Recently, it was reported that the activity of rabbit P450 1A2 is markedly increased at elevated salt concentration (Yun, C-H., Song, M., Ahn, T., and Kim, H. (1996) J. Biol. Chem. 271, 31312–31316). The activity increase of P450 1A2 coincides with the raised \( \alpha \)-helix content and decreased \( \beta \)-sheet content. The presence of phospholipid magnified this effect. Here, possible structural change of rabbit P450 1A2 accompanying the phospholipid-induced increase in its enzyme activity was investigated by circular dichroism, fluorescence spectroscopy, and absorption spectroscopy. Studies with the reconstituted system supported by cumene hydroperoxide or NADPH showed that the P450 1A2 activities were found to be dependent on the head group and hydrocarbon chain length of phospholipid. Phosphatidylcholines having short hydrocarbon chains with a carbon number of 8–12 were very efficient for reconstitution of the P450-catalyzed reactions supported by both cumene hydroperoxide and NADPH. It was found that the phospholipid increased the \( \alpha \)-helix content and lowered the \( \beta \)-sheet content of P450. Intrinsic fluorescence intensity is also increased in the presence of phospholipid. The low spin iron configuration of P450 1A2 shifted toward the high spin configuration by most of the phospholipids in the endoplasmic reticulum. Some synthetic phospholipids having short hydrocarbon chains with a carbon number of 10–12 caused a shift in the spin equilibrium of P450 1A2 toward low spin. The effect of detergents on the activity and conformation of P450 1A2 was also studied. It was found that the addition of detergents to P450 1A2 solution increased the enzyme activity of P450 1A2. Detergents also increased the \( \alpha \)-helix content and lowered the \( \beta \)-sheet content of P450 1A2. Intrinsic fluorescence emissions also increased with the presence of detergents. Octyl glucoside and deoxycholate caused a shift toward high spin. On the other hand, cholate caused a shift toward low spin. It was found that the activity increase of rabbit P450 1A2 coincides with the conformational change including raised \( \alpha \)-helix content. It is proposed that the interaction with the phospholipid molecules surrounding P450 1A2 in the endoplasmic reticulum is important for a functional conformation of P450 1A2 in a monooxygenase system including NADPH-P450 reductase.

The microsomal monooxygenase metabolizes a variety of endogenous and xenobiotic compounds. This enzyme system includes cytochrome P450 (P450) enzymes, NADPH-P450 reductase, and phospholipids. Cytochrome \( b_5 \) and NADH-cytochrome \( b_5 \) reductase may also contribute to the electron flow (2). P450-dependent activities can be reconstituted by mixing P450, NADPH-P450 reductase, and phosphatidylcholine (PC) (3, 4). P450 and NADPH-P450 reductase seem to be distributed randomly on the plane of membranes, and they interact through lateral diffusion (5, 6). However, the organization of constituent proteins in phospholipid membranes and their mechanism of interaction are not fully understood yet. P450 and NADPH-P450 reductase have been reported to interact by forming a functional complex for the electron transfer (7). On the other hand, P450 is present in the membrane in large excess over the reductase, with the molar ratios ranging from 10:1 to 25:1, depending on treatment with inducers (8, 9). Since NADPH-P450 reductase is the limiting component in microsomes, different P450 enzymes must compete for the available reductases.

Phospholipid molecules in the immediate vicinity of P450 in rabbit liver microsomes have been known to be highly organized as compared with those in bulk membrane (10). The functional properties of many proteins in biological membranes, including the endoplasmic reticulum membranes, seem to be closely related to the microenvironment provided by membrane lipids (11, 12). It was proposed that the increase of P450 activities in a reconstituted system by the addition of phospholipid or detergent was mainly due to the increased interaction between P450 and NADPH-P450 reductase. It was also suggested that the interaction of phospholipid with P450 may be necessary for maintaining an active protein conformation and its ability to interact with NADPH-P450 reductase and necessary for efficient electron transfer (13). The P450 1A2 is one of P450 enzymes that is induced by the

