Biochimica et Biophysica Acta, 692 (1982) 287–295
Elsevier Biomedical Press

BBA 71345

THERMOTROPIC BEHAVIOUR OF PHOSPHOLIPID VESICLES RECONSTITUTED WITH RAT LIVER MICROSOMAL CYTOCHROME *P*-450

A.A. AKHREM, V.T. ANDRIANOV, S.B. BOKUT, Z.A. LUKA, M.A. KISSEL, T.G. SKORNYAKOVA and P.A. KISSELEV

Institute of Bioorganic Chemistry, B.S.S.R. Academy of Sciences, 220600 Minsk (U.S.S.R.)

(Received December 9th, 1981)
(Revised manuscript received June 3rd, 1982)

Key words: Cytochrome P-450; Phospholipid vesicle; Thermotropic behavior; Differential scanning calorimetry; (Rat liver)

The interaction of rat liver microsomal cytochrome *P*-450 with phospholipids has been investigated employing differential scanning microcalorimetry. It is shown that the thermotropic behaviour of phospholipid vesicles reconstituted with cytochrome *P*-450 depends on liposome composition, protein concentration and the mode of reconstituted system preparation. From the ΔH dependence on protein concentration in proteoliposomes it was calculated that one cytochrome *P*-450 molecule influence 350 ± 50 dimyristoylphosphatidylcholine (DMPC) molecules. The electrostatic interaction of cytochrome *P*-450 and negatively charged phospholipid, phosphatidylinositol (PI), mixed with DMPC involves the temperature stabilization of proteoliposomes at a phase transition of phospholipid bilayers. The thermal denaturation temperature is increased due to negatively charged PI added.

Introduction

The hydroxylation of many compounds in mammalian liver, including fatty acids, steroids, and a variety of drugs is controlled by the multienzyme system consisting of cytochrome *P*-450 (EC 1.14.14.1), flavoprotein, NADPH-cytochrome *P*-450 reductase (EC 1.6.4.1), and phospholipids. It is demonstrated that phospholipids are necessary for reconstituting catalytic properties of the enzyme system [1–3]. There are molecular and supermolecular levels of interaction of the phospholipid component with the cytochrome *P*-450-containing hydroxylation system. The interaction of phospholipids with cytochrome *P*-450 on the molecular level involves an increase in the α-helix content of the protein molecule as well as by a shift in the spin equilibrium towards a high-spin form of hemoprotein [4–6]. A certain specific effect of the phospholipids on the properties of cytochrome *P*-450 has been revealed. It has been recently shown that the oxidase activity and the high-spin cytochrome *P*-450 content correlate with the charge of the phospholipids added [7,8]. The role of phospholipids in the supermolecular structure of the enzyme system is to improve the interaction of cytochrome *P*-450 with its reductase and lipophylic substrates. This involves an increase of the spectral change intensity and a maximal rate of the oxidation reaction [9].

Investigation of the enzymatic activity of the cytochrome *P*-450-containing microsomal system over a wide temperature range points to a break in the Arrhenius plot at about 20°C, this being attributed to the change in the phase state of phospholipids [10,11]. The breaks in the plots for the

Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; PI, phosphatidylinositol from baker's yeast; T_c, phase transition temperature; ΔH, phase transition enthalpy.

thermotropic behaviour of microsomes are also registered at 18–22°C or at 28–33°C by using a number of physico-chemical methods [12–14]. However, the microcalorimetric study of phase transition of the microsomal membrane lipids did not detect any temperature-induced change above 5°C [9]. The discrepancy in the data obtained is sometimes attributed to the presence of an 'annular' layer of rigidly organized phospholipids [13] or associated with a change in the activation energy of the reactions catalysed by cytochrome *P*-450 with its structural rearrangement [15].

One of the approaches to a solution of the problem on the structure of mixed-function oxidase is the study of model systems containing individual protein components of the enzyme system and synthetic phospholipids. Since the synthetic phospholipids exhibit a well-defined composition and distinct phase transition, the scanning differential microcalorimetry may turn to be an effective method for their investigation. It is worth noting that similar investigations have been made with other membrane proteins [16–19].

In this paper the scan microcalorimetry assay is used to investigate the interaction of highly purified cytochrome *P*-450 from phenobarbital-pretreated rat liver microsomes with synthetic phospholipid, DMPC, or with DMPC/PI mixture.

Materials and Methods

Preparation of microsomes. Wistar male rats, 100–120 g body weight, were injected with sodium phenobarbital (75 mg/kg of body weight) for 4 days before sacrifice. Livers were homogenized with a motor-driven Teflon pestle in 2 vol. 1.15% KCl. The microsomal subfraction was washed in 10 mM EDTA and 1.15% KCl and stored in 0.25 M sucrose at −20°C.

