MODELING THE STRUCTURE OF THE FATTY ACYL CHAIN REGION OF CARDIOLIPIN*

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Cardiolipin has been shown to be the most effective activator of cholesterol side chain cleavage activity of cytochrome P450SCC, and evidence has been provided for a lipid effector site on the enzyme. Results suggested the headgroup of cardiolipin as major determinant of lipid interaction with P450SCC (Lambeth, J. D. (1981) J. Biol. Chem. 256, 4757-4762). The role of unsaturation is contradictory and open to question (Igarashi, Y. and Kimura, T. (1986) Biochemistry 25, 6461-6466). We synthesized phosphatidylcholines with fully saturated branched fatty acyl chains substituted in the 2-positions of the main chains and studied the influence of these lipids on the activity and other properties of P450SCC in vesicle-reconstituted systems. These saturated branched lipids, with regard to the fatty acyl moiety in molecular shape similar to cardiolipin but with the headgroup of phosphatidylcholines retained, showed a stimulatory efficiency higher than any other phospholipid and at least comparable to cardiolipin. Activation is sensitive to the acyl chain structure and composition. Results suggest that the shape of the molecule at least partially plays an important role in the process of stimulation of the activity of P450SCC. Because binding of cholesterol was increased by the branched lipids monitored optically by the fraction of P450SCC in the high spin form, it was concluded that these lipids, like cardiolipin and other lipids, exert their effects by regulating the binding of cholesterol to P450SCC. These data suggest that polymorphic lipids such as branched phosphatidylcholines and cardiolipin might influence P450SCC function by maintenance of the membrane curvature at a value optimal for activity.

Cytochrome P450SCC (P450SCC), located at the matrix side of the inner mitochondrial membrane in cells of the adrenal cortex and other steroid-producing tissues catalyzes the side chain cleavage (SCC) of cholesterol to yield pregnenolone, the common precursor of all steroid hormones. Cholesterol SCC activity of P450SCC can be reconstituted in phospholipid vesicles whereby the activity was found to be dependent on lipid constituents used, the method of reconstitution, and the size of the vesicles (for a review see Ref. 1, and citations therein). P450SCC systems appear to exhibit specific lipid-protein interactions. It was found that some lipids exert a stimulatory effect by increasing the interaction between cholesterol and P450SCC. For instance, cardiolipin (to date the most potent activator lipid) binds to the enzyme in a 1:1 complex, thereby activating the enzyme by enhancing the binding of cholesterol (2).

The major determinant of the CL specificity is the headgroup (3), but the fatty acyl chain composition also has large effects on activity (4, 5). In general, activity correlates with the degree of unsaturation, but somehow contradictory observations were reported with certain PCs. Saturated phospholipids exhibited an inhibition for steroidogenic activity, and a particularly important role has been attributed to certain polyunsaturated configurations, e.g. to the adrenoyl (C22:4) group. Taken together, there is no doubt that both head and fatty acid groups attribute to the stimulation, yet the relative importance of both groups and perhaps other previously unconsidered structural properties of the phospholipid molecules remain to be solved.

Cardiolipin, while poor in (C22:4), appears exceptional in its stimulatory efficiency. Moreover, being a potent activator of SCC activity, it proved a potent inhibitor of another mitochondrial P450, P45011b (6). With regard to the molecular structure CL sharply differs from other phospholipids of the inner mitochondrial membrane, both in the polar and hydrophobic region of the molecule. Three residues of glycerol and two phosphate groups are included in the CL polar group, resulting in a double negative net charge, and the hydrophobic area is formed by means of four fatty acid chains (see Fig. 1). Mitochondrial CLs represent a heterogeneous mixture with regard to the fatty acid composition. For instance, bovine heart CL has fatty acid chains with 18 C-atoms as most abundant component (C18:0, C18:1, C18:2, and C18:3, comprising nearly 80% of total content) followed by (C20:0) and (C20:3) ones. CL from eukaryotic cells are characterized by a relative high degree of unsaturation (4).