### Conformational Change of Cytochrome P450 1A2 Induced by Phospholipids and Detergents*

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The microsomal monooxygenase metabolizes a variety of endogenous and xenobiotic compounds. This enzyme system includes cytochrome P450 (P450) enzymes, NADPH-P450 reductase, and phospholipids. Cytochrome \( b_5 \) and NADH-cytochrome \( b_5 \) reductase may also contribute to the electron flow (2). P450-dependent activities can be reconstituted by mixing P450, NADPH-P450 reductase, and phosphatidylcholine (PC) (3, 4). P450 and NADPH-P450 reductase seem to be distributed randomly on the plane of membranes, and they interact through lateral diffusion (5, 6). However, the organization of constituent proteins in phospholipid membranes and their mechanism of interaction are not fully understood yet. P450 and NADPH-P450 reductase have been reported to interact by forming a functional complex for the electron transfer (7). On the other hand, P450 is present in the membrane in large excess over the reductase, with the molar ratios ranging from 10:1 to 25:1, depending on treatment with inducers (8, 9). Since NADPH-P450 reductase is the limiting component in microsomes, different P450 enzymes must compete for the available reductases.

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1 The abbreviations used are: P450, cytochrome P450 (EC 1.14.14.1), also termed heme-thiolate protein P450 by the Enzyme Commission (1); PC, phosphatidylcholine(s); PE, phosphatidylethanolamine(s); DCPC, \( \alpha \)-1,2-dicaprylyloxyphosphatidylcholine (C8:0); DDPC, \( \alpha \)-1,2-didecanoyloxyphosphatidylcholine (C16:0); DUPC, \( \alpha \)-1,2-dioleoyloxyphosphatidylcholine (C18:0); DLPC, \( \alpha \)-1,2-dilauroyloxyphosphatidylcholine (C12:0), DMPC, \( \alpha \)-1,2-dimyristoyloxyphosphatidylcholine (C14:0); DPPC, \( \alpha \)-1,2-dipalmitoyloxyphosphatidylcholine (C16:0); DSPC, \( \alpha \)-1,2-distearyloxyphosphatidylcholine (C18:0); DAPC, \( \alpha \)-1,2-diarachidoyloxyphosphatidylcholine (C20:0); DBPC \( \alpha \)-1,2-dibenzyloxyphosphatidylcholine (C22:0); DLPE, \( \alpha \)-1,2-dilauroylphosphatidylethanolamine (C12:0); DMPE, \( \alpha \)-1,2-dimyristoyloxyphosphatidylethanolamine (C14:0); DPPPE, \( \alpha \)-1,2-dipalmitoyloxyphosphatidylethanolamine (C16:0); DSPPL, \( \alpha \)-1,2-distearyloxyphosphatidylethanolamine (C18:0); BBPE, bovine brain phosphatidylethanolamine; BOBE, bovine brain phosphatidylycholine; CL, bovine heart cardiolipin; lyso-PC, \( \alpha \)-lyso-phosphatidylcholine; CuOOH, cumene hydroperoxide; CHAPS, 3-[[(cholamidopropyl)dimethylammonio]-1-propanesulfonic acid.
Polycyclic aromatic hydrocarbon. It was demonstrated that the stimulated activity of P450 1A2 by the high concentration of salt accompanies structural change of P450 1A2 in the presence of phospholipid (14). It seems worthwhile, therefore, to study the structural changes of P450 1A2 induced by phospholipid in detail to elucidate the relationship between the structure and the activity. The catalytic reactions by cumene hydroperoxide (CuOOH) are studied to evaluate the role of the protein conformation in the catalytic function and to see how the environment of the heme is influenced by the conformational change induced by phospholipid and detergent. The relationship between phospholipid- or detergent-induced conformational changes and activities of the P450 is discussed. Here, the hydroperoxide system instead of NADPH-P450 reductase and NADPH is used to follow the change in the P450 1A2 conformation without a complicating effect of another protein, NADPH-P450 reductase.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Sodium cholate, sodium deoxycholate, CHAPS, octyl glucoside, CuOOH, and most of the phospholipids (DDPC, DPPC, DLPC, DMPC, DSPC, DAPC, DBPC, DLPE, DMPE, DPPE, DSPE, BBPE, BBPC, BBPS, PS, PA, PI, PG, CL, and lso-PC) were from Sigma. DCPC was obtained from Doosan Sardery Research Laboratories (Englewood Cliffs, NJ). 7-Ethoxyresorufin, Triton X-100, Triton X-102, and Tween 20 were obtained from Aldrich. 7-Ethoxyresorufin and resorufin were obtained from Molecular Probes, Inc. (Eugene, OR). Emulgen 911 and 913 were kindly provided by Dr. T. Shimada (Osaka Prefectural Institute of Public Health, Osaka, Japan). Other chemicals were of the highest grade commercially available.