Preparation of cytochrome P-450. The cytochrome *P*-450 used in these experiments was prepared from phenobarbital-pretreated rat liver microsomes by the procedure [20] as modified in Ref. 21. The specific content of cytochrome *P*-450 was as high as 15.1–15.4 nmol/mg of protein. The purity of preparations was controlled by polyacrylamide gel electrophoresis.

Preparation of liposomes. 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine was prepared from *sn*-glycero-3-phosphocholine as $CdCl_2$ complex according to the procedure of Khorana et al. [22]. Phosphatidylinositol was isolated from baker's yeast as described in Ref. 23. DMPC and its mixture with PI in chloroform solution were taken to absolute dryness under nitrogen. The residue was dispersed in Tris-HCl buffer (pH 7.5) at a temperature above that of the lipid bilayer phase transition. Phospholipid suspensions were sonicated using a UZDN-sonicator (U.S.S.R.). Following the electron microscopy data, 30–50-nm diameter liposomes were obtained after sonication.

Reconstitution of cytochrome P-450/DMPC vesicles. Two methods for the incorporation of cytochrome *P*-450 into phospholipid vesicles were used; the cholate dialysis method previously described [24] and a method involving incubation of protein with sonicated vesicles for 24 h at 12–15°C.

Microcalorimetric study. The microcalorimetric study of samples was carried out employing the differential scanning microcalorimeter DASM-1 M (U.S.S.R.) [25]. The scanning rate was 1 K/min. The phospholipid content in the samples was 0.5 mg/ml.

Electron microscopy. The specimens were examined by the negative staining method in the electron microscope EMV-100 L (U.S.S.R.) at a magnification of ×32000 as described in Ref. 26.

Other procedures. The purity of phospholipids was checked by the TLC method. The phospholipid concentration in the preparations was determined according to Ref. 27. Protein concentrations were measured by the Bradford method [28]. The cytochrome *P*-450 content was determined from the CO difference spectrum of the reduced hemoprotein [29]. Polyacrylamide gel electrophoresis was performed under denaturing conditions in the presence of sodium dodecyl sulfate as described by Laemmli [30]. Protein bands were detected by staining with Coomassie Blue G-250 [31].

Results

Interaction of cytochrome P-450 with DMPC liposomes

Fig. 1. shows the thermograms that reflect the effect of cytochrome *P*-450 on the phase transition

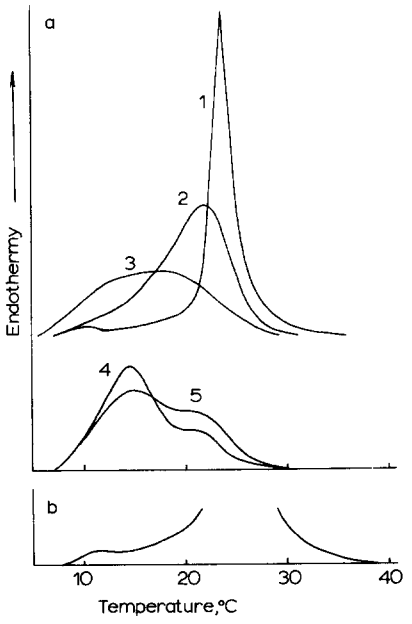

Fig. 1. Microcalorimetry curves of the thermotropic behaviour of proteoliposomes reconstituted from cytochrome *P*-450 and DMPC in the absence of sodium cholate (a) 1, DMPC; 2, 0.007 mol% cytochrome *P*-450; 3, 0.07 mol% cytochrome *P*-450; 5, 0.5 mol% cytochrome *P*-450; 4, rescanning of sample No. 5 after heating to 33°C. (b) The bottom of curve 1 is increased.

of DMPC. It is seen that the behaviour of the sonicated dispersion of DMPC vesicles in the absence of protein is characterized by a pretransition at 12.5°C and a main peak at 24.5°C. The addition of cytochrome *P*-450 causes a decrease in the phase transition temperature (Fig. 2) and is accompanied by a decrease in the enthalpy of the lipid phase transition from 6.9 kcal/mol for pure DMPC to 3.8 kcal/mol for liposomes containing 0.09 mol% of protein (Fig. 3). As cytochrome *P*-450 content in the incubation mixture increases, two partially overlapping peaks appear with T_c values of 15°C and 20–22°C (0.5 mol% of protein). A repeated scanning of the sample being heated up to 33°C results in the growth of the peak amplitude at 15°C and a corresponding decrease of heat absorption at 22°C. Note that the addition of cytochrome *P*-450 (from 0.007 mol% to 0.5 mol%) to the sonicated DMPC vesicles removes the pretransition of liposomes in the thermogram. These data support the formation of the protein-lipid complexes [32].