The present paper is aimed at the elucidation of the possible importance of such a lipid configuration for the process of cholesterol side chain cleavage activity of P450SCC. For this purpose, the α-branched phosphatidylcholines 1,2-di-(2'-hexyl-decanoyl)-sn-glycero-3-phosphocholine (PC(10,6)) and 1,2-di-(2'-octyl-decanoyl)-sn-glycero-phosphocholine (PC(12,8)) have been synthesized and used for reconstitution of the SCC activity of P450SCC using various phosphatidylcholine vesicle
systems. Synthesis and some properties of these unusual lipids have been described previously (7). We proceed from the fact that branching of fatty acid residues at the second carbon nucleus (2-position) makes the structure of the hydrophobic region of the synthesized PC similar to the structure of CL, while retaining the differences at the level of polar headgroup. To facilitate comparison with the aforementioned studies using CL as activator lipid, we studied the influence of the branched 1,2-diacyl PCs on the enzymatic activity and other properties of P450SCC in vesicles made from mixtures of these branched PCs with DMPC and DOPC, respectively. The present study provides evidence that 1,2-diacyl PCs with fatty acid chains substituted at the 2-position of the main fatty acyl chains are more effective stimulators of the SCC activity of P450SCC than other activator PCs, and at least comparable to or even more effective than CL. Moreover, our results suggest the potential usefulness of P450SCC incorporated in vesicle membranes containing branched diacyl PCs as model system to investigate in a more systematic way, i.e. with regard to the number, length, and degree of unsaturation of the fatty acid group (without changing the headgroup of the lipid) resultant effects on the activity and other functions of enzymes bound to biological membranes.

**EXPERIMENTAL PROCEDURES**

Materials—[14C]Cholesterol was from Amersham. DOPC and DMPC were from Sigma, and bovine heart cardiolipin, cholesterol, and glucose oxidase from Serva (Germany). Catalase was from Merck (Darmstadt, Germany) at 150-watt power setting (30-min incubation). The resulting products were separated by gel chromatography using Sepharose 4B or Sephacryl S-1000 (Pharmacia, Sweden) (not shown).

Enzymatic Activity Assay—Activity of P450SCC was determined as side chain cleavage activity of cholesterol to produce pregnenolone addition of 0.25 μM P450SCC and 7 mM NADPH to standard buffer were incubated at 37°C for 10 min with vesicles consisting of 400 μM phosphatidolipid with 6 mol% cholesterol including [14C]cholesterol in a total volume of 0.5 ml. 0.25 μM AR was added, and the reaction was initiated by addition of 2.5 mM NADPH (to final concentration of 250 μM). After 5 min the reaction was terminated by addition of 0.1 ml of 0.5 M HCl. The residual substrate and the product were extracted with 2 × 2 ml of methylene chloride, and pregnenolone was separated from unreacted cholesterol by thin layer chromatography on silica gel (0.25 mm, 2 × 20 cm; Merck, Darmstadt, Germany) using a solvent mixture of n-hexane/petroleum ether/acetic acid (15:15:1, v/v/v). Analysis was done by counting the 14C radioactivity of cholesterol and pregnenolone using a Linear Analyzer LB284 (Berthold, Wildbad, Germany). Each analysis was done three times to ensure reproducibility within a standard error of about less than 7%.

Stopped-flow Kinetics of Reduction of P450SCC—Stopped-flow experiments to monitor the transfer of the first electron from AD to P450SCC were conducted at 21°C using a computer-controlled sequential stopped-flow ASVD Spectrofluorimeter DX-17 MV (Applied Photonics, Leatherhead, United Kingdom). For stopped-flow measurements, solution A in syringe A contained 1 μM P450SCC, 1200 μM phosphatidolipid(s), 6 mol% cholesterol in standard buffer. Syringe B contained 1 μM AR, 15 μM AD, and 2.5 mM NADPH in the same buffer. An oxygen-consuming system consisting of 50 units/ml glucose oxidase, 10000 units/ml catalase, and 100 μM glucose was added to both mixtures, which were then mixed with CO for 10 min at room temperature just before the measurement. Reduction of P450SCC was followed by monitoring the absorbance increase at 450 nm caused by the reduced P450SCC-CO complex. Rate constants and the extent of reduction were obtained by exponential fits of the experimental data using the software provided with the fluorimeter.

