Biochimica et Biophysica Acta, 688 (1982) 341–348
Elsevier Biomedical Press

BBA 71184

INTERACTION OF PHOSPHOLIPASE A_2 AND PHOSPHOLIPID BILAYERS

MAHENDRA K. JAIN [a,b,*], MAARTEN R. EGMOND [a], HUBERTUS M. VERHEIJ [a], RAFAEL APITZ-CASTRO [b,**], RUUD DIJKMAN [a] and GERARD H. DE HAAS [a]

[a] *Department of Biochemistry, State University of Utrecht, Padualaan 8, NL-3584 CH, Utrecht (The Netherlands)* [b] *Department of Chemistry, University of Delaware, Newark, DE 19711 (U.S.A.)*

(Received November 19th, 1981)

Key words: Phospholipase A_2; Phospholipid bilayer; Protein-membrane interaction

Binding of phospholipase A_2 from porcine pancreas and from *Naja melanoleuca* venom to vesicles of 1,2-di(tetradecyl)-*rac*-glycero-3-phosphocholine (diether-PC_{14}) is studied in the presence and absence of 1-tetradecanoyl-*sn*-glycero-3-phosphocholine and myristic acid. The bound enzyme coelutes with the vesicles during gel filtration through a nonequilibrated Sephadex G-100 column, modifies the phase transition behavior of bilayers, and exhibits an increase in fluorescence intensity accompanied by a blue shift. Using these criteria it is demonstrated that the snake-venom enzyme binds to bilayers of the diether-PC_{14} alone. In contrast, the porcine enzyme binds only to ternary codispersions of dialkyl (or diacyl) phosphatidylcholine, lysophosphatidylcholine and fatty acid. Binding of the pig-pancreatic enzyme to vesicles of the diether-PC_{14} could not be detected even after long incubation (up to 24 h) below, at, or above the phase-transition temperature, whereas the binding in the presence of products is almost instantaneous and observed over a wide temperature range. Thus incorporation of the products in substrate dispersions increases the binding affinity rather than increase the rate of binding. The results are consistent with the hypothesis that the pancreatic enzyme binds to defect sites at the phase boundaries in substrate bilayers induced by the products. The spectroscopically obtained hyperbolic binding curves can be adequately described by a single equilibrium by assuming that the enzyme interacts with discrete sites. The binding experiments are supported by kinetic studies.

Introduction

Phospholipases A_2 from a variety of sources act preferentially on their substrates at an organized interface formed by dispersions of the phospholipid substrates in water. The concept 'quality of the interface' has been introduced [1] to explain differences in binding and catalysis by the enzyme on different forms of the substrate interface. Factors that change the quality of the interface include surface pressure in monolayers [1,2], comicellization of substrate with detergents [3], incorporation of medium-chain alkanols in substrate bilayers [4,5], preparation of unannealed substrate bilayers below their phase transition temperature [6,7], and substrate bilayers at their phase transition temperatures [7–10]. Studies on the steady-state kinetics of phospholipase A_2 action have shown that for

* All correspondence may be addressed to Mahendra K. Jain at the University of Delaware, Newark, DE 19711, U.S.A.
** Permanent address: Centro de Biofisica y Bioquimica, IVIC, Caracas. Venezuela.

Abbreviations: dialkylphosphatidylcholine, 1,2-di(tetradecyl)-*rac*-glycero-3-phosphocholine; lysophosphatidylcholine, 1-tetradecanoyl-*sn*-glycero-3-phosphocholine; diacylphosphatidylcholine, 1,2-di(tetradecanoyl)-*sn*-glycero-3-phosphocholine.

0005-2736/82/0000–0000/$02.75

the various aggregated forms of a substrate the rate of hydrolysis is initially slow but becomes faster as products are formed, thus giving rise to a latency period in the progress curves of product formation [1,6,7,11]. In monolayers the latency period is found to depend upon the composition, surface pressure and charge [1], and in bilayers it depends upon the phase-transition temperature and other determinants leading to defects in bilayers [6,7]. Also, for enzymes from different sources the latency period is found to be different [1,6,7]. This initial presteady-state step can be slow for one or more of the following reasons: (a) the binding of the enzyme to the interface is slow; (b) the enzyme undergoes a slow conformational change when bound to the interface; (c) binding is fast but the actual amount of enzyme bound depends on the presence of the products; or (d) catalytic steps are affected by products.

