed. The reduction of cytochrome b_5 by NADH-cytochrome b_5 reductase, is, therefore, a diffusion-limited reaction, and the average distance between the two proteins determines the electron transfer reaction as in the case of the system described with cytochrome P-450/NADPH-cytochrome P-450 reductase.

Effect of membrane fluidity on monooxygenase

In order to elucidate the role of the membrane in the monooxygenase system in detail, the effect of the change in membrane fluidity on the monooxygenase reaction was investigated. For that purpose, a synthetic phospholipid with a defined fatty acid chain, namely, dimyristoylphosphatidylcholine, was used as the lipid component. This phospholipid shows a clear gel to liquid crystalline phase transition approximately at 23 °C due to melting of the fatty acid chain portion, and the fluidity changes drastically at the phase transition temperature.

Cytochrome P-450 and NADPH-cytochrome P-450 reductase were incorporated into liposomes of dimyristoylphosphatidylcholine by the cholate dialysis method and the monooxygenase activity was deter-

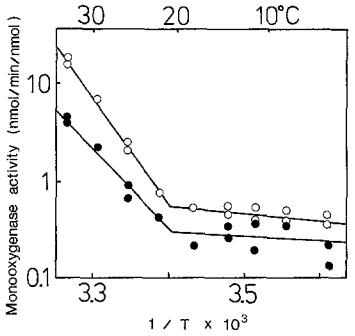

Fig. 3. Arrhenius plot of NADPH-dependent monooxygenase activities in the liposomal reconstituted system with dimyristoylphosphatidylcholine. Cytochrome P-450 and its reductase were incorporated into the liposomes of dimyristoylphosphatidylcholine as described in *Materials and methods*. The reconstituted system consisted of cytochrome P-450, reductase, and the phospholipid at a molar ratio of 1:1:400. The NADPH-dependent benzphetamine (○) and *N*-methylaniline *N*-demethylase (●) activities were measured at indicated temperatures

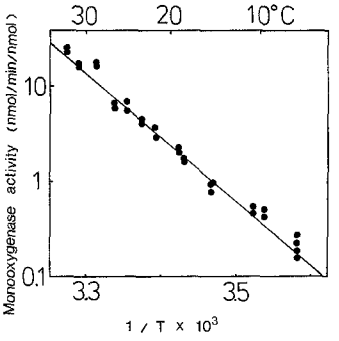

Fig. 4. Arrhenius plot of NADPH-dependent monooxygenase activities in the liposomal reconstituted system with egg yolk phosphatidylcholine. Cytochrome P-450 and its reductase were incorporated into the liposomes of egg yolk phosphatidylcholine. The reconstituted system consisted of cytochrome P-450, reductase, and phospholipid at a molar ratio of 1:1:400. The NADPH-dependent benzphetamine *N*-demethylase activity was determined at indicated temperatures

mined using two different substrates at various temperatures. Surprisingly, the Arrhenius plot of the monooxygenase reaction showed nonlinear behavior (Fig. 3). The activation energy changed rather abruptly at approximately 20 °C in both activities measured with benzphetamine and *N*-methylaniline as substrates. On the other hand, the monooxygenase system reconstituted in liposomes of egg yolk phosphatidylcholine, which shows no phase transition and remains fluid in the temperature range studied, showed a linear Arrhenius plot (Fig. 4). The change in the activation energy was, therefore, clearly caused by the fluidity change of the liposomal membrane around the phase transition temperature. The change in the activation energy can be interpreted as the result of a

Table 1. The apparent dissociation constants of benzphetamine obtained for cytochrome P-450 in various states. The dissociation constants were determined from the substrate-induced spectral change of cytochrome P-450 with purified liposome-bound and microsome-bound cytochrome P-450 at 25°C

Preparation	$K_{apparent}$ (μM)
Purified	200
Liposome	25
Microsome	20

conformational change of cytochrome P-450 induced by the membrane fluidity change. Membrane fluidity, therefore, also plays an important role in maintaining a certain conformation of cytochrome P-450.