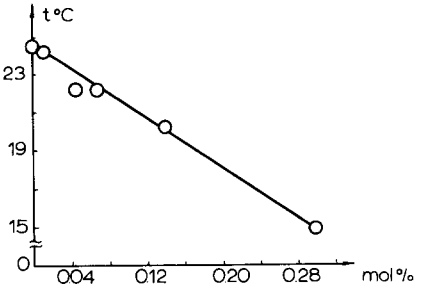

Fig. 2. Phase transition temperature of proteoliposomes versus the cytochrome *P*-450 content after DMPC protein incubation.

It follows from the present authors' data that an increase in the mole content of cytochrome *P*-450 in DMPC liposomes involves a decrease in ΔH and T_c. Such an effect of cytochrome *P*-450 on the thermotropic behaviour of liposomes allows its assignment to type II proteins (according to the classification in Ref. 16) capable of both hydrophobic and hydrophilic interactions with phospholipids.

Fig. 3 is a plot of the total enthalpy vs. cytochrome *P*-450 content in liposomes. The plot is linear over a protein concentration range from 0 to 0.09 mol%, and then a break is observed. The latter may be associated with 'saturation' of liposomes with cytochrome *P*-450 and a predominant sorption of the excess protein on vesicle surfaces due to hydrophilic interactions only. The assumption agrees well with the electron microscopy data. The incubation of the increasing cytochrome *P*-450 concentrations with DMPC is found to lead primarily to a dimensional increase of phospholipid vesicles, and then to their fusion into multilamellar structures (results not shown).

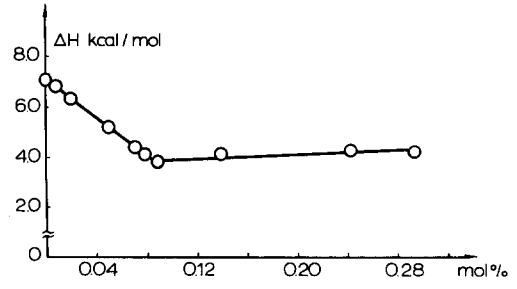

Fig. 3. Phase transition enthalpy of proteoliposomes versus the cytochrome *P*-450 content after DMPC protein incubation.

Effect of sodium cholate on phase transition of DMPC

The cholate dialysis method is one of the ways to reconstitute the model membranes [24]. In the present work the effect of different cholate concentrations on the thermotropic behaviour of DMPC-liposomes has been studied. No changes have been observed in either T_c or ΔH in liposomes at a detergent concentration below 0.002%. An increase in sodium cholate concentration up to 0.01% was accompanied by the separation of the phase transition region into two partially overlapping peaks with maximum amplitudes at 21.5°C and 26°C. A repeated scanning of the sample led to an increase in the peak amplitude at 21.5°C and a relative decrease of the second peak intensity (Fig. 4). This may show the redistribution of the detergent between two liposome structures above the T_c. Removal of sodium cholate via dialysis of the probe for 12 h with constant stirring of the solution caused the reconstitution of the primary liposome structure.

Reconstitution of the cytochrome P-450-containing phospholipid vesicles by the cholate dialysis method

Reconstitution of the proteoliposomes by the cholate dialysis method entails three types of effects in DMPC phase transition, namely, a decrease in T_c, the heat absorption peak widening and a decrease of phase transition enthalpy ΔH (Fig. 5). Note that the modification of the sample preparation conditions causes two partially overlapping peaks whose total ΔH is less than that of pure DMPC. We attribute this effect to incomplete removal of the detergent whose rate of removal during dialysis in the presence of protein may drop significantly, as illustrated in Ref. 33.

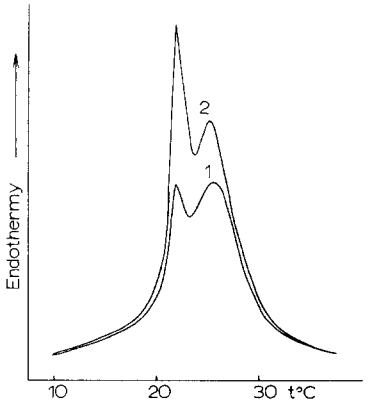

Fig. 4. Microcalorimetry curves of the thermotropic behaviour of DMPC liposomes in the presence of 0.01% sodium cholate. 1, first scan; 2, second scan.

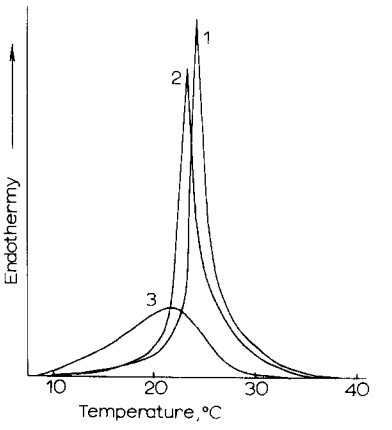

Fig. 5. Microcalorimetry curves of cytochrome *P*-450/DMPC proteoliposomes reconstituted by the cholate dialysis method 1, DMPC after dialysis; 2, 0.007 mol% cytochrome *P*-450; 3, 0.08 mol% cytochrome *P*-450.