**Lipid Vesicles**—The phospholipid vesicles or micelles were prepared by hydrating a dry films in 100 mM potassium phosphate buffer (pH 7.4), followed by sonication under nitrogen gas while cooling on ice. Titanium particles and any residual multilamellar structures were removed by centrifugation. The phospholipid concentration was determined as described previously (15). The vesicles were freshly prepared and added to the P450 solution just before the activity assay and the spectral studies. It has been shown that P450 1A2 can be incorporated into preformed phospholipid vesicles (16).

**Enzyme Preparation**—P450 1A2 was purified from liver microsomes of 5,6-benzoflavone-treated rabbits as described (17). The P450 1A2 was electrochemically homogeneous and had a specific P450 content of 17 nmol/mg protein. NADPH-P450 reductase was purified to an apparent homogeneity from phenobarbital-treated rabbits as described (18).

For the assay, two different approaches were taken to examine the effect of phospholipid and detergent on the P450 1A2 activity using the method described elsewhere with slight modification (14). For the NADPH-P450 reductase-supported reactions, 0.5 nmol of P450 1A2, 0.6 nmol of NADPH-P450 reductase, 15 μg of phospholipid or detergent, and an NADPH-generating system were mixed together. The volume of the reaction mixture in potassium phosphate buffer (100 mM, pH 7.4) was 0.5 ml. Reactions were initiated by adding 2 μl of either 7-ethoxyresorufin (to 50 μM) or 7-ethoxyresorufin (to 5 μM) as the substrates.

When the reaction was supported by CuOOH, each incubate contained 0.5 nmol of P450 1A2 and 15 μg of phospholipid or detergent in addition to the same concentrations of the substrates and phosphate buffer as described above for the NADPH-P450 reductase-supported reactions. The reaction was initiated by the addition of 2 μl of CuOOH (to 0.1 mM).

The reaction mixtures were incubated at 25 °C for 10 min, and the products were estimated fluorometrically as described elsewhere for the assay of 7-ethoxyresorufin O-deethylation (19) and 7-ethoxyresorufin O-deethylation (20).

Protein was assayed using a bicinchoninic acid procedure according to the manufacturer’s directions (Pierce). P450 concentrations were determined by Fe2+-CO versus Fe3+ difference spectroscopy (21).

**Spectroscopy**—All spectroscopic experiments were done in 100 mM potassium phosphate buffer (pH 7.4) at 25 °C using the methods described elsewhere with slight modification (14). Fluorescence intensity was measured on a Shimadzu RF-5301 PC spectrofluorometer (Shimadzu Corp., Tokyo) in a thermostated cuvette. Circular dichroic spectra were recorded on a Jasco J700 spectropolarimeter (Japan Spectroscopic, Tokyo) in a thermostated cuvette. Blanks (buffer with or without phospholipid/detergent) were routinely recorded and subtracted from the original spectra. Absorption spectra were recorded with a Perkin-Elmer Lambda 16 spectrophotometer (Norwalk, CT).

Stock solutions of P450 1A2 and NADPH-P450 reductase contained 20% glycerol, but the final glycerol concentrations in all experiments were kept below 0.2%, since glycerol affects the NADPH-P450 reductase-mediated reactions (22) and the protein structure (14, 23).

**RESULTS**

**Effects of Phospholipid and Detergent on the Catalytic Activities of P450 1A2**—To investigate the effect of the head group and acyl chain length of phospholipids on the enzyme activity of P450 1A2, we examined the P450 1A2-catalyzed reaction in the presence of different types of phospholipids. The enzymatic activity of P450 1A2 was quantified by measuring its ability to catalyze the O-deethylation of 7-ethoxyresorufin and 7-ethoxyresorufin. The activities toward both 7-ethoxyresorufin and 7-ethoxyresorufin, as determined in the presence of CuOOH in place of the reductase and NADPH, increased by 10–38% when BBPC or BBPE was added to the P450 1A2 solution (Table I). When lyso-PC was added, there were no changes in enzymatic activities. In the presence of negatively charged lipids, PS, PI, or PG, a substantial increase in the P450 1A2 activities (38–86%) was observed. PA and CL, on the other hand, inhibited P450 1A2-catalyzed reactions by 40–78%.