Analytical Methods—The concentration of P450SCC was determined from reduced CO minus reduced difference spectra using a difference extinction coefficient of 91 mM⁻¹ cm⁻¹ (A₂₅₀ – A₄₅₀) according to Ref. 15. The concentration of AR and AD were determined using extinction coefficients of 10.9 mM⁻¹ cm⁻¹ at 450 nm and 11 mM⁻¹ cm⁻¹ at 415 nm, respectively (16). Cholesterol was quantitated using 14C-labeled cholesterol. Absorption spectra were recorded at room temperature on a spectrophotometer UV2101 PC (Shimadzu, Japan).

Phospholipid phosphorus was determined by the method of Ames and Dubin (17). The ashing was performed according to the method of Hess and Derr (18). Briefly, samples containing lipoprotein (200 μl) were dried down in glass tubes and 30 μl of 10% H₂SO₄ and 90 μl of 60% perchloric acid were added. The tubes were heated at 130°C until the samples stopped fuming. Water (0.9 ml) was added, followed by the addition of 21 ml of 0.42 M ammonium molybdate × 4H₂O in 1 ml H₂SO₄,10% ascorbic acid (6.1 M mixture). The solutions were incubated at 45°C for 30 min, and the absorbance at 660 nm was determined. A standard curve for calibration was constructed by using inorganic phosphorus in the range of 0.1–10 mg.

**RESULTS**

Effect of Branched 1,2-Diacyl PCs on the Catalytic Activity of P450SCC in DMPC Vesicles—Fig. 1 shows the chemical structures of the 1,2-diacyl-sn-glycerol-3-phosphocholines containing hexyl and octyl substituents in the 2-position of their decanoyl and dodecanoyl fatty acid chains, respectively (branched 1,2-diacyl PCs), used in the present studies. For comparison and discussion of the results the molecular structure of CL was included in the scheme, too. Mitochondrial CL represents a heterogeneous mixture with regard to the fatty acyl chain composition; here we show a CL molecule with two 18:2 acyl chains, which compromise the most abundant fatty acid component of bovine heart mitochondrial CL (4).

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According to their effects on the catalytic activity of P450SCC, lipids have been divided into two groups: lipids that activate the SCC activity of P450SCC (activator lipids) and nonactivator lipids (5). PCs containing saturated fatty acid chains (in particular DMPC) belong to the nonactivator lipids and have been often used as "host lipids" in the construction of proteoliposomes to study the influence of activator lipids on the rate of side chain cleavage by P450SCC (1, 19, 20). To facilitate direct comparison of our results with those mentioned, we studied PC vesicles made from mixtures of activator phospholipids (the branched 1,2-diacyl PCs) and a nonactivator lipid (DMPC). In all experiments the cholesterol concentration of the vesicle membranes constituted 6 mol% of total phospholipid, which approximately corresponds to its content in the membrane of mitochondria (21).

Fig. 2 shows the activity of P450SCC in vesicle preparations containing pure DMPC and mixtures of DMPC with either PC(10,6) or PC(12,8), respectively. The results demonstrate the effect of varying the membrane content of the branched 1,2-diacyl PC. In each case the total phospholipid content remained the same. To facilitate direct comparison with previous experimental data, results were normalized to 100% maximum activity corresponding to the region in the activity plot where the activity reaches a plateau. Note that the 100% value corresponds to 3.7 nmol of PG/min/mmol of P450SCC for PC(10,6) and 3.9 nmol of PG/min/mmol of P450SCC for PC(12,8), respectively. As can be seen from Fig. 2, inclusion of the branched PCs in the DMPC vesicles results in a more than 6-fold enhancement of the SCC activity of P450SCC. As the ratio of activator to nonactivator lipid is increased, activity increases strongly until it reaches a plateau value. It can clearly be seen that the activity curves have a sigmoidal shape, in contrast to results obtained with CL as activator lipid using the same vesicle system (2). Comparing the two branched PCs, we see that the activity is sensitive to the acyl chain structure. As the length of the acyl chains and the branched chains increase, a smaller amount of PC(12,8) as compared to PC(10,6) is required for reaching the limiting (plateau) value.