Fig. 6 is a plot of T_c vs. cytochrome *P*-450 content. T_c decreases from 24.5°C for pure DMPC to 20°C for vesicles containing 0.14 mol% cytochrome *P*-450. A change of the phase transition enthalpy as a function of the cytochrome *P*-450 content in mol% is shown in Fig. 7. The plot is seen to be a straight line. Its extrapolation to $\Delta H = 0$ allows the estimation of the amount of DMPC molecules which are withdrawn from the cooperative transition per cytochrome *P*-450 molecule [19,34]. As calculated at the point of intersec-

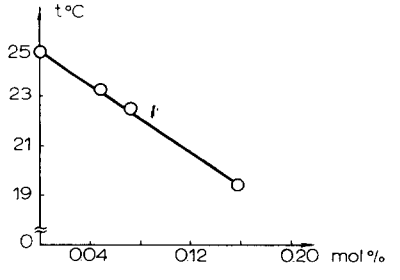

Fig. 6. Phase transition temperature of proteoliposomes reconstituted by the cholate dialysis method versus the cytochrome *P*-450 content.

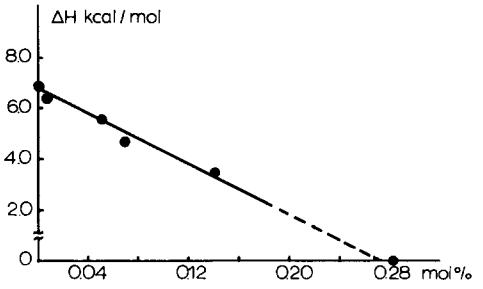

Fig. 7. Phase transition enthalpy of proteoliposomes reconstituted by the cholate dialysis method versus the cytochrome *P*-450 content.

tion of the plot with axis X, the number of the DMPC molecules connected with one cytochrome *P*-450 molecule is equal to 350 ± 50. Indeed, we have not detected any phase transition in reconstitued phospholipid vesicles in experiments with the protein content exceeding 0.28 mol%. The decrease of the phase transition enthalpy, illustrated in Fig. 7, supports protein-DMPC interaction rather than vesicles diminishing. This conclusion is very important because a heavier liposome surface curvature may also cause essential alterations in thermotropic characteristics [35]. In fact, the cytochrome *P*-450 incorporation into DMPC vesicles by the cholate dialysis method, as in the case of protein incubation with pure DMPC, involves an increase of phospholipid vesicles. At a higher protein/lipid ratio, DMPC vesicles fuse into multilamellar structures. A dimensional increase of the liposome vesicles was also observed under the interaction of rabbit liver microsomal cytochrome *P*-450 with natural phosphatidylcholine [36]. A partial fusion of the DMPC vesicles into large bilayer structures has been shown in the presence of spectrin [17]. The process is markedly enhanced by addition of negatively charged dimyristoylphosphatidylserine [18].

From comparing Figs. 2, 3, 6 and 7 it may be concluded that for low protein/lipid ratios the manner of proteoliposome reconstitution does not significantly affect the T_c and ΔH dependence on the concentration of the protein incorporated. However, an increase in the cytochrome *P*-450 content in liposomes above 0.09 mol% brings about different changes in the thermotropic properties of both systems. When the cholate dialysis method for protein incorporation is used, a further linear decrease of ΔH is observed. The incubation of high concentrations of cytochrome *P*-450 with DMPC gives rise to two low temperature peaks (Fig. 1). This behaviour of the system under study is, in our opinion, due to various mechanisms of cytochrome *P*-450 incorporation.

Microcalorimetric study of DMPC/phosphatidylinositol mixtures

Sonicated vesicles of phosphatidylinositol and DMPC mixtures did not show phase separation at molar ratios up to 1/2, although followed by the increase in the peak asymmetry and the decrease in the phase transition cooperativity. The resulting mixtures were stable, and their characteristics were completely reproduced upon heating up to 100°C and repeated scanning. As seen from Fig. 8, an increase in the PI content in the mixtures resulted in a gradual decrease if T_c. In experiments on cytochrome *P*-450 incorporation, a PI/DMPC mixture at a molar ratio of 1/5 and a T_c of 21°C were employed.