Although mixed PC with various acyl chain lengths has been known to be an essential component of the microsomal P450 monooxygenase system, DLPC, a synthetic phospholipid, has been widely used for the reconstituted system (3, 4). We investigated the effect of the acyl chain length of phospholipids on the P450 1A2 activities using PC with a chain carbon number of 8–22 (Table I). PC is the major phospholipid in the endoplasmic reticulum. DCPC, DPPC, DPC, and DLPC, having short hydrocarbon chains with a carbon number of 8–12, were very efficient for reconstitution of the CuOOH-supported reaction, and the activities increased by 50–124%. DMPC, DAPC, and DBPC having a chain carbon number of 14–22 were less efficient than PC with short hydrocarbon chains and showed the increase of activities by 32–48%. We also checked the chain length effect of PE, another major phospholipid in the endoplasmic reticulum. The effect of hydrocarbon chain length of PE is similar to that of PC. These results indicate that the chain length of both PC and PE exerts an effect on the P450 1A2-catalyzed reaction.

When the reconstituted system contained P450 1A2, NADPH-reductase, and NADPH, it was observed that the activities were also dependent upon the type of phospholipids (Table I). In the presence of BBPC, there was a 24–32% reduction in the activities of P450 1A2. When BBPE was added, there was no change in the activities. The addition of the negatively charged phospholipids to P450 1A2 solution caused the 1.4–3.1-fold increase in the activities. PI among them was the most efficient to reconstitute the P450 1A2-catalyzed reactions. These results indicate that certain anionic phospholipids may be important for the reconstitution. The effect of hydrocarbon chain length of PC on the P450 1A2 activities was also shown. When the synthetic PC with a chain carbon number of 8–12 was added to the P450 1A2 solution, the activities increased 1.6–4.2-fold. DPPC was the most efficient at reconstituting the P450 1A2 activities. The phospholipids with a chain carbon number of 14–22 were less efficient. Some of them inhibited up to 32% of the P450 1A2 activities. It is possible that an inefficient transfer of electrons from NADPH to P450 1A2 in the presence of PC vesicles with long hydrocarbon chains may cause the decreased catalytic activities.

We also investigated the effect of detergents on the P450 1A2 activities, because several detergents have been used for the P450 purification procedure and for the P450 reconstitution system (24, 25). The stimulatory effect of detergents with the exception of deoxycholate was routinely observed and provided...
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The experimental protocols were set up as outlined in the general procedure described under “Experimental Procedures” for 7-ethoxycoumarin O-deethylation and 7-ethoxycoumarin O-deethylation. Rates are shown (means of duplicate experiments) for the reconstituted P450 1A2 system supported by NADPH-P450 reductase or CuOOH set up in the presence of the indicated phospholipid.

| Phospholipid | CuOOH  | NADPH CuOOH | NADPH  |
|--------------|--------|-------------|--------|
| None         | 0.136  | 0.120       | 0.080  | 0.182  |
| BBPC         | 0.149  | 0.082       | 0.101  | 0.138  |
| DCPC         | 0.211  | 0.295       | 0.152  | 0.511  |
| DDPC         | 0.279  | 0.508       | 0.179  | 0.706  |
| DUPC         | 0.257  | 0.373       | 0.163  | 0.599  |
| DLPC         | 0.204  | 0.225       | 0.131  | 0.297  |
| DMPC         | 0.185  | 0.103       | 0.106  | 0.173  |
| DPPC         | 0.189  | 0.103       | 0.111  | 0.155  |
| DSPC         | 0.188  | 0.108       | 0.110  | 0.146  |
| DAPC         | 0.184  | 0.082       | 0.115  | 0.142  |
| DBPC         | 0.185  | 0.096       | 0.118  | 0.144  |
| Lyso-PC      | 0.129  | 0.239       | 0.083  | 0.495  |
| BBPE         | 0.163  | 0.122       | 0.110  | 0.186  |
| DLFPE        | 0.188  | 0.101       | 0.096  | 0.173  |
| DMPPE        | 0.165  | 0.088       | 0.106  | 0.138  |
| DPPE         | 0.189  | 0.130       | 0.101  | 0.129  |
| DSPE         | 0.182  | 0.113       | 0.110  | 0.164  |
| FS           | 0.231  | 0.179       | 0.149  | 0.260  |
| PI           | 0.227  | 0.371       | 0.112  | 0.479  |
| PA           | 0.072  | 0.304       | 0.048  | 0.411  |
| PG           | 0.190  | 0.178       | 0.110  | 0.264  |
| CL           | 0.041  | 0.212       | 0.018  | 0.351  |