The activator capability of PC(10,6) and PC(12,8) can be quantitatively compared to each other and to results obtained earlier by other groups using their $K_{1/2}$ values for half-maximum activation. $K_{1/2}$ values are not equal for both 1,2-diacyl PCs. On a weight basis activation by the branched PC with the shorter acyl chain, the half-maximal value is shifted toward a higher content of the activator lipid. Table I summarizes the values obtained; stimulation of activity is half-maximal when about 12.5 (13.9) weight % of the membrane phospholipid is branched 1,2-diacyl PC. Taking into account the differences in the molecular weights of the both branched PCs, the observed chain dependence of the activating efficiency for the both branched PCs is even more significant; 10.6 mol% of PC(12,8) are required to half-maximum activation, compared to 13.4 mol% for PC(10,6). Compared to other activator lipids, the branched PCs used in the present study are even more effective stimulators of the SCC activity of P450SCC; moreover, they show stimulation even higher compared to CL from beef heart and comparable with the activation by CL from bacteria reported by Lambeth (1981). To study the chain dependence in greater detail, it would be interesting to use branched PCs with even longer acyl chain substituents in the 2-position; such experiments are now under way.

Effect of Branched 1,2-Diacyl PCs on the Catalytic Activity, the Rate of Reduction, and the Optical Properties of P450SCC in Vesicles Consisting of Mixtures of DOPC and PC(10,6) or PC(12,8)—Recently, for investigation of various aspects of

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$K_{1/2}$ values are expressed as the weight ratio of activator lipid (branched 1,2-diacyl-PC) to total phospholipid (including cholesterol) required to half-maximum stimulation of the SCC activity, where the maximal velocity was taken in our case as the rate obtained from the plateau value extrapolated to infinite activator lipid concentration.
P450SCC-lipid interaction, vesicles made on the basis of DOPC were often used; applying these a rather high rate of enzymatic activity as compared to DMPC could be observed (1, 19, 22). Thus, it may be of interest to see to what extent α-branched PCs are capable of exerting their influence on the various properties of P450SCC reconstituted into vesicles from DOPC. Figs. 3 and 4 show the influence of the branched PCs on the properties of P450SCC, namely enzymatic activity, absorption spectrum, and kinetics of reduction of P450SCC by NADPH via its physiological electron transfer partners AR and AD. In spite of the fact that, according to the classification in Ref. 5, DOPC also belongs to the activator lipids, addition of 33 weight % PC(10, 6) and PC(12, 8) to DOPC vesicles results in approximately 2-fold enhancement of the cholesterol SCC reaction rate, i.e. PG formation (Fig. 3). Thus, comparing these results with previous ones obtained by other groups regarding the stimulation of SCC activity of P450SCC in DOPC, α-branched PCs used in our study manifest themselves as effective activators close to CL, as well as to another negatively charged lipid, namely phosphorylcholine (20).

The kinetics of reduction of P450SCC in DOPC vesicles and its mixtures with PC(10, 6) and PC(12, 8), respectively, was measured under conditions in which reduced AD, together with its reducing equivalents and AR, were mixed with P450SCC vesicles as described under “Experimental Procedures.” In all systems studied the reaction could be characterized by two phases, a fast and a slow phase, respectively. The amount of P450SCC reduced within the fast phase of the reaction accounts to about 52–57% and scarcely depended on the lipid composition. The values of the rate of fast phase are shown in Fig. 3, together with the corresponding enzymatic activities. Obviously, inclusion of the α-branched PCs in the membrane of DOPC vesicles is accompanied by practically the same increase of both parameters. The rate constant of the slow phase was equal to 0.66, 0.78, and 0.80 s⁻¹ for vesicles made from DOPC, DOPC/PC(10, 6), and DOPC/PC(12, 8), respectively.