To elaborate these possibilities it is of interest to examine the effect of both the products of hydrolysis upon the binding and kinetic properties of diacylphospholipids. A rationale for this may be found in the following observations. We have observed that binary codispersions of lysophosphatidylcholine and fatty acid form bilayers [12], whereas dispersions of either one of these components alone form micelles. Furthermore, our recent studies on ternary mixtures of diacylphosphatidylcholine, lysophosphatidylcholine and fatty acid have demonstrated [13] that these codispersions have unique phase-transition properties and do not ideally mix in bilayers. In this paper we report that phospholipid bilayers containing both the reaction products are considerably better substrates for the pig-pancreatic phospholipase A_2; that no latency period is observed in the presence of the products; that the binding of the pancreatic phospholipase A_2 to substrate bilayers is enhanced more than 30-fold in the presence of products; that the apparent binding and kinetic constants are comparable; and that none of these effects is observed when only one of the products is added to the substrate bilayer. These observations demonstrate that in phospholipid bilayers the apparent autocatalytic reaction progress curve for pig-pancreatic phospholipase A_2 is due to product-assisted binding of the enzyme, and the fraction of the total enzyme bound to the bilayer depends upon the mole proportion of the products in the substrate bilayer. Thus in the presteady-state phase of the reaction progress curve, the rate of reaction is determined by the fraction of the total enzyme bound to the substrate bilayer.

Materials and Methods

Pure phospholipase A_2 from pig pancreas [14] and *Naja melanoleuca* fraction DE-III [15] were isolated according to the published procedures. All phospholipids used in this study were synthesized as described elsewhere [16]. All other reagents were of analytical grade.

Phospholipid dispersions were prepared by sonicating the dried film of premixed phospholipid(s) with appropriate buffer in a closed tube in a bath-type sonicator. Standard buffer consisted of 50 mM Tris/100 mM KCl/25 mM $CaCl_2$ at pH 8.0. The vesicles were 'annealed' by keeping them at more than 10°C above the transition temperature of the dispersions for 2 h. For all studies reported here we have used the dispersions that are obtained after equilibration for more than 10 h.

Differential scanning calorimetry was performed with a Perkin-Elmer DSC-2 instrument on samples placed in sealed aluminum pans [17]. Each sample pan contained about 1–2 μmol lipid in 15 μl buffer. Kinetics of phospholipase hydrolysis were followed by pH-stat titration of the released fatty acids [6,14]. Freeze-fracture electron microscopy was done on samples quenched from 25°C according to the published procedures [18].

Gel filtration studies were performed on a thermostatically controlled Sephadex G-100 column (40 × 1.5 cm). These studies were performed under non-equilibrium binding conditions on the column. Thus, a mixture of phospholipase (100–500 μg) and phospholipid dispersion (1.5–3.5 mg phospholipid) in 100–300 μl buffer was applied to the column and then eluted with the equilibration buffer. About 5–6-ml fractions were collected to ensure that separation between enzyme (elution volume about 60 ml) and phospholipid dispersions (elution volume about 25 ml) was adequate. The elution rate was about 10–15 ml/h.

Fluorescence measurements were made on a Perkin-Elmer MPF-3 spectrophotometer essen-

tially as described by Van Dam-Mieras et al. [19]. The excitation wavelength was set at 280 nm. Proteins (5–7 μM) were dissolved in standard buffer. The observed change in the fluorescence intensity (expressed as percentage change relative to initial intensity) and the blue shift of the emission maximum were stable within 5 min and were used to derive binding parameters.