Membrane serves as a pool for the hydrophobic substrates of cytochrome P-450

The binding of substrate to cytochrome P-450 can be determined from the spectral change of cytochrome P-450 upon the binding of substrates. The apparent dissociation constant of benzphetamine was determined in the microsome-bound state, in the purified soluble state, and in the liposome-bound state. With purified soluble cytochrome P-450, the apparent dissociation constant determined was one order of magnitude larger than that in the microsome-bound state (Table 1). The incorporation of cytochrome P-450 into liposomal membranes restored the apparent high affinity binding of the substrate to cytochrome P-450. Since the addition of detergents or dilauroylphosphatidylcholine, which has a shorter fatty acid chain and does not form a bilayer at the concentrations used, did not change the apparent dissociation constant obtained with the purified enzyme (data not shown), the change in the substrate binding upon incorporation into liposomal membranes cannot be due to the conformational change of cytochrome P-450 induced by protein-lipid interactions, but was probably due to the condensation effect of the membrane, in which the rather hydrophobic substrates of cytochrome P-450 were dissolved at higher concentrations than in the water phase (Parry et al. 1976).

Discussion

The cytochrome P-450-dependent monooxygenase system resides in cellular membranes such as the endoplasmic reticulum and the nuclear envelope. The meaning of this specific localization in membrane fractions, however, has not yet been fully realized. Several advantages of the membrane localization are conceivable. Firstly, lipophilic substrates are mainly condensed into the hydrophobic phase of the phos-

pholipid bilayer membrane. Cytochrome P-450 catalyzes the first step of the detoxification cascade, which transforms hydrophobic substrates into hydrophilic water soluble metabolites. If the substrate binding site of the enzyme directly faces the hydrophobic inner side of the bilayer membrane, the enzyme can bind substrates directly from the membrane phase, and can utilize the condensation effect. The data presented in this work suggest that this is actually the case with cytochrome P-450. Microsome-bound and liposome-bound reconstituted cytochrome P-450 showed higher apparent affinity than the solubilized membrane-free enzyme. We have previously shown that the apparent binding constant of substrates is affected by the change in the partition coefficient of substrates between membrane and water phases (Taniguchi et al. 1984 b). These observations clearly show that the substrate binding site of cytochrome P-450 directly faces the inner phase of the membrane. This aspect seems especially useful for the microsomal monooxygenase system. Thus, cytochrome P-450 seems to have very broad substrate specificity, and the only common feature of the various substrates is hydrophobicity. In order to bind such a broad range of substrates efficiently, the condensation effect of lipophilic substrates into hydrophobic membranes is very useful.

Secondly, the interactions between two proteins can be more effective in two-dimensional membranes than in three-dimensional solutions. Cytochrome P-450 accepts electrons from NADPH-cytochrome P-450 reductase and cytochrome b_5 alternatively (Taniguchi et al. 1984 b), and effective interactions between these membrane-bound enzymes are necessary. The finding that cytochrome P-450 and other protein components of the monooxygenase system can diffuse easily on the plane of membranes (Taniguchi et al. 1979, 1984 b, 1987) also seems advantageous, since the electron transfer system proteins (the two flavoprotein reductases and cytochrome b_5) should interact not only with excess amounts of cytochrome P-450, which is known to exist in various isoenzymes, but also with other various terminal enzymes such as fatty acid desaturase. The amounts of each cytochrome P-450 isoenzyme, too, vary drastically depending on the treatment of the animals. The formation of stable complexes between the component proteins, therefore, is not adequate for such a versatile system. The membrane-bound system, in which component proteins are all membrane-bound and free to move and to interact on the two-dimensional plane of membranes, seems to be ideal for the monooxygenase system. In addition, membranes also seem necessary to keep the right orientation of proteins, since the reconstituted activity is found to be very low in the absence of lipids or detergents.