Pember et al. (3) proved that the stimulation of the SCC activity of P450SCC by various PCs and CL is correlated with the fraction of P450SCC in the high spin state, reflecting a stronger binding of the substrate cholesterol to the enzyme. Based on absorption spectra, we found that the branched 1,2-diacyl PCs showed a similar effect (Fig. 4). In (pure) DOPC vesicles P450SCC was only 30% high spin; on the other hand, the high spin content was enhanced to about 84% and 82% when the vesicles contained 33 weight % PC(10,6) and PC(12,8), respectively. The increased high spin forms of P450 were observed only in vesicles containing (equally) 6 mol% cholesterol. Measurements were made with water in the reference cuvette. Thus, although both cholesterol and the total lipid content were kept equally, in each case the absorbance was different, probably caused by differences in the average size and degree of aggregation of the vesicles in the various vesicle suspensions. In conclusion, the optical spectra indicate that the branched PCs probably realize their stimulatory role

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TABLE I

| Activator lipid | Cholesterol / lipid | K_L2 |
|-----------------|---------------------|------|
|                 | (mol/mol)          | % (by weight) |
| DOPC            | 0.05               | 85*  |
| CL (b.h.)       | 0.02               | 19.4*|
| CL (bac.)       | 0.02               | 13.8*|
| PC(10,6)        | 0.06               | 13.9 |
| PC(12,8)        | 0.06               | 12.5 |

* Data are taken from Lambeth (1981) for beef heart (b.h.) and bacterial (bac.) cardiolipin.

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**Fig. 3.** Effect of branched 1,2-diacyl phosphatidylcholines on the SCC activity and the rate of reduction of P450SCC by NADPH in DOPC vesicles. In each case the vesicles contained 6 mol% cholesterol and the same amount of total phospholipid. P450SCC was reconstituted into the DOPC vesicles (see "Experimental Procedures") containing either 0% (left bar), 33 weight % PC(10,6) (middle), or 33 weight % PC(12,8) (right), respectively.

**Fig. 4.** Effect of branched 1,2-diacyl phosphatidylcholines on the absorption spectra of P450SCC in DOPC vesicles. In each case the vesicles contained 6 mol% cholesterol and the same amount of total phospholipid. P450SCC was reconstituted into the DOPC vesicles (see "Experimental Procedures") containing either 0% (---), 33 weight % PC(10,6) (-----), or 33 weight % PC(12,8) (-----), respectively. Spectra were recorded with water in the reference cuvette.

Temperature Dependence of the Catalytic Activity of P450SCC in Vesicles Consisting of Mixtures of DOPC and Branched 1,2-Diacyl PCs—The reaction of cholesterol conversion to pregnenolone in P450SCC reconstituted into DOPC vesicles containing 33 weight % PC(10,6) or PC(12,8) have been studied within the temperature range 15-42 °C. Fig. 5 demonstrates the effects of temperature on the SCC activity of...
P450SCC; it can clearly be seen from the data presented that, in the whole temperature region investigated, the rate of pregnenolone formation is much higher for DOPC vesicles containing 33 weight % of PC(10,6) and PC(12,8) as compared to (pure) DOPC vesicles. All Arrhenius plots are characterized by breaks at temperatures localized in the region of 27–30 °C. Obviously, the existence of the break is determined by properties of the protein component in the system, since it is well known that at least DOPC does not undergo any phase changes in this physiological temperature region.

The value of activation energy $E_{a1}$ of the enzymatic process of cholesterol conversion to pregnenolone in DOPC liposomes could be determined in the region below the break temperature to about 11.9 kcal/mol and thus is compatible with values known for a number of other membrane-bound enzymatic systems (23). In contrast, a value of nearly zero observed for $E_{a2}$ above the break temperature is unusual and requires further and more detailed experiments for its interpretation. In general, it is clear that at least at the weight ratio of 1:2 inclusion of the branched PCs into the membrane of DOPC is not accompanied by essential changes in the activation energy. However, the $E_{a1}$ value in the mixture of DOPC and PC(10,6) in the region below the temperature break is somewhat decreased and becomes about 8 kcal/mol. In the presence of PC(12,8), $E_{a2}$ in the temperature region above the break point increases to about 1.6 kcal/mol. We found a very large difference in the activation energy and the temperature break point in the SCC reaction catalyzed by P450SCC for the branched PCs in DOPC compared to results reported for CL in DOPC vesicles (24).

Decrease of the activation energy by 2 times and the observed shift of the break temperature by 7–10 °C toward lower temperatures in the presence of the branched PCs might be indicative of the formation of a rather "less rigid" protein-lipid configuration in the case of branched PC-containing DOPC vesicles.