Activity

nmol product/min/nmol P450

about 1.2–1.9-fold enhancement depending on the head group type and chain length when CuOOH was present (Table II). With the reconstitution system containing P450 1A2, NADPH-P450 reductase, and NADPH in addition to detergent, it was observed that the activities increased up to 2.4-fold. In the case of cholate and octyl glucoside, there were no apparent changes of turnover numbers. These results may be due to the differential effect of each detergent on the conformation of P450 1A2 itself and also on the interaction of P450 1A2 with NADPH-P450 reductase.

CD Spectra—It was reported that the absorption spectrum of P450 1A2 is sensitive to its own concentration and to the ionic strength of the solution (26). When the concentration of P450 1A2 was 1 μM and the concentration of potassium phosphate buffer (pH 7.4) was 100 mM, the spectrum was almost entirely originated from the absorption by the oxidized P450 1A2 in the presence of phospholipid or detergent without light scattering due to aggregation. Therefore, all of the spectroscopic studies were performed under these conditions.

The effect of phospholipid and detergent on the secondary structure of the P450 1A2 was studied by CD in the far UV region. Fig. 1A shows the CD spectra of P450 1A2 in 100 mM potassium phosphate buffer (pH 7.4) in the presence of various types of PC and PE, the major phospholipids in the endoplasmic reticulum (27). The CD spectra were curve-fitted by the least squares method into the reference spectra obtained from five proteins: myoglobin, lysozyme, ribonuclease A, papain, and lactate dehydrogenase (28). Analysis of the CD spectrum for P450 1A2 in the absence of phospholipid and detergent yielded 32% α-helix, 24% β-sheet, 23% β-turns, and 21% random structure (Table III). When various types of PC and PE, which have been known to have a high affinity on P450, were added, the α-helix content generally increased, while the amounts of β-sheet, β-turns, and random structure decreased (Fig. 1A and Table III). The CD spectra of P450 1A2, obtained in the presence of synthetic PC with a chain carbon number of 8–12, showed an increase of α-helix content by 11–16% (Fig. 1A and Table III). However, the effect of PC and PE with a chain carbon number of 8–12 was smaller than that of these phospholipids with a chain carbon number of 14–22. Overall, PE seems to be more effective in increasing the α-helix content of P450 1A2 than PC. Lyso-PC was also efficient at increasing the α-helix content and decreasing β-sheet and random structures of P450 1A2.

To investigate the lipid specificity for the induction of secondary structure of the P450 1A2, a comparison was also made between zwitterionic (PC and PE) and anionic phospholipids (PS, PI, PA, PG, and CL). Fig. 1B shows the CD spectra of P450 1A2 in 100 mM potassium phosphate buffer in the presence of different anionic phospholipids. For both PS and PA, the spectra with two minima at 208 and 222 nm were observed, and the percentage of α-helix was calculated to be 42 and 48%, respectively (Table III). Although PS induced a substantial increase in α-helical structure (Fig. 1B, Table III), it is less than what PC, PE, and PA brought about. The observed difference in α-helical content of the P450 1A2 upon interaction with different phospholipids is most probably due to the head group specificity of the interaction. However, upon interaction with other negatively charged phospholipids, PG, CL, and PI, a decrease in CD spectral intensity of P450 1A2 was shown. It was not possible in these cases to curve fit the CD spectra to estimate the secondary structures using the reference spectra (28). It is possible that the P450 1A2 aggregates in the presence of PG, CL, or PI with an apparent molecular weight larger than its basic oligomeric structure of P450 1A2 (29).

Fig. 1C shows the effect of detergents on the CD spectra of P450 1A2. All of the detergents showed an effect on the CD spectra of P450 1A2 similar to that of most phospholipids. All of the detergents increased the α-helix content by 14–20% and lowered β-sheet content by 2–11% (Table III).