Cytochrome P-450 interaction with a binary mixture

Fig. 9 shows thermotropic behaviour of PI/DMPC vesicles incubated with cytochrome *P*-450. The latter (0.04 mol% as counted for a total lipid content), when added to sonicated PI/DMPC vesicles, induced an increase in T_c of the system from 21°C to 35°C. In this case, a single peak is observed. The elevation of the cytochrome *P*-450 content in liposomes causes a downshift in T_c and division of the phase transition region into two partially overlapping peaks with their maximum

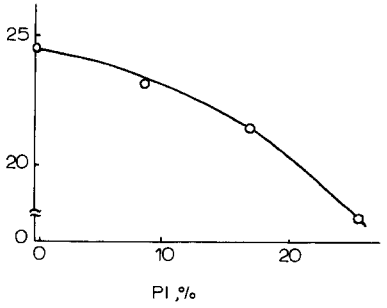

Fig. 8. Phase transition temperature of a binary mixture versus the PI content in the liposomes.

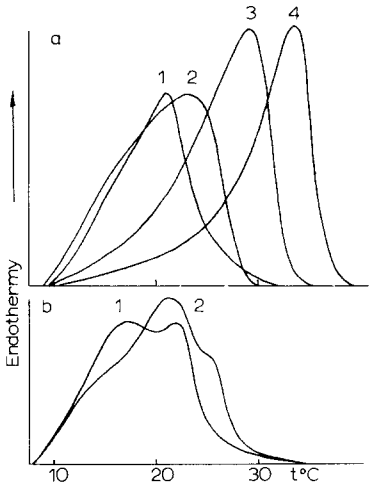

Fig. 9. Microcalorimetry curves of the thermotropic behaviour of a DMPC/PI binary mixture in the presence of cytochrome *P*-450. The PI content is 16.5%. (a) 1, DMPC/PI mixture; 2, 0.28 mol% cytochrome *P*-450; 3, 0.092 mol% cytochrome *P*-450; 4, 0.04 mol% cytochrome *P*-450. (b) 1, 0.55 mol% cytochrome *P*-450; 2, rescanning of sample 1b after heating to 100°C.

amplitudes at 16°C and 21°C. Heating of the sample (0.135 mol% cytochrome *P*-450) up to 100°C brings about, when repeatedly scanned, the following changes on the heat absorption curve for the binary mixture phase transition: a decrease in the peak amplitude at 16°C and development of a new peak at 26°C (Fig. 9b). Simultaneously, heat absorption at 21°C is increased. This does not cause any changes in the total phase transition enthapy within experimental error. As shown in Fig. 9a, the peak at 21°C specifies the binary mixture phase transition in the absence of protein. These data allow the assumption that the heat absorption enhancement in this temperature range is associated with the cytochrome *P*-450 denaturation causing a partial release of the previously bound phospholipid molecules from the protein/lipid complex. This assumption is supported by the decrease of the peak amplitude at about 16°C and the appearance of a new peak at 26°C, which may correspond to a lipid complex with the denaturated form of cytochrome *P*-450; this is in agreement with [19].

The electrostatic interaction of protein with negatively charged phospholipids is known to increase the T_c of liposomes [15,37]. A similar effect is caused by divalent metal ions. The influence of pH and Ca^{2+} and Mg^{2+} on the liposomes formed by acidic phospholipids, and the mechanism of the process have been studied [38–40]. The systems of Ca^{2+} (or Mg^{2+})/acidic lipid exhibit an increase in ΔH and T_c of phase transition at molar ratios less than 1/2, while at higher ratios structures completely impermeable to K^+ are observed. The same results have been obtained for the bilayers consisting of the mixture of negatively charged and neutral phospholipids after their neutralization by Ca^{2+}. These data offer an explanation to the temperature stabilization of PI/DMPC liposomes in the presence of cytochrome *P*-450. In fact, negatively charged phospholipids increase the catalytic activity of cytochrome *P*-450 in the oxidation reactions of different compounds [41–43]. A correlation between the catalytic properties of this protein and the charge of phospholipids is obtained [7]. It is postulated that the acidic lipids bound by electrostatic forces change the protein conformation [4,8]. Thus, the electrostatic interaction of cytochrome *P*-450 with liposomes exerts a pronounced influence both on the behavior of the protein itself and on the structure of proteoliposomes. Our results show that the most significant increase in T_c of the system occurs when the cytochrome *P*-450 content is 0.04 mol%. A further increase in the protein concentration levels this effect evidently at the expense of other lipid-cytochrome *P*-450 interactions.