Since phospholipid dispersions could contribute to the emission spectrum by scattering and filtering the incident and the emitted light, such a contribution was evaluated as follows. By measuring the emission intensity of a suspension at 340 nm (excitation at 280 nm) and at 420 nm (excitation at 360 nm) a calibration curve was generated from which the contribution due to scattering in the emission spectrum at 335–340 nm could be obtained by measuring the emission intensity at 420 nm (excitation at 360 nm) for the phospholipid and phospholipase mixture. The contribution from scattering varies significantly for different dispersions. Therefore, calibration curves were generated for each phospholipid dispersion. A scattering contribution of under 5% of the total fluorescence intensity was considered acceptable for all the binding isotherm data. The binding curves were fitted to the equation outlined in the Results section by nonlinear regression analysis. Concentrations of lipid and enzyme were corrected for dilution. The binding isotherm is characterized by three parameters as defined later. The goodness of fit is expressed as standard deviation. Covariances of the three parameters were also evaluated to establish independence and reliability of these parameter values.

Results

Gel filtration behavior of phospholipid and phospholipase A_2 dispersions on Sephadex G-100 column

We studied the gel filtration behavior of mixtures of phospholipase A_2 with the various nonsubstrate phospholipid dispersions in order to ascertain binding without catalysis. As shown in Table I, venom phospholipase A_2 binds tightly to vesicles of pure dialkylphosphatidylcholine and also to vesicles of the ternary mixture. In contrast, the porcine-pancreatic enzyme binds only to vesicles of the ternary mixture.

TABLE I

FRACTION OF PHOSPHOLIPASE A_2 COELUTED WITH PHOSPHOLIPID DISPERSIONS ON A SEPHADEX G-100 COLUMN

Enzyme	Lipid mixture	Percent bound
Naja	dialkylphosphatidylcholine	95
Naja	ternary [a]	98
Pig	dialkylphosphatidylcholine	2
Pig	lysophosphatidylcholine + myristic acid (1 : 1)	5
Pig	dialkylphosphatidylcholine + myristic acid (1 : 1)	5
Pig	dialkylphosphatidylcholine + lysophosphatidylcholine (1 : 1)	5
Pig	ternary [a]	98

[a] Ternary: equimolar mixture of dialkylphosphatidylcholine, lysophosphatidylcholine and myristic acid.

Thermotropic transition characteristics of phospholipid dispersions in the presence of phospholipases

The hydrophobic binding of phospholipases to phospholipid dispersions could change their thermotropic gel-to-liquid crystalline transition behav-

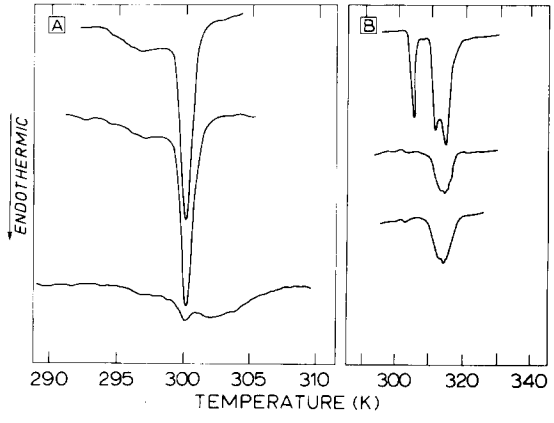

Fig. 1. Phase transition profiles. A. Of dialkylphosphatidylcholine in the absence (upper curve) and in the presence of porcine pancreatic enzyme (molar ratio, 40 : 1, middle curve) and in the presence of venom enzyme (molar ratio 40 : 1, lower curve). B. Of an equimolar mixture of dialkylphosphatidylcholine, lysophosphatidylcholine and myristic acid containing no additive (upper), porcine pancreatic enzyme (molar ratio 40 : 1, middle) and venom enzyme (molar ratio 40 : 1, lower).

ior. As shown in Fig. 1, the thermotropic transition behavior of dialkylphosphatidylcholine dispersions is indeed altered drastically in the presence of *N. melanoleuca* enzyme. In contrast, the transition profile remains essentially unchanged in the presence of pig-pancreatic phospholipase A_2. The thermotropic transition behavior of the ternary mixtures is somewhat unique in the sense that they exhibit multiple transitions suggesting phase separation. The precise nature of the various phases giving rise to this complex transition is not known (see, however, Ref. 13). It is striking to note that both the venom and the pancreatic enzymes modify the transition profile of the ternary dispersions. These results are in accord with the gel-filtration data.