Intrinsic Fluorescence—The intrinsic fluorescence of P450 1A2 reflects mainly the individual environments of its intrinsic fluorophores, Trp, which are spaced evenly throughout its sequence (30). The intrinsic fluorescence spectra of P450 1A2 obtained in the presence of various types of phospholipids are shown in Fig. 2. The fluorescence intensity increased in the
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Table II
Effect of detergents on the catalytic activities of P450 1A2

| Detergent      | 7-Ethoxycoumarin O-deethylation | 7-Ethoxyresorufin O-deethylation |
|----------------|----------------------------------|----------------------------------|
|                | CuOOH NADPH                      | CuOOH NADPH                      |
|                | Activity (nmoles product/min/nmol P450) | Activity (nmoles product/min/nmol P450) |
| None           | 0.136                            | 0.137                            |
| Deoxycholate   | 0.136                            | 0.080                            |
| Cholate        | 0.162                            | 0.131                            |
| Tween 20       | 0.218                            | 0.254                            |
| Lubrol WX      | 0.212                            | 0.233                            |
| CHAPS          | 0.233                            | 0.146                            |
| Octyl glucoside| 0.169                            | 0.115                            |
| Emulgen 911    | 0.203                            | 0.277                            |
| Emulgen 913    | 0.211                            | 0.242                            |
| Triton X-100   | 0.228                            | 0.245                            |
| Triton X-102   | 0.236                            | 0.210                            |

The detergents also increased the fluorescence intensity of P450 1A2 at $\lambda_{max}$ = 330 nm (Fig. 2C).

These results suggest that the stimulation of catalytic activity by phospholipid and detergent involves the increased secondary structure as well as the change of the overall conformation of P450 1A2.

Spin State of P450 1A2—The P450 1A2 exists as a mixture of high and low spin iron forms with corresponding absorption peaks at 395 and 414 nm, respectively. Fig. 3 gives the absorption spectra of P450 1A2 complexed with different types of phospholipids (Fig. 3, A and B) and detergents (Fig. 3C). The spectra obtained in the absence of phospholipids and detergents are also given for comparison. When BBPC, BBPE, or lyso-PC was present, the absorption spectra shifted toward the high spin state in the spin equilibrium (Fig. 3, A and D). But the spectra shifted toward the low spin in the presence of synthetic PC or PE with a chain carbon number of 10–12. When the negatively charged phospholipids, PS, PI, PG, and CL, were added to the P450 1A2 solution, the absorption spectra shifted toward the high spin state (Fig. 3, B and E). Fig. 3, C, and F, show the effects of detergents on the P450 1A2 spin state. Deoxycholate and octyl glucoside shifted the equilibrium toward the high spin state, but cholate shifted the equilibrium toward the low spin state. In contrast, no apparent changes were noted in the spin equilibrium in the case of CHAPS, Tween 20, and Lubrol WX.

We could not obtain the spectra in the presence of Triton X-100, Triton X-102, Emulgen 911, or Emulgen 913 because of the high light scattering of the detergents (data not shown).

Discussion

The importance of the interaction of P450 with phospholipids in microsomes was recognized early on (3, 4). PC has been known to be an essential component in a reconstituted P450 monooxygenase system. The effect of phospholipids on P450 activities seems to be the result of their influences on the interaction between P450 and NADPH-P450 reductase for the electron transfer (31, 32). Although synthetic DLPC and microsomal PC with varying lengths of acyl chain have been known to be very effective in the reconstituted P450 monooxygenase system (4), the effect of phospholipid on the P450 conformation without a complicating effect of NADPH-P450 reductase is not fully understood yet. Although some attempts were made to see the effect of phospholipid on the P450 conformation (33–35), these were apparently overshadowed by glycerol, which is usually used to stabilize the P450. The addition of glycerol to the P450 1A2 solution induced an appreciable increase in the $\alpha$-helix and decrease in the $\beta$-sheet content (14).