Thermal denaturation of cytochrome P-450 in the protein-lipid complexes

The extension of the scanning range enables one to detect the heat absorption region starting at 40–45°C and ending at about 70°C. The temperature corresponding to the maximum peak amplitude depends both on the liposome composition and the cytochrome *P*-450/lipid ratio, and ranges from 55°C to 65°C (Fig. 10). Under our experimental conditions, the maximum peak temperature is registered for 0.094 mol% cytochrome *P*-450 in PI/DMPC liposomes. No high temperature peak is detected by a repeated scanning of the sample; in the PT region of liposomes the above alterations are observed (Fig. 9). It is shown [4,44,45] that the 45–65°C temperature range is characterized by: (i) a decrease of the helix content

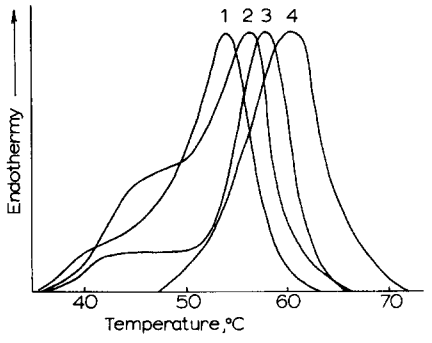

Fig. 10. Microcalorimetry curves of the heat denaturation of cytochrome *P*-450 in the presence of phospholipids. 1, 0.8 mol% cytochrome *P*-450 in DMPC liposomes; 2, 0.3 mol% cytochrome *P*-450 in DMPC liposomes; 3, 0.28 mol% cytochrome *P*-450 in DMPC/PI liposomes (the PI content is 16.5%); 4, 0.04 mol% cytochrome *P*-450 in DMPC/PI liposomes (the PI content is 16.5%). The curves are normalized to 0.5 mol% cytochrome *P*-450 and 0.5 mg/ml phospholipid concentration.

of cytochrome *P*-450; (ii) an increase in light scattering, and (iii) a decrease or complete absence of the catalytic activity of cytochrome *P*-450 both in the reconstituted and in microsomal systems. Thus, it may be concluded that the peak about 55–65°C characterizes protein heat denaturation. In this case, the shift of the denaturation temperature from 55°C in the presence of DMPC to 65°C for DMPC/PI proteoliposomes can be attributed to the protein temperature stabilization due to the addition of negatively charged PI.

Discussion

The data presented in this paper suggest that the mechanism of interaction of phenobarbital-induced rat liver microsomal cytochrome *P*-450 with phospholipid bilayers depends on the liposomal composition, protein concentration and, sometimes, on the mode of reconstituted system preparation.

It is well known that cytochrome *P*-450 easily aggregates [1–3]; however, the addition of detergents promotes its disaggregation. According to our unpublished data, the decrease in the protein concentration involves an increase in the portion of cytochrome *P*-450 monomers. In view of these results and by comparing the cytochrome *P*-450 effect on ΔH and T_c of DMPC liposomes with or without sodium cholate, it may be supposed that at a protein concentration of up to 0.09 mol%, the latter in the monomer form interacts with phospholipids and then penetrates the hydrophobic core of liposomes. In the absence of detergents, the increase in the cytochrome *P*-450 concentration causes its aggregation that decreases the accessibility of hydrophobic sites. This leads to a predominant sorption of protein aggregates on liposomal surfaces and to a T_c decrease [16]. In our experiments, in the absence of sodium cholate, the increase of the cytochrome *P*-450 concentration in the incubation medium from 0.09% mol% to 0.5 mol% did not induce a change in ΔH. This may be attributed to the limited liposomal 'capacity' [46]. It should be noted that the optimum catalytic activity of the reconstituted microsomal hydroxylating system is achieved by using approximately the same cytochrome *P*-450/lipid ratios [2,7,36]. In the presence of sodium cholate, there is no break in the plot of the phase transition enthalpy ΔH versus cytochrome *P*-450 content but a linear decrease in ΔH upto complete disappearance of the liposomal phase transition is observed instead. This may be accounted for by the destruction of the original liposome composition due to the addition of sodium cholate and then, as detergent is removed, the formation of a model membrane from individual components. In this case, the amount of cytochrome *P*-450 incorporated is not limited by the initial 'capacity' of the liposomes used.

The results obtained indicate that one cytochrome *P*-450 molecule influences the phase transition of about 350 ± 50 DMPC molecules; data which agrees with Ref. 34. In other reconstituted systems that have been investigated using various techniques, the number of phospholipid molecules interacting with a single protein molecule is lower, ranging between 20 and 120. Perhaps this may be explained both by the different nature of the proteins studied and by the inadequacy of the methods employed [2,47–49]. In contrast to other proteins, cytochrome *P*-450 is characterized by an extremely high content of hydrophobic amino acid residues achieving 74–78% in the N-terminal region [50]. It is possible that two or more phospholipid layers may form around a cytochrome

P-450 molecule as shown for another system [47]. As considered in a number of works [19,51,52], there is no rigidly organized layer of annular phospholipids in reconstituted systems involving (Ca^{2+} + Mg^{2+})-ATPase and proteins from *Halobacterium halobium*. This is confirmed by NMR studies [53–55], from which opposing results obtained by the spin and fluorescence labels may be explained by the limited label mobility due to its direct contact with protein. On the other hand, these observed conditions may be attributed to different time scales of the methods applied. The authors' results display essential disturbances in DMPC liposomes induced by the cytochrome *P*-450 incorporation. This is supported by the decrease in ΔH of the proteoliposome phase transition.