Binding studies by fluorescence enhancement

Binding of venom phospholipase A_2 to vesicles of pure dialkylphosphatidylcholine can be followed by the change in fluorescence intensity [19]. In accordance with the results summarized in the preceding section, no fluorescence change was found for pancreatic enzyme, whereas the venom enzyme shows a fluorescence change upon binding to the vesicle of pure diether-PC_{14}. Binding of the pancreatic phospholipase A_2 was not seen even after long periods of incubation (up to 24 h) with diether-PC_{14} vesicles at the phase transition temperature. Additional experiments not shown here demonstrate that Ca^{2+} is required for binding, and the affinity for Ca^{2+} increases with increasing pH. These experiments demonstrate that Ca^{2+} is required for binding of the enzyme to the substrate, and incorporation of both the products favors the binding of the pancreatic enzyme, presumably by increasing the number of binding sites.

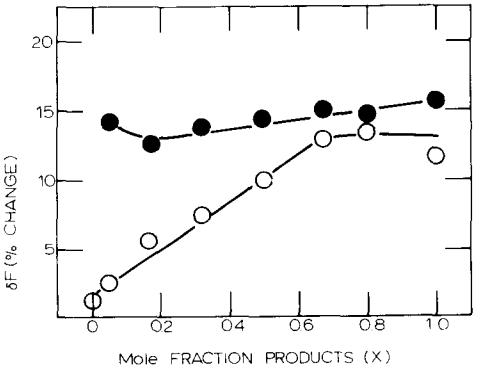

Fig. 2. Change in fluorescence intensity of *N. melanoleuca* (●—●) and pig pancreatic phospholipase (○———○) upon addition of dialkylphosphatidylcholine dispersions containing varying mole fractions, *X*, of lysophosphatidylcholine/myristic acid mixture (1 : 1). Total lipid concentration for each run was 100 μM as the sum of diether-PC_{14} or diether-PC_{14} + products.

The effect of varying the mole fraction of products in the ternary codispersions on the fluorescence intensity of the porcine and the venom enzyme is shown in Fig. 2. It demonstrates that the presence of products hardly influences the binding of the venom enzyme, whereas the binding of the pancreatic enzyme changes drastically as a function of the mole fraction of the products. It should be pointed out that, based on the increase in fluorescence intensity, binding of both the enzymes to the appropriate substrate is fast (half-time less than 10 s), suggesting that the rate of penetration of the enzyme is not a rate-limiting step in the reaction progress curves (see below).

Characterization of the binding isotherm: definition of the binding parameters

Quantitative information about the interaction of phospholipase A_2 with phospholipid bilayers can be adduced from a binding isotherm obtained by measuring the change in fluorescence intensity at various lipid concentrations. For the interpretation of such data we used the following approach [20]. Suppose an enzyme molecule, E, interacts with an interface binding site, I, according to equilibrium 1:

$$E + I \overset{K_d}{\rightleftharpoons} EI \qquad (1)$$

It is further assumed these sites are independent and composed of *n* lipid molecules. Therefore,

$$[I]_T = \frac{[L]_T}{n}$$

where $[I]_T$ is the total concentration of binding sites I and $[L]_T$ is the total concentration of lipid added.

Since $[E]_T = [E] + [EI]$, where $[E]_T$, $[E]$ and $[EI]$ are total, free and bound enzyme concentrations, respectively, we define the dissociation constant for the equilibrium expression 1 as:

$$K_d = \frac{[\text{free enzyme}][\text{free lipid}]}{[\text{complex}]} \qquad (2)$$

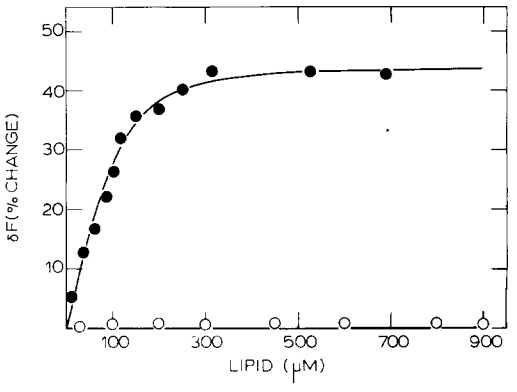

Fig. 3. Change in fluorescence intensity (δF) of *N. melanoleuca* venom phospholipase (●——●) and of the phospholipase from pig pancrease (○——○) in the presence of dialkylphosphatidylcholine vesicles at 20°C.