![Fig. 1. Effect of phospholipid and detergent on the circular dichroism spectra of P450 1A2. CD spectra of 1 $\mu$m P450 1A2 in 100 mM potassium phosphate buffer, pH 7.4, were recorded in the presence of various types of PC and PE (A), negatively charged phospholipids (B), or detergents (C). The final concentration of the phospholipid or detergent was 30 $\mu$g/ml. P450 1A2 was incubated with phospholipid or detergent for 5 min at 25 °C prior to the measurement of CD spectra.](image)
It was reported that P450 1A2-catalyzed reactions in the detergent system proceeded at a rate more than twice that in the micellar DLPC system (36). It was also suggested that the negative charge of the membrane is important for the interaction of P450 with NADPH-P450 reductase. It was also reported that some typical P450 substrates, inhibitors, cytochrome \( b_5 \), or high salts can cause a shift in the spin equilibrium of P450 1A2 toward high spin (24, 39, 40). It has also been reported that detergents, several P450 substrates, or alcohols influence the conversion of rabbit and rat P450 1A2 from a high to low spin iron configuration (26, 41, 42). Interestingly, the effect of most of the phospholipids, cholate and deoxycholate were found to be rather ineffective. It was suggested that the role of phospholipid is physical rather than chemical and that both DLPC and certain detergents form micelles of appropriate dimensions to facilitate interaction between P450 and NADPH-P450 reductase. It was also reported that the negative charge of the membrane is important for the catalytic activity of P450 2B4 (33).

Recently, it has been shown that some typical P450 substrates, inhibitors, cytochrome \( b_5 \), or high salts can cause a shift in the spin equilibrium of P450 1A2 toward high spin (24, 39, 40). It has also been reported that detergents, several P450 substrates, or alcohols influence the conversion of rabbit and rat P450 1A2 from a high to low spin iron configuration (26, 41–43). Interestingly, the effect of most of the phospholipids,
except the PC with a small chain carbon number of 10–12, on P450 1A2 is very similar to their effect on P450 2B4. The effect includes the configurational change toward the high spin state (33). It has been reported that there is no correlation between the spin state and the catalytic activity of the rat P450s in reconstituted systems and that not all of the P450s exhibited a relationship between the spin state and reduction potential (42).

In the present study, we have investigated the possible correlation between the increased enzyme activity and the conformational change of the P450 1A2 when the protein interacts with various types of phospholipids or detergents. The conformation of P450 1A2 should be highly dependent on the presence of phospholipid and detergent. The results clearly show that all of the phospholipids and detergents tested here can increase the α-helix content and lower the β-sheet content of P450 1A2. Intrinsinc fluorescence emissions also increased with the presence of all of the phospholipids (except for PS) and detergents. These phospholipid- or detergent-induced conformational change coincided with elevated activity of P450 1A2 in addition to the stimulated interaction of the P450, allowing coupling with a reductase. The results also clearly show that all of the phospholipids and detergents tested here can increase the α-helix content and lower the β-sheet content of P450 1A2. Intrinsinc fluorescence emissions also increased with the presence of all of the phospholipids (except for PS) and detergents. These phospholipid- or detergent-induced conformational change coincided with elevated activity of P450 1A2-catalyzed reactions. These structural studies revealed a strong interaction of the P450 1A2 with phospholipid and detergent. It was impossible to determine whether the high α-helix content of P450 1A2 induced by phospholipid and detergent was the cause of the increased catalytic activity or if the high α-helix contents were simply parallel phospholipid- or detergent-induced phenomena without direct connection. Since the P450 is in close contact with phospholipids in the endoplasmic reticulum, the conformation of P450 1A2 induced by phospholipid may actually be the physiologically active form, which has high affinity for the NADPH-P450 reductase. The stimulation of the activity by phospholipid is consistent with the conformational change of P450 1A2 in addition to the stimulated interaction between P450 and NADPH-P450 reductase. Changes in the lipid microenvironment are likely to control how one of many P450s in the endoplasmic reticulum couples with a reductase present in relatively small amounts. It is also possible that the binding of a specific substrate alters the α-helix:β-sheet ratio of P450, allowing coupling with a reductase.

The picture now emerging from this and previous studies (14) on the interaction of the P450 1A2 and phospholipid is that the P450 1A2 binds preferentially to phospholipids present in the membrane. The P450 1A2 with high α-helix content and low β-sheet content appears to have a high affinity to NADPH-P450 reductase. The conformation of P450 1A2 should be important for the enzymatic activity and the interaction with NADPH-P450 reductase for an efficient electron transfer.

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