The electrostatic interaction of cytochrome *P*-450 with PI mixed with DMPC is accompanied by the temperature stabilization of the whole system. As shown in Fig. 9, T_c of the model membrane depends on the protein/lipid ratio. Indeed, additional investigations are necessary, the fact itself being of no doubt. It has been reported that acidic phospholipids, as well as PI, are important components of the microsomal membrane [8]. Our data on model systems suggest the appearance of local regions with increased T_c in the microsomes at a certain cytochrome *P*-450/lipid ratio. These regions are detected by differently distributed spin and fluorescence labels in the membrane and are not observed on the scanning microcalorimetric curves due to cooperation of microsomal T_c. This may account for the differences of T_c obtained by various techniques and for microsomes differing in their composition.

References

1 Lu, A.Y.H. and Coon, M.J. (1968) J. Biol. Chem. 243, 1331–1332
2 Coon, M.J., Haugen, D.A., Guengerich, F.P., Vermillion, J.L. and Dean, W.L. (1976) in Structural Basis of Membrane Function (Hatefi, Y. and Djavadi-Ohanience, L., eds.), pp. 409–427, Academic Press, New York
3 Lu, A.Y.H. and Levin, W. (1974) Biochim. Biophys. Acta 344, 205–240
4 Chiang, Y.L. and Coon, M.J. (1979) Arch. Biochem. Biophys. 195, 178–187
5 Akhrem, A.A., Kisselev, P.A., Kissel, M.A., Usanov, S.A. and Metelitza, D.I. (1979) Dokl. Akad. Nauk. S.S.S.R. 245, 234–238
6 Gibson, G.G., Cinti, D.L., Sligar, S.G. and Schenkman, J.B. (1979) Biochem. Soc. Trans. 7, 1289–1290
7 Ingelman-Sundberg, M., Johansson, I., Brunström, A., Ekström, G., Haaparanta, T. and Rydström, J. (1980) in Biochemistry, Biophysics and Regulation of Cytochrome *P*-450 (Gustafsson, J.-Å., Carlsted-Duke, J., Mode, A. and Rafter, J., eds.), pp. 299–306, Elsevier/North-Holland Biomedical Press, Amsterdam
8 Ruckpaul, K., Rein, H., Ballou, D.P. and Coon, M.J. (1980) Biochem. Biophys. Acta 626, 41–50
9 De Pierre, J.W. and Dallner, G. (1975) Biochim. Biophys. Acta 415, 411–472
10 Duppel, W. and Ullrich, V. (1976) Biochim. Biophys. Acta 426, 399–407
11 Yang, C.S., Strickhard, F.S. and Kicha, L.P. (1977) Biochim. Biophys. Acta 465, 362–370
12 Annaev, B., Koltover, V.K., Mamedniassov, O.N., Reichman, Z.M. and Rozantzev, E.G. (1972) Biophysica 17, 224–232
13 Stier, A. and Sackmann, E. (1973) Biochim. Biophys. Acta 311, 400–408
14 Maricić, S., Vuk-Pavlović, S., Benko, B. and Porok, J. (1977) Croat. Chem. Acta 49, 323–331
15 Lu, A.Y.H. and Miwa, G.T. (1980) in Biochemistry, Biophysics and Regulation of Cytochrome *P*-450 (Gustafsson, J.-Å., Carlsted-Duke, J., Mode, A. and Rafter, J., eds.), pp. 125–128, Elsevier/North-Holland Biomedical Press, Amsterdam
16 Papahadjopoulos, D., Moscarello, M., Eylar, E.H. and Isac, T. (1975) Biochim. Biophys. Acta 401, 317–335
17 Mombers, C., Van Dijk, P.W.M., Van Deenen, L.L.M., De Gier, J. and Verkleij, A.J. (1977) Biochim. Biophys. Acta 470, 152–160
18 Mombers, C., Verkleij, A.J., De Gier, J. and Van Deenen, L.L.M. (1979) Biochim. Biophys. Acta 551, 271–281
19 Gomez-Fernandez, J.C., Goni, F.M., Bach, D., Restall, C.J. and Chapman, D. (1980) Biochim. Biophys. Acta 598, 502–516
20 West, S.B., Huang, M.-T., Miwa, G.T. and Lu, A.Y.H. (1979) Arch. Biochem. Biophys. 193, 42–50
21 Elshourbagy, N.A. and Guzelian, P.S. (1980) J. Biol. Chem. 255, 1279–1285
22 Gupta, C.M., Radhakriohnan, R. and Khorana, H.G. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 4315–4319
23 Trevelyan, W.E. (1969) J. Lipid Res. 7, 445–452
24 Dawson, R.M. (1963) Biochem. J. 88, 414–417
25 Privalov, P.L. (1974) FEBS Lett. 405, 140–153
26 Parsons, D.F. (1963) J. Cell Biol. 16, 620–624
27 Yaskovsky, V.E., Kostetsky, E.Y. and Yasendin, J.M. (1975) J. Chromatogr. 114, 129–141
28 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254
29 Omura, T. and Sato, R. (1964) J. Biol. Chem. 239, 2370–2378
30 Laemmli, O.K. (1970) Nature 227, 680–685
31 Reisner, A.H., Nemes, P. and Bucholtz, C. (1975) Anal. Biochem. 64, 509–516
32 Curatolo, W., Sakura, I.D., Small, D.M. and Shipley, G.G. (1977) Biochemistry 16, 2313–2319
33 Van Cauwelaert, F.H., Hanssens, I. and Herreman, W. (1980) Biophys. Struct. Mech. 6, Suppl. 91