or

$$K_d = \frac{([E]_T - [EI])([L]_T/n - [EI])}{[EI]} \quad (3)$$

or

$$\frac{[E]_T}{[EI]} = 1 + \frac{nK_d}{[L]_T - n[EI]} = \frac{\delta F_{max}}{\delta F} \quad (4)$$

and $\delta F_{max} = C \cdot [E]_T$, where δF is the observed change in the fluorescence intensity, δF_{max}, the value of δF under saturation conditions and C the molar fluorescence change. $n \cdot K_d$ describes an apparent dissociation constant expressed as lipid monomer concentration.

On the basis of expression 4 a titration or binding curve of the type shown in Fig. 3 can be used to generate not only C but also n and the dissociation constant K_d of the enzyme-interface (site) complex. The binding isotherms of the type shown in Fig. 3 were fitted to expression 4 by nonlinear regression analysis. The values of parameters n, and K_d for serveral systems are presented in Table II. These values show that K_d and n for the two enzymes are different. The significance of these two parameters can be elaborated by the following experiments.

Binding of phospholipase A_2 to bilayers as a function of temperature

Since the changes in the fluorescence intensity of the venom enzyme, but not of the pancreatic enzyme, are reasonably high for systems containing pure acyl- or alkylphosphatidylcholines, only the binding of the former enzyme was studied at several temperatures. As shown in Fig. 4, the maximal change in fluorescence intensity (C) upon lipid binding decreases only slightly as a function of temperature. Both K_d and n change appreciably both below and above the transition temperature, whereas these parameters remain fairly constant over the range of thermotropic phase transition. Thus, n decreases with increasing temperature, whereas K_d increases rather steeply with increasing temperature both below and above the phase-transition temperature range.

TABLE II

BINDING AND KINETIC PARAMETERS FOR PHOSPHOLIPASE A_2

All experiments were conducted at 25°C. Binding experiments were done with 4–7 μM protein. Kinetic experiments were done with 5 nM enzyme. Diether is 1,2-ditetradecyl-3-glycerophosphocholine; ternary-1: equimolar mixture of diether, myristoyllysophosphatidylcholine and myristic acid; ternary-2: equimolar mixture of didecanoylphosphatidylcholine, myristoyllysophosphatidylcholine and myristic acid. The dispersions for kinetic experiments contain hydrolyzable *sn*-3-ester analogs. The binding experiments were done with the ether or the *sn*-1-ester analogs. Both K_d and K_m are given as μM. As discussed in the text, the apparent dissociation constant, $n \cdot K_d$, is expected to be comparable to K_m if binding of the enzyme depends upon the substrate concentration during the catalysis. The K_m was determined as reported elsewhere [5,6,29].

Lipid	Phospholipase source	K_d	n	K_m
Diether	*N. melanoleuca*	0.15–0.25	24	17.8
Ternary-1	*N. melanoleuca*	0.5 –0.8	25–35	38.4
Ternary-1	Pig pancreas	1.5–3.5	54–60	450
Ternary-2	Pig pancreas	2 –3	40–50	400

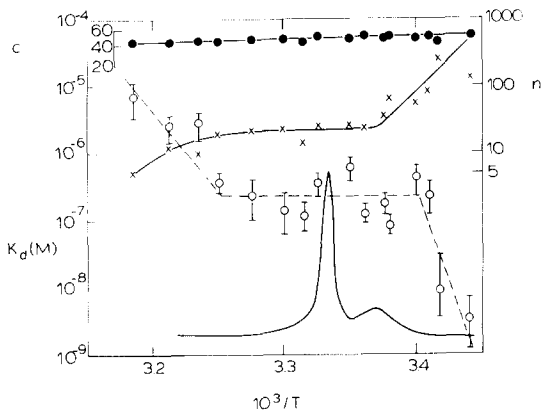

Fig. 4. Dependence of the binding parameters for *N. melanoleuca* phospholipase to dialkylphosphatidylcholine vesicles on temperature; dissociation constant, K_d (O——O); the number of phospholipid molecules per enzyme molecule in the complex, n (×——×). Inset: the maximal change in fluorescence intensity (%) when all the protein is bound to the bilayers, C (●——●). The lower curve is the thermotropic phase transition profile of dialkylphosphatidylcholine vesicles drawn on the reciprocal temperature scale. Note that the temperature range for the phase transition profile of the vesicles (as shown here) is much narrower compared to the temperature range for the discontinuity in the Arrhenius plots for K_d or for n. As shown in Fig. 1, incorporation of the venom phospholipase A_2 into dialkylphosphatidylcholine vesicles raises their phase transition temperature, and the temperature range of the phase transition is also wider.

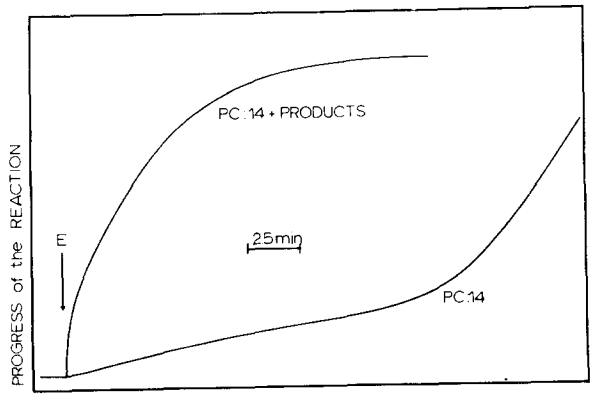

Fig. 5. Reaction progress curves for the hydrolysis of dimyristoylphosphatidylcholine vesicles containing 0 or 20 mol% preincorporated products. The detailed features of such reaction progress curves depend upon a variety of factors as described in the following paper. The curves reproduced here were obtained at 37°C and pH 8.0 with pig-pancreatic phospholipase A_2 (175 ng/5 ml) and 250 μM substrate.

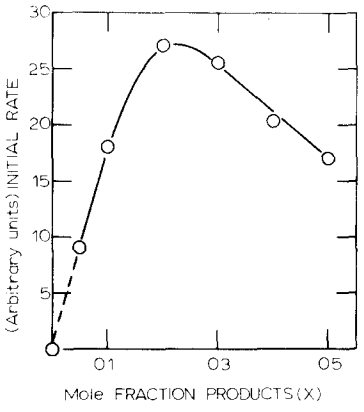

Fig. 6. Initial rates of hydrolysis of diacylphosphatidylcholine vesicles containing varying mole fractions (X) of lysophosphatidylcholine + myristic acid (1 : 1). Total lipid concentration, 250 μM; T, 20°C; Pig-pancreatic phospholipase A_2 concentration, 250 ng/5 ml.

Kinetics of hydrolysis of the substrate in bilayers

Direct binding studies described so far demonstrate that the porcine-pancreatic phospholipase binds to bilayers only in the presence of both the products. Since binding of the enzyme must precede its catalytic action, one would expect a dependence of the rate of hydrolysis on the mole fraction of the products in the substrate bilayer. Fig. 5 shows reaction progress curves for the hydrolysis of dimyristoylphosphatidylcholine vesicles in the presence and absence of both the products: neither of the products alone eliminates the latency. The latency period seen in the absence of products depends upon the enzyme, substrate and the product concentrations (Ref. 6, and unpubslihed data). Furthermore, as shown in Fig. 6, the initial rate of hydrolysis increases with the mole fraction of the products up to about 25%. At higher mole fractions the rate of hydrolysis decreases, probably due to substrate dilution at the interface. The Michaelis-Menten kinetic constant for several phospholipid dispersions are compared with the corresponding binding constants in Table II. The apparent binding constant ($n \cdot K_d$) and the kinetic constant are in reasonable agreement.

Discussion

The results reported in this paper demonstrate that a correlation exists between the bilayer binding properties and the catalytic properties of phospholipase A_2. The data show substantial differences between pancreatic and snake-venom enzymes in their lipid-binding properties. As is evident from the phase-transition profiles in Fig. 1, the venom enzyme binds to vesicles of the substrate analog dialkylphosphatidylcholine and modifies the bilayer structure. In contrast, porcine pancreatic enzyme does not bind to vesicles of the pure substrate analog, but it does bind when both lysophosphatidylcholine and fatty acid are present as well. Although the ternary disperisons have been shown to consist of lipid bilayers, phase separation occurs [13]. Increased binding of the porcine enzyme in the presence of products is due to a more favorable binding equilibrium rather than an increase in the rate of binding. This is probably due to an increase in the number of enzyme-binding sites on the bilayer. It is likely that these sites are at the phase boundaries in the ternary phase separated bilayers.

The conclusions derived from the gel-filtration and differential scanning calorimetry experiments are confirmed by fluorescence spectroscopy studies (Fig. 2). Binding of the snake venom enzyme to pure substrate analog is indicated by the increase in flurescence intensity, while no effect is observed for the porcine enzyme, as expected. The binding data for the snake venom enzyme can be further evaluated by adopting the model in which the enzyme is considered to bind micelles of phospholipid analogs [21]. By assuming that phospholipase A_2 binds to discrete and independent sites in bilayers, we have used the same formalism. When the fluorescence data are fitted by non-linear regression analysis to the equation derived for the model, one obtains the dissociation constant, K_d, the number of lipid molecules per protein in the lipid-protein complex, n, and the maximum fluorescence signal per protein concentration, C. Fig. 4 summarizes the effect of temperature on the binding parameters, C, n and K_d. The maximum fluorescence signal, C, remains constant over the whole temperature range, indicating that temperature has no influence on the enzyme itself over the range investigated. The other two parameters are affected: K_d increases, while the stoichiometry factor n, decreases with increasing temperature. Interestingly, both of these parameters remain fairly constant over the range of phase transition. It appears that the state of lipid molecules in the coexisting phases remains unchanged while temperature affects only the relative proportions of lipid molecules in the different phases. Outside the phase transition range the parameters n and K_d change as expected for hydrophobic interaction. One can only speculate on the exact nature of the lipid-protein complexes formed. The simplest thermodynamic interpretation is that n lipid molecules provide the interaction energy for binding of an enzyme molecule. The data are consistent with several possibilities that are not mutually exclusive: binding of one enzyme molecule modifies the conformational state of n lipid molecules; n lipid molecules on the average provide one bonding site; $(EL_n)_x$ type of complex is formed which may persist in a bilayer or disrupt into micelles. It is quite likely that the interaction energy arises from *gauche* to *trans* conformational change in n acyl chains.

The binding properties of porcine pancreatic phospholipase A_2 is correlated with the kinetics of hydrolysis of diacylphophatidylcholine bilayers. Both the extent of binding (cf. Fig. 2) and the rate of hydrolysis (Fig. 6) increase with the increasing mole fraction of the products in bilayers. A decrease in the rate of hydrolysis above 25 mol% products is most probably due to surface dilution of the substrate. For a detailed study of temperature dependence of the reaction progress curve, see the next paper [29]. It is, however, certain that there is little increase in the binding of the enzyme to the pure substrate bilayer at the phase transition temperature. An increase in binding is seen only in the presence of both the products. The observed minimum in the latency phase at the phase-transition temperature [6] is probably due to an increased rate of lateral diffusion, or intervesicular exchange of the products, or due to an increased turnover of the enzyme bound to bilayers at the phase-transition temperature.

It has been suggested that the binding of phospholipase A_2 to bilayers is facilitated by a negative charge on the substrate interface [22] or by an

increase in the phase-transition temperature of the substrate bilayer [23]. Our observation that the incorporation of fatty acid alone increases neither the binding nor the rate of hydrolysis essentially rules out these suggestions, since the phospholipid bilayers containing fatty acids satisfy both of these conditions (see also Ref. 5). Similarly, the data presented in this paper invalidate the postulate [7,24] that the products of hydrolysis of phospholipase A_2 induce desorption of the enzyme bound to the interface. In fact, the product-induced formation of organizational defects in bilayers and possible modulation and regulation of membrane proteins [25–28] by such sites is important for the activation of phospholipase A_2, and it offers a new dimension in regulation of membrane functions by phospholipase A_2.