Thirdly, the activity of membrane-bound enzymes can be modulated by a change in the membrane fluidity. A change in the membrane fluidity directly affects the conformation of membrane-bound enzymes, and, in the case of the multienzyme system consisting of more than two membrane-bound proteins, the fluidity change affects the interaction between the component proteins. As for the cytochrome P-450-dependent monooxygenase system, the two mechanisms seem to be effective. The change in the membrane fluidity drastically affects the interaction between cytochrome P-450 and its reductase (Taniguchi et al. 1987). The two-dimensional concentration of the proteins, i.e., the lipid-to-protein ratio, also determines the effective activity of the total monooxygenase system. As we have reported in the present work, the membrane fluidity affects the conformation of cytochrome P-450, too. The NADPH-cytochrome P-450 reductase, on the other hand, seems not to be affected (Taniguchi et al. 1987). The rapid control of the monooxygenase activity through the fluidity change of microsomal membranes, therefore, might be possible in addition to the rapid control of the monooxygenase activity by the posttranslational modification of the protein components (Pyerin et al. 1984, 1987; Taniguchi and Pyerin 1987).

Finally, biological membranes act as a barrier between two different cellular compartments. As for the endoplasmic recticulum, this nature of membrane is utilized for various purposes such as calcium storage, and protein secretion. Does the barrier nature of biomembranes, then, play a role in the monooxygenase system? Such a possibility is suggested by the postulated luminal localization of UDP-glucuronyltransferase (Van Stapel and Blanckaert 1987). This so called phase-two enzyme catalyzes one of the conjugation reactions in which hydroxylated metabolites of the cytochrome P-450 system are conjugated with a large hydrophilic glucuronic acid. Like secretory proteins, metabolites are thus effectively separated from the cytosol, and easily secreted from the cell. However, this hypothesis, although intriguing, has not yet been substantiated. Detailed studies on the topological locations of the component proteins of the monooxygenase system and other phase-two enzymes will elucidate the role of the endoplasmic reticulum membrane in this respect.

In conclusion, biological membranes are actively involved in the process of membrane-bound enzyme-catalyzed activities (Fig. 5). The monooxygenase reaction involves the binding of substrates, the introduction of the first and second electrons from the reductase and cytochrome b_5, the binding of molecular oxygen, and the actual monooxygenation of the substrate. The endoplasmic reticulum membrane takes

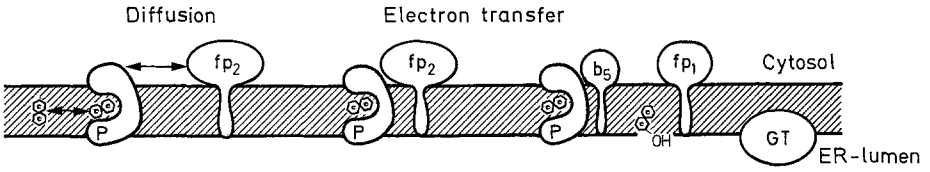

Fig. 5. Schematic presentation of the roles of endoplasmic reticulum membrane in the monooxygenase reaction. The membrane acts as a hydrophobic pool of substrates, and interaction between the component proteins is controlled by the membrane fluidity. The diffusion process of the proteins plays an important role in the monooxygenase system. The luminal localization of UDP-glucuronyltransferase is postulated. P, cytochrome P-450; fp_2, NADPH-cytochrome P-450 reductase; b_5, cytochrome b_5; fp_1, NADH-cytochrome b_5 reductase; GT, UDP-glucuronyl-transferase; ⬡, substrate; ⬡–OH, product

part in most of these phases of the monooxygenase reaction catalyzed by cytochrome P-450, and plays a decisive role in the detoxification and the activation of chemical carcinogens.

References

Bösterling B, Trudell JR, Trevor AJ, Bendix M (1982) Lipid-protein interactions as determinants of activation or inhibition by cytochrome b_5 of cytochrome P-450-mediated oxidations. J Biol Chem 257:4375–4380

French JS, Guengerich FP, Coon MJ (1980) Interactions of cytochrome P-450, NADPH-cytochrome P-450 reductase, phospholipid and substrate in the reconstituted liver microsomal enzyme system. J Biol Chem 255:4112–4119

Hashimoto-Yutsudo C, Imai Y, Sato R (1980) Multiple forms of cytochrome P-450 purified from liver microsomes of phenobarbital- and 3-methylcholanthrene preteated rabbits. II. Spectral properties. J Biochem 88:505–516

Iyanagi T, Mason HS (1973) Some properties of hepatic reduced nicotinamide adenine dinucleotide phosphate-cytochrome c reductase. Biochemistry 12:2297–2308