34 Petri, W.A., Estep, T.N., Pal, R., Thompson, T.E., Biltonen, R.L. and Wagner, R.R. (1980) Biochemistry 19, 3088–3091
35 Van Dijk, P.W.M., De Kruijff, B., Aarts, P.A.M.M., Verkleij, A.J. and De Gier, J. (1978) Biochim. Biophys. Acta 506, 183–191
36 Bachmanova, G.I., Karuzina, I.I., Mengazetdinov, D.A., Archakov, A.I., Galushchenko, I.V. and Obraztsov, V.V. (1978) Biochimia (Russian) 44, 1361–1368
37 Verkleij, A.J., De Kruijff, B., Ververgaert, P.H.I.T., Tocanne, I.F. and Van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 339, 432–437
38 Newton, C., Pangborn, W., Nir, S. and Papahadjopoulos, D. (1978) Biochim. Biophys. Acta 506, 281–287
39 Van Dijk, P.W.M., De Kruijff, B., Verkleij, A.J., Van Deenen, L.L.M. and De Gier, J. (1978) Biochim. Biophys. Acta 512, 84–96
40 Mandersloot, J.G., Gerritsen, W.J., Lennissen-Bijvelt, J., Van Echteld, C.J.A., Noordam, P.C. and De Gier, J. (1981) Biochim. Biophys. Acta 640, 106–113
41 Ingelman-Sundberg, M. (1977) Biochim. Biophys. Acta 488, 225
42 Ingelman-Sundberg, M. and Johansson, I. (1980) Biochemistry 19, 4004–4011
43 Metelitza, D.I., Akhrem, A.A., Erjomin, A.N., Kissel, M.A. and Usanov, S.A. (1979) Acta Biol. Med. Germ. 39, 511–518
44 Uvarov, V.Yu., Bachmanova, G.I., Archakov, A.I., Suhomudrenko, A.G. and Majasoedova, K.N. (1980) Biochimia (Russian) 45, 1463–1468
45 Erjomin, A.N., Metelitza, D.I., Usanov, S.A. and Akhrem, A.A. (1980) Biophysika (Russian) 25, 71–74
46 Swaney, J.B. and Chang, B.C. (1980) Biochemistry 19, 5637–5644
47 Knowles, P.F., Watts, A. and March, D. (1979) Biochemistry, 18, 4480–4487
48 Massey, J.B., Gotto, A.M., Jr. and Pownall, H.J. (1980) J. Biol. Chem. 255, 10167–10173
49 Lenaz, G. (1979) Sub-Cell. Biochem. 6, 233–243
50 Dus, K.M. (1980) in Biochemistry, Biophysics and Regulation of cytochrome *P*-450 (Gustafsson, J.-Å., Carlsted-Duke, J., Mode, A. and Rafter, J., eds.), pp. 129–132, Elsevier/North-Holland Biomedical Press, Amsterdam
51 Cherry, R.J., Muller, V. and Schneider, G. (1977) FEBS Lett. 80, 465–469
52 Moore, B.M., Lents, B.R. and Meissner, G. (1978) Biochemistry 17, 5248–5255
53 Oldfield, E., Gilmore, R., Glaser, M., Gutowsky, H.S., Hshung, J.C., Kang, S.J., King, E., Meddows, M. and Rice, D. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 4657–4660
54 Seelig, A. and Seelig, J. (1978) Hoppe-Seylers, Z. Physiol. Chem. 359, 1747–1756
55 Rice, D.M., Meadows, M.D., Scheinman, A.O., Goni, F.M., Gomez, J.C., Moscarello, M.A., Chapman, D. and Oldfield, E. (1979) Biochemistry 18, 5893–5903