Acknowledgment

This work was supported by NATO grant No. 051.81.

References

1 Verger, R. and De Haas, G.H. (1976) Annu. Rev. Biophys. Bioeng. 5, 77–117
2 Pattus, F., Slotboom, A.J. and De Haas, G.H. (1979) Biochemistry 18, 2691–2697
3 Dennis, E.A. (1973) J. Lipid Res. 14, 152–159
4 Jain, M.K. and Cordes, E.H. (1973) J. Membrane Biol. 14, 101–118
5 Upreti, G., Rainier, S. and Jain, M.K. (1980) J. Membrane Biol. 55, 97–112
6 Upreti, G. and Jain, M.K. (1980) J. Membrane Biol. 55, 113–123
7 Tinker, D.O. and Wei, J. (1979) Can. J. Biochem. 57, 97–107
8 Op den Kamp, J.A.F., De Gier, J. and Van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 345, 253–258
9 Op den Kamp, J.A.F., Kauerz, M.Th. and Van Deenen, L.L.M. (1975) Biochim. Biophys. Acta 406, 169–177
10 Wilschut, J.C., Regts, J., Westenberg, H. and Scherphof, G. (1978) Biochim. Biophys. Acta 508, 185–196
11 Rosenthal, A.F. and Pousada, M. (1968) Biochim. Biophys. Acta 164, 226–237
12 Jain, M.K., Van Echteld, C.J.A., Ramirez, F., De Gier, J., De Haas, G.H. and Van Deenen, L.L.M. (1980) Nature 284, 486–487
13 Jain, M.K. and De Haas, G.H. (1981) Biochim. Biophys. Acta 642, 203–211
14 Nieuwenhuizen, W., Kunze, H. and De Haas, G.H. (1974) Methods Enzymol. 32B, 147–154
15 Joubert, F.J. and Van der Walt, S.J. (1975) Biochim. Biophys. Acta 379, 317–328
15 Slotboom, A.J., Verheij, H.M. and De Haas, G.H. (1973) Chem. Phys. Lipids 11, 295–317
17 De Kruyff, B., Van Dijck, P.W.M., Demel, R.A., Schuijff, A., Brants, F. and Van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 356, 1–7
18 Ververgaert, P.H.J.Th., Verkleij, A.J., Elbers, P.F. and Van Deenen, L.L.M. (1973) Biochim. Biophys. Acta 311, 320–329
19 Van Dam-Mieras, M.C.E., Slotboom, A.J., Pieterson, W.A. and De Haas, G.H. (1975) Biochemistry 14, 5387–5394
20 Hille, J.D.R., Donné-Op den Kelder, G.M., Sauve, P., De Haas, G.H. and Egmond, M.R. (1981) Biochemistry 20, 4068–4073
21 De Araujo, P.S., Rosseneu, M.Y., Kremer, J.M.H., Van Zoelen, E.J.J. and De Haas, G.H. (1979) Biochemistry 18, 580–586
22 Dawson, R.M.C. (1969) Methods Enzymol. 14, 633–648
23 Kensil, C.R. and Dennis, E.A. (1979) J. Biol. Chem. 254, 5843–5848
24 Tinker, D.O., Purdon, A.D., Wei, J. and Mason, F. (1978) Can. J. Biochem. 56, 552–558
25 Linden, C.D., Wright, K.L., McConnell, H.M. and Fox, C.F. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 2271–2275
26 Jain, M.K. and White, H.B. (1977) Adv. Lipid Res. 15, 1–60
27 Swaney, J.B. and Chang, B.C. (1980) Biochemistry 19, pp. 5637–5644.
28 Boggs, J.M., Clement, I.R. and Moscarello, M.A. (1980) Biochim. biophys. Acta 601, 134–151
29 Apitz-Castro, R., Jain, M.K. and De Haas, G.H. (1982) Biochim. Biophys. Acta 688, 349–356