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# Dehydration of the lipid–protein microinterface on binding of phospholipase $A_2$ to lipid bilayers

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**A novel method is described to demonstrate inaccessibility to the bulk aqueous phase of the microinterface between pig pancreatic phospholipase $A_2$ and lipid bilayers to which this protein is bound. The method is based on the fact that the fluorescence emission quantum yields of the tryptophan residue of the protein and of a 5-dimethylaminonaphthalene-1-sulfonyl (dansyl) chromophore attached to a lipid are lower in water as compared to that in deuterated water. The fluorescence emission quantum yield of these chromophores is measured in water and in deuterated water under conditions where the protein is either bound or not bound to the surface of a lipid bilayer containing the dansyl chromophore. Under conditions where the protein is tightly bound to the surface of the bilayer, desolvation of both fluorophores abolishes the observed effect of deuterated water. The tryptophan residue in the bound phospholipase $A_2$ also becomes inaccessible to fluorescence quenching by acrylamide or succinimide. Desolvation of the microinterface is observed only under conditions that are significant for the catalytic action of phospholipase $A_2$ in the scooting mode and not in the hopping mode. Also, under similar conditions, binding of pro-phospholipase $A_2$ to anionic vesicles does not cause dehydration of the microinterface. The mechanistic significance of these observations for lipid–protein interactions, in general, and for interfacial catalysis and interfacial activation, in particular, is discussed.**

## Introduction

During the catalytic action of phospholipase $A_2$ on vesicles of anionic phospholipids in the scooting mode, the binding of the enzyme to the bilayer surface is essentially irreversible [1–4]. In order to account for such a high affinity of phospholipase $A_2$ to the bilayer it was postulated that the binding is accompanied by desolvation of the microinterface between the enzyme and the bilayer, and the resulting phospholipase $A_2$-bilayer complex facilitated the formation of an interfacial Michaelis-Menten complex between the bound enzyme and the monomeric substrate in the interface [4]. In this report we demonstrate that a dehydration of the microinterface between phospholipase $A_2$ and the bilayer does indeed occur under conditions that are significant for the catalytic action of phospholipase $A_2$ in the scooting mode.

Abbreviations: dansyl-, 5-dimethylaminonaphthalene-1-sulfonyl-; HPE, hexadecylphosphorylethanolamine; Trp-3, the tryptophan-3 residue in phospholipase $A_2$ from pig pancreas; TTGPE, 1,2-ditetradecyl-*rac*-3-phosphatidylethanolamine; TTGPMe, ditetradecylphosphatidylmethanol.

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The photophysical basis of the method for establishing dehydration of the protein–lipid microinterface is based on the observation that the fluorescence quantum yields of tryptophan [2,5] and dansyl [6,7] chromophores are lower in water compared to their quantum yields in deuterated water. This difference is attributed to specific interactions between the solvent and the chromophore in the excited state [5–8], and it has been used to demonstrate the accessibility of protein-bound chromophores to the bulk solvent [7]. In this paper we present evidence which shows that both Trp-3 of the protein and lipid-attached dansyl chromophore in the microinterface between the protein and the bilayer have a considerably reduced solvent accessibility as compared to that in the free bilayer or free protein.

## Materials and Methods

Synthesis of phospholipids, preparation of vesicles, sources of reagents and phospholipase $A_2$ from pig pancreas, and other general protocols used in this work have been described elsewhere [1–4]. Dansyl-HPE was synthesized by reaction of 0.1 mmol dansylchloride (Sigma, > 95% pure) with HPE (0.095 mmol) in the presence of triethylamine (0.15 mmol) in 10 ml tetrahydrofuran at room temperature for 48 h. The residue, after removal of the solvent was purified on silica gel to yield (0.09 mmol) homogeneous dansyl-HPE. Dansyl-TTGPE was synthesized similarly from TTGPE.

All fluorescence measurements reported in this paper were taken at 25 °C in 10 mM Tris-HCl at pH 8.0 (or 7.6 for solutions in deuterated water) on a Hitachi F4000 or SLM 4800 spectrofluorimeters. All spectra are corrected. Although most of the studies have been repeated with dansyl-TTGPE, only the results with dansyl-HPE are reported here. Results with dansyl-TTGPE were essentially identical. The experimental advantage of using dansyl-HPE is the fact that it can be added to preformed vesicles where it distributes only in the outer monolayer. Specific experimental conditions are given in the figure legends.

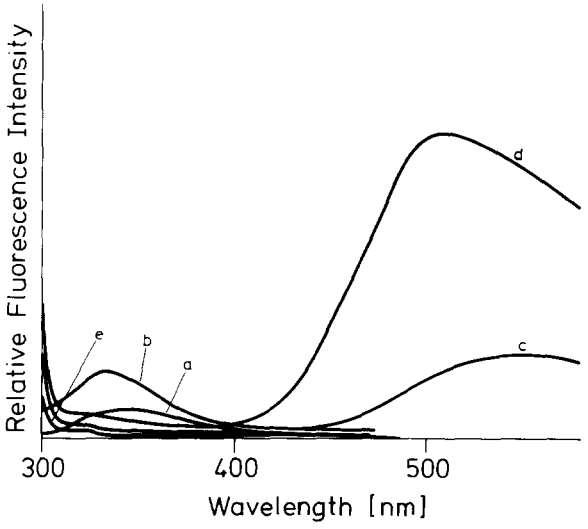



Fig. 1. Corrected fluorescence emission spectra of (a) 3.7 $\mu$M phospholipase $A_2$ (PLA), (b) 3.7 $\mu$M PLA + 0.3 mM TTGPMe, (c) 0.3 mM TTGPMe + dansyl-HPE (molar ratio of 16 : 1), (d) 3.7 $\mu$M PLA + 0.3 mM TTGPMe + dansyl-HPE (molar ratio of 16 : 1), and (e) 0.3 mM TTGPME in 10 mM Tris -HCl/0.5 mM $CaCl_2$/pH 8.0 at 25 °C. Excitation at 290 nm, excitation and emission bandpasses were 5 nm. The contribution of scatter is shown by curve (e).

## Results

Upon binding of PLA to vesicles of TTGPMe containing dansyl-HPE (or dansyl-TTGPE), at a molar ratio of 16 : 1, several effects are observed. First, resonance energy transfer occurs between the Trp-3 in the protein (as donor) and the dansyl group on the lipid bilayer surface (as acceptor) when the Trp-3 is excited at 290 nm. The occurrence of such energy transfer can be used to monitor binding of proteins to lipid bilayers in general [9]. As shown in Fig. 1, on binding of PLA to dansyl-HPE-containing TTGPMe vesicles the Trp-3 fluorescence emission at 333 nm (excitation at 290 nm) is less than 3% of what it would have been in the absence of dansyl-HPE. The calculated efficiency of energy transfer is > 95% which suggests that the protein Trp-3 and the dansyl chromophore at the lipid bilayer interface are separated by a distance of < 8 Å based on a critical transfer distance of 17–18 Å for the Trp-3-dansyl pair [7,9]. Control experiments, the results of which are not shown here, demonstrate that the decrease in fluorescence intensity at 335 nm and the increase in intensity at 500 nm is a monotonic function of dansyl-HPE concentration

in the system. This effect is qualitatively the same in deuterated water and water. This means that (1) the probe does not bind specifically to phospholipase $A_2$, (2) deuterated water has no anomalous effect, and (3) the probe is randomly distributed in the bilayers.

Second, a spectral blue-shift of the fluorescence emission of the dansyl chromophore is observed upon binding of PLA to the lipid vesicle surface. This shift, from 545 nm in the absence of the protein to 493 nm when all of the surface of the lipid vesicle is covered by the protein, suggests that the polarity of the microenvironment of the dansyl chromophore is lower at the phospholipase $A_2$-bilayer microinterface as compared to the bilayer-water interface [7]. The blue shift is independent of whether the excitation of the dansyl chromophore is done at 290 or 346 nm (as shown later in Figure 2). This blue shift could be due to removal of water from the PLA-lipid microinterface, as shown to be the case below, or it could also occur if that part of the PLA which adsorbs to the surface of the lipid bilayer is an environment of a relatively low dielectric constant. The marked time-dependent shifts in the emission energy of the dansyl chromophore due to solvent reorientational processes (10) complicates the interpretation of this observation. All these possibilities are not mutually exclusive.

Third, as seen in Fig. 2, the fluorescence emission quantum yield of the dansyl group of dansyl-HPE either alone in the aqueous solution or in vesicles of TTGPMe is higher in deuterated water than in water. This difference is due to a specific interaction of the excited state of the dansyl chromophore with its solvent cage and does not involve excited state proton transfer reactions [6–8]. When phospholipase $A_2$ completely covers the surface of TTGPMe vesicles containing dansyl-HPE the fluorescence quantum yield of the dansyl group in water or deuterated water is the same (Fig. 2). This lack of effect of deuterated water on the dansyl fluorescence emission quantum yield in the protein-lipid vesicle complex is interpreted to be the result of desolvation of the dansyl chromophore in this system. It may be noted that in Fig. 2 excitation was at 346 nm (where Trp-3 does not absorb) but the effect is also observed if excitation is at 290 nm (as shown in Fig. 1).

It is of interest to note that the fluorescence quantum yield of Trp-3 shows a similar behaviour

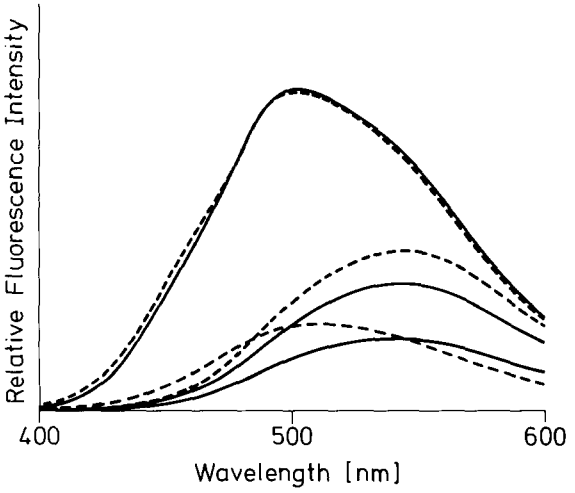



Fig. 2. Fluorescence emission spectra (from bottom) for dansyl-HPE (6 $\mu$M) in water (solid line) and in deuterated water (dotted line), dansyl-HPE (6 $\mu$M + TTGPMe (0.15 mM) in water (solid line) and deuterated water (dotted line), and dansyl-HPE (6 $\mu$M) + TTGPMe (0.15 mM) + phospholipase $A_2$ (12 $\mu$M) in water (solid line) and deuterated water (dotted line). Excitation was at 345 nm. Other conditions as in Fig. 1. The data points given in Fig. 5 were obtained from curves of the type shown in this figure.

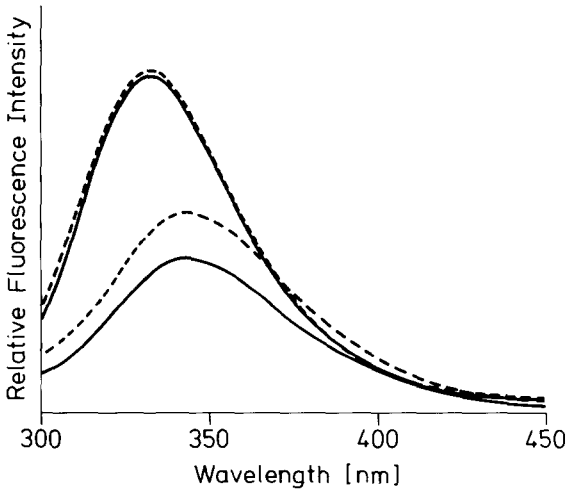



Fig. 3. Fluorescence emission spectra (from bottom) for phospholipase $A_2$ (PLA) in water (solid line) and in deuterated water (broken line), for PLA + TTGPMe (1:100) in water (solid line) and in deuterated water (broken line). Excitation was at 290 nm. Other conditions were as in Fig. 1.

to that of the dansyl group [2]. As seen in Fig. 3, the fluorescence quantum yield of Trp-3 when phospholipase $A_2$ is not bound to a lipid bilayer surface is lower in water than it is in deuterated water. There is, however, no difference in the quantum yield of Trp-3 when the phospholipase $A_2$ is bound to the surface of TTGPMe vesicles. The effect of deuterated water on tryptophan fluorescence is complex [5] (see also Ref. 2) but could also be accounted for by a desolvation of the Trp-3 residue upen phospholipase $A_2$ binding to the vesicle surface.

The inaccessibility of Trp-3 in phospholipase $A_2$ bound to TTGPMe vesicles was also monitored with water-soluble quenchers like acrylamide and succinimide. As shown in Fig. 4, Trp-3 in the free phospholipase $A_2$ or pro-phospholipase $A_2$ is readily accessible to acrylamide. Although the Stern-Volmer plot is complex the limiting quenching constant, derived from the linear part of the plot, is only slightly smaller than that which is expected for a tryptophan residue that is freely accessible to the aqueous phase. On the other hand, on binding of phospholipase $A_2$ to TTGPMe vesicles the Trp-3 is almost inaccessible to quenching by acrylamide.

With the preceeding results we have provided evidence for desolvation of the phospholipase

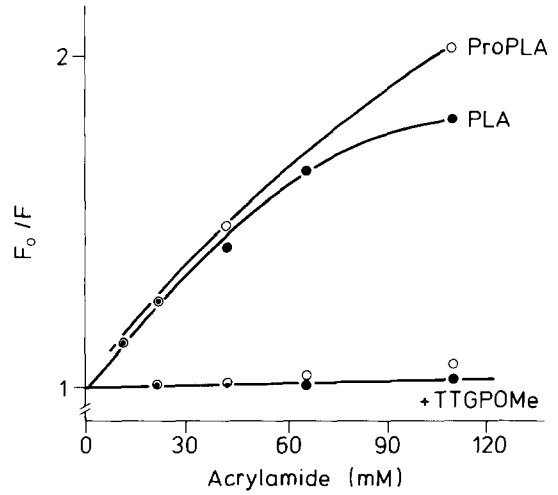



Fig. 4. Stern-Volmer plots for the quenching of tryptophan-3 in phospholipase $A_2$ (PLA) (excitation at 300 nm, emission at 350 nm) by acrylamide. Other conditions as in Fig. 1.

$A_2$–lipid microinterface as sensed by chromophores attached to both the protein and the lipid partners in the interaction.

Energy transfer between tryptophan residues in proteins and lipid-bound dansyl groups is useful for titration of lipid-protein association. In the case under study a decrease in the Trp-3 fluorescence emission intensity at 333 nm (excitation at 290 nm) is seen when phospholipase $A_2$ binds to TTGPMe vesicles which contain dansyl-HPE. The dansyl fluorescence emission also increases upon protein binding to the vesicles in addition to the blue shift in fluorescence (Fig. 2). The increase in dansyl emission intensity (followed at 495 nm) is partly due to energy transfer from the protein Trp-3 and partly due to the fact that the environment of the dansyl group in the protein–lipid microinterface is more apolar as described above. The Trp-3 to dansyl energy transfer occurs only when excitation is done at 290 nm, a wavelength where Trp-3 absorbs (Fig. 1). When excitation is at 346 nm (Fig. 2) the increase in dansyl fluorescence emission intensity is only due to environmental effects on the dansyl emission. Monitoring the decrease in Trp-3 fluorescence emission at 333 nm or the increase in dansyl fluorescence emission at 495 nm provides titration curves which allow an estimation of the protein-lipid binding stoichiometry and equilibrium binding constants. From the data in Fig. 5 we obtain a lipid-protein molar stoichiometry of about 51 (increase in dansyl fluorescence without energy transfer). We have previously obtained a PLA/lipid stoichiometry of about 60 by monitoring the increase in Trp-3 fluorescence emission upon binding of phospholipase $A_2$ to TTGPMe bilayer vesicles [3]. These values of stoichiometry relate to the total lipid in the vesicles. Therefore, the actual number of lipid molecules in contact with the protein would be about 35–40.

The high-affinity binding of phospholipase $A_2$ to anionic vesicles and the desolvation of the microinterface described above could, as articulated in the model for catalysis in the scooting mode [4], be meaningful for understanding the mechanism of interfacial catalysis by phospholipase $A_2$. This is further substantiated by the following observations that emphasize the functional role in the high-affinity binding of phospholipase $A_2$ to the substrate membrane surface.

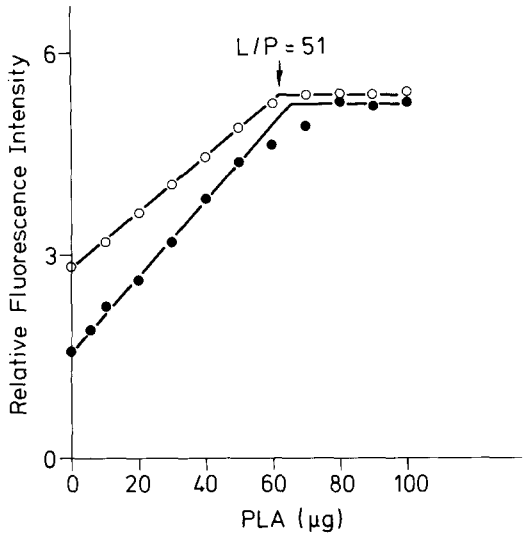



Fig. 5. Fluorescence emission intensity at 500 nm of TTGPMe (250 nmol) with dansyl-HPE (10 μg added to preformed vesicles 15 min before titration) as a function of phospholipase $A_2$ (PLA) added, in water (lower) and in deuterated water (upper). Excitation at 345 nm. The TTGPMe to PLA ratio for saturation is 51. Other conditions as in Fig. 1.

(a) The spectroscopic effects reported here are not artifacts of the introduction of dansyl-HPE into the bilayer. It has been shown elsewhere [11] that insertion of molecules analogous to the probe in the vesicles is very rapid and that the half-time for their transbilayer movement is about 8 h. We therefore believe that under our experimental conditions almost all of the dansyl-HPE is located in the external monolayer of the bilayer vesicles. Dansyl-HPE and dansyl-TTGPE exhibit essentially similar effects with the exception that the latter probe has to be introduced into the vesicles before sonication and is, therefore, located in both monolayers of the vesicles. On addition of PLA to the vesicles containing dansyl-TTGPE only that part of the probe in the external monolayers (roughly 2/3) shows the effects reported for dansyl-HPE above, whereas that probe (roughly 1/3) which is in the internal monolayers shows the same effects only after sonic or freeze-thaw induced rupture of the vesicles in the prescence of an excess of PLA. Further, the effects of deuterated water are also observed with the fluorescence emission properties of the protein Trp-3 in the absence of the dansyl probes. Also, the kinetics of hydrolysis of dimyristoylphosphatidylmethanol is not noticeably altered in the presence of over 15 mol% dansyl-HPE.

(b) Based on kinetic and binding experiments we have shown that phospholipase $A_2$ does not bind to vesicles of zwitterionic phospholipids [3,12]. This is confirmed by the lack of significant resonance energy transfer ($< 5\%$) from Trp-3 of the phospholipase $A_2$ to dansyl-HPE in vesicles of ditetradecylphosphatidylcholine (data not shown).

(c) There is ample evidence that phospholipase $A_2$ binds to micelles of alkylphospholines [13,14] and it has been shown that the relative quantum yields of Trp-3 in the presence of these micelles in water and in deuterated water are significantly different [2] implying that desolvation of the microinterface does not occur in this case. This difference in the behaviour of the phospholipase $A_2$-alkylphosphocholine micelle complex implies a difference in the quality of binding of phospholipase $A_2$ to micelles of the alkylphosphocholines.

(d) Calcium is required for interfacial catalysis by phospholipase $A_2$. All the effects related to desolvation discussed earlier essentially disappear when calcium is removed by addition of EGTA or when the binding studies are done in a medium that contains no calcium. However, energy transfer from Trp-3 to dansyl with a lower transfer efficiency is still observed but without the blue shift of the dansyl fluorescence.

(e) In phospholipase $A_2$ bound to anionic vesicles, the rate of accessibility of Trp-3 to covalent modification by 2-hydroxy-5-nitrobenzyl bromide is drastically slower (more than 20-fold) compared to the rate in the aqueous phase alone (data not shown).

(f) Catalytically meaningful binding of phospholipase $A_2$ to anionic vesicles is appreciably reversed in the presence of anions in the aqueous phase [2]. This is consistent with the observation that the efficiency of energy transfer from Trp-3 to the dansyl chromophore is reduced by increasing the sodium chloride concentration in the aqueous phase.

(g) Both kinetic and binding studies have shown that only the phospholipid molecules in the outer monolayer of vesicles are accessible to phospholipase $A_2$ added to preformed vesicles [1,3]. This is also supported by the observation that the maximum fluorescence intensity arising from

binding and energy transfer from Trp-3 of externally added phospholipase $A_2$ to dansyl-HPE codispersed with TTGPMe vesicles increases further on sonication or freeze-thaw treatment. This is because, after these treatments, the probe in the inner layers becomes accessible to the excess phospholipase $A_2$ in the external aqueous phase.

(h) The energy transfer occurring between Trp-3 of vesicle-bound phospholipase $A_2$ and a lipid-bound dansyl chromophore in the vesicles can be used to monitor the rate of exchange of the phospholipase $A_2$ between vesicles. Based on the measurement of the release of self-quenching, the intervesicle exchange time for dansyl-HPE is found to be about 10 min and for dansyl-TTGPE it is well over 5 h. Thus, by monitoring the change in dansyl-TTGPE fluoresence intensity in vesicles of TTGPMe with bound phospholipase $A_2$ upon addition of unlabelled TTGPMe vesicles, it is possible to measure the rate of intervesicle exchange of bound phospholipase $A_2$. Such experiments showed that the rate of intervesicle exchange of phospholipase $A_2$ bound to TTGPMe is well over 1 h. However, the rate of intervesicle exchange increases significantly for phospholipase $A_2$ bound to vesicles of a charged lipid that can act as a substrate for this enzyme.