Lu AYH, Coon MJ (1968) Role of hemoprotein P-450 in fatty acid ω-hydroxylation in a soluble enzyme system from liver microsomes. J Biol Chem 243:1331–1332

Lu AYH, Junk KW, Coon MJ (1969) Resolution of the cytochrome P-450-containing ω-hydroxylation system of liver microsomes into three components. J Biol Chem 244:3714–3721

Lu AYH, Levin W, Kuntzman R (1974) Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds and endogenous substrates. VII. Stimulation of benzphetamine *N*-demethylation by lipid and detergent. Biochem Biophys Res Commun 60:266–272

Mihara K, Sato R (1976) Purification and properties of the intact form of NADH-cytochrome b_5 reductase from rabbit liver microsomes. J Biochem 78:1057–1073

Morgan ET, Coon MJ (1984) Effects of cytochrome b_5 on cytochrome P-450 catalyzed reactions: studies with manganese-substituted cytochrome b_5. Drug Metab Dispos 2:358–364

Nash T (1953) The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochem J 55:416–421

Ortiz de Montellano (ed) (1986) Cytochrome P-450, Structure, Mechanism and Biochemistry. Plenum Press, New York

Parry G, Palmer DN, Williams SJ (1976) Ligand partitioning into membranes: its significance in determining K_M and K_S values for cytochrome P-450 and other membrane bound receptors and enzymes. FEBS Lett 67:123–129

Pyerin W, Taniguchi H, Stier A, Oesch F, Wolf CR (1984) Phosphorylation of rabbit liver cytochrome P-450 LM_2 and its effect on monooxygenase activity. Biochem Biophys Res Commun 122:620–626

Pyerin W, Horn F, Taniguchi H (1987) Posttranslational modifications of the cytochrome P-450 monooxygenase system. J Cancer Res Clin Oncol 113:155–159

Schenkman JB, Kupfer D (eds) (1982) Hepatic cytochrome P-450 monooxygenase system. Pergamon Press, Oxford

Strittmatter P, Velick SF (1956) The isolation and properties of microsomal cytochrome. J Biol Chem 221:253–264

Strobel HW, Lu AYH, Heidema J, Coon MJ (1970) Phosphatidylcholine requirement in the enzymatic reduction of hemoprotein P-450 and in fatty acid, hydrocarbon, and drug hydroxylation. J Biol Chem 254:4851–4854

Taniguchi H, Pyerin W (1987) The effects of phosphatase on the components of the cytochrome P-450-dependent microsomal monooxygenase. Biochim Biophys Acta 912:295–302

Taniguchi H, Imai Y, Iyanagi T, Sato R (1979) Interaction between NADPH-cytochrome P-450 reductase and cytochrome P-450 in the membrane of phosphatidylcholine vesicles. Biochim Biophys Acta 550:341–356

Taniguchi H, Imai Y, Sato R (1980) Protein-protein and lipid-protein interactions in a reconstituted liver microsomal monooxygenase system. Coon MJ, Conney AH, Estabrook RW, Gelboin HV, Gilette JR, O'Brien PJ (eds) Microsomes, drug oxidations, and chemical carcinogenesis. Academic Press, New York, pp 537–540

Taniguchi H, Imai Y, Sato R (1984a) Substrate binding site of microsomal cytochrome P-450 directly faces membrane lipids. Biochim Biophys Res Commun 118:916–922

Taniguchi H, Imai Y, Sato R (1984b) Role of the electron transfer system in microsomal drug monooxygenase reaction catalyzed by cytochrome P-450. Arch Biochem Biophys 232:585–596

Taniguchi H, Imai Y, Sato R (1987) Protein-protein and lipid-protein interactions in a reconstituted cytochrome P-450-dependent microsomal monooxygenase. Biochemistry 26:7084–7090

Von Stapel F, Blanckaert N (1987) Endogenous esterification of bilirubin by liver microsomes. Evidence for an intramicrosomal pool of UDP-glucose and luminal orientation of bilirubin UDP-glycosyl-transferase. J Biol Chem 262:4616–4623

Received July 2, 1987/Accepted August 25, 1987