The stimulation of the SCC activity by the saturated branched PCs accounts to more than a factor of 6. For comparison, it should be mentioned that under similar conditions with regard to cholesterol content, protein-protein and protein/lipid relationship, ionic strength, lipid composition, and pH of buffer, the catalytic activity of P450SCC was increased by bovine adrenal cortex CL by 4–5 times (20). In Ref. 2, absolute values for the activity in DMPC vesicles containing CL from various sources were not reported, making comparison based on absolute activities difficult. However, for comparison, quantitation of the stimulatory efficiency of different PCs is possible based on the $K_{1/2}$ values for half-maximum stimulation. Comparing the data in Table I and taking into account the 2-fold increase of the reaction rate after inclusion of PC(10,6) or PC(12,8) in DOPC vesicles (see Fig. 3) leaves no doubt that the effectiveness of stimulation of the P450SCC activity by saturated $\omega$-branched fatty acid chains in 2-position of the (saturated) main fatty acyl chains and used them in the reconstitution of the SCC activity of P450SCC in phospholipid vesicles. In our opinion, branching of fatty acids in 2-position best approaches the structure of the hydrophobic part of CL (compare chemical structures in Fig. 1).

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present paper, vesicles were prepared from mixtures of a non-activator (host) lipid (DMPC) and an activator lipid (branched 1,2-diacyl PC). Fig. 2 shows the experimental and theoretical activity data. As can be seen, the experimental data for both branched 1,2-diacyl PC systems could be satisfactorily fitted to the theoretical sigmoidal curves. The theoretical curve, which corresponds to a hyperbolic dependence not observed experimentally for the both branched PCs under investigation. This is in strict contrast to CL, for which a hyperbolic dependence was reported. The best fit for PC(10,6) was reached with \( n = 3 \); for PC(12,8), \( n = 4 \) gave the best fit; other exponents led to less good fits (not shown).

How do the \( \alpha \)-branched PCs exert their stimulatory role on the enzymatic activity? It could be shown for CL (as well as for other activator lipids) that its binding to P450SCC caused an increase in cholesterol binding (3). The same could be expected to be the case for the branched PCs. This is most probably because the optical measurements clearly relate the presence of branched PCs in DOPC vesicles with a significantly enhanced content of the high spin form of P450SCC, which is connected with a higher portion of P450SCC to be complexed with its substrate cholesterol. However, this conclusion must be justified by experiments studying directly the effect of branched PCs on the cholesterol binding to P450SCC.

As follows from the stopped-flow kinetics experiments, in the presence of branched PCs a \( -2 \)-fold increased rate of electron transfer to P450SCC could be observed. However, the relative high value of this rate both in the absence (3.3 s\(^{-1}\)) and in the presence (6–6.3 s\(^{-1}\)) of branched PCs transfer of the first electron cannot limit the rate of the enzymatic reaction as a whole. Thus, the observed correlation between the increase of electron transfer and SCC activity most probably indicates only that the change of both parameters might have the same reason.

In accordance with the binding equilibrium model elaborated in Ref. 3, cholesterol and CL on the one side and AD and cholesterol on the other show mutually facilitated binding to P450SCC. Thus, assuming an analogous action for the branched PCs beside from the lipid-induced favorable cholesterol binding, these PCs show an additional influence on P450SCC resulting in an enhanced AD-P450SCC interaction in such a way as to cause the increased reduction rate of P450SCC in DOPC vesicles containing branched PCs.

According to Refs. 3 and 5, one can consider three general classes of mechanism by which fatty acyl moieties of phospholipids might affect the binding of cholesterol to P450SCC. First, some physical property of the lipid phase or the membrane, e.g., fluidity, nonbilayer phase, or phase transition temperature of the lipid, regulates the activity of P450SCC. Because we have until now investigated only mixtures and not the pure lipid system, it is not possible to consider this possibility decisively. The second mechanism assumes for each lipid a given affinity for cholesterol competing with P450SCC for cholesterol binding; this possibility was excluded because the order of affinity of various lipids for cholesterol is not correlated inversely with the affinity of P450SCC for cholesterol (for further discussion see Ref. 5). A third possibility, that the cholesterol binding is regulated by direct interaction of the lipid(s) with the P450SCC, seems most consistent with the new data obtained by studying the effect of branched PCs on the activity of P450SCC. 1–2 molecules of CL or 3–4 branched PCs, respectively, bind highly specific to P450SCC at an effector site distant from the cholesterol binding site with a resultant stabilization of an optimal cholesterol-binding conformation of P450SCC. Although it is possible to consider such an explanation for the interpretation of our results, too, we believe that another model than the specific allosteric binding model is more consistent with the available data. Remembering the structural similarity of the hydrophobic region of the branched PCs and CL, we propose that the molecular shape of the lipid molecule may be a partial determinant for the interaction between P450SCC and lipids. Both CL as well as the branched PCs belong to a class of lipids (so-called polymorphic lipids) that have a great tendency to adopt nonbilayer structures under certain conditions (25–27). Currently there is a considerable experimental effort and discussion about the possible functions of polymorphic lipids in biological membranes. For example, Gruner (28) and Hui and Sen (29) have suggested that curvature strain by polymorphic lipids may modulate the activity of some membrane proteins. Following such a hypothesis, one can suppose that some polymorphic lipids are necessary to accommodate the P450SCC in an optimal conformation to the bilayer. Such a model is supported by the finding that the incorporation of P450SCC into octylglucoside-reconstituted (relatively large) PC/PE/CL vesicles and its mobility in the membrane depend critically on the CL content of the membrane (30). Furthermore, it has been reported previously that reconstitution by both octylglucoside and cholate dialysis results in the formation of two main populations of vesicles, larger and smaller ones with the smaller vesicles containing significantly more P450SCC (31, 32). Recently it was reported that the size of the vesicles, which in turn determines the vesicle curvature, has a large influence on the activity (22). Taken together, these findings all suggest that the curvature of the membrane plays a role for the incorporation of P450SCC and thus support our view that polymorphic lipids like CL and the branched PCs could influence P450SCC by maintaining the membrane curvature at a value optimal for P450SCC activity. It is too early to speculate on the possible physiological relevance of these results. However, it should be mentioned that PE also is a lipid which is able to adopt nonbilayer phases (27) and that PE together with CL comprise \( \geq 50\% \) of lipids of the inner mitochondrial membrane. In relation to CL from mitochondria with its fatty acyl chains equal to or even longer than 18 C-atoms, it would be interesting to study the influence of longer than hexyl- and octyl substituents as used by us in the present study. Synthesis of such lipids is now under way.

Our results are not in accordance with conclusions on the importance of unsaturated fatty acid groups of phospholipids with regard to their steriodogenic activity. In Ref. 4, a strong correlation of the SCC activity of P450SCC with the degree of unsaturation in the fatty acid moiety, regardless of headgroup, was reported. Moreover, saturated phospholipids exhibited inhibition of steriodogenic activity. No such simple relation between the degree of chain unsaturation and activity was reported in Ref. 5, but generally the activity was found relative low in saturated PCs. Our results clearly show that the inhibition of stimulation is not related to saturation of the fatty acid components. As shown, fully saturated PCs as the branched PCs used lead to a very high stimulation. Taking into account the roughly comparable stimulation by predominantly saturated bacterial and unsaturated bovine CL, respectively (2), and our results, it seems that unsaturation of the fatty acyl chain region is of less importance. The sometimes higher activity found for several unsaturated PCs, which did not correlate with the number of double bonds in the various chains, also might be indicative of a decisive role of the lipid conformation in connection with a higher propensity of these lipids to adopt nonbilayer phases. This is supported by a significant promotion of hexagonal phase formation of fatty acid-PE mixtures by increasing unsaturation of fatty acids reported recently (33).

In conclusion, by application of the branched 1,2-diacyl PCs, it was possible to provide for the first time direct evidence for a
specific lipid-P450SCC interaction with an apparently important role of the saturated hydrophobic fatty acyl chain region of the lipid. The results suggest polymorphic lipids as CL and branched PCs might be important in control of P450SCC activity. Finally, our data highlight the potential usefulness of P450SCC vesicles containing branched 1,2-diacyl PCs in studies of the effect of polymorphic lipids on the biological activity of membrane-bound enzymes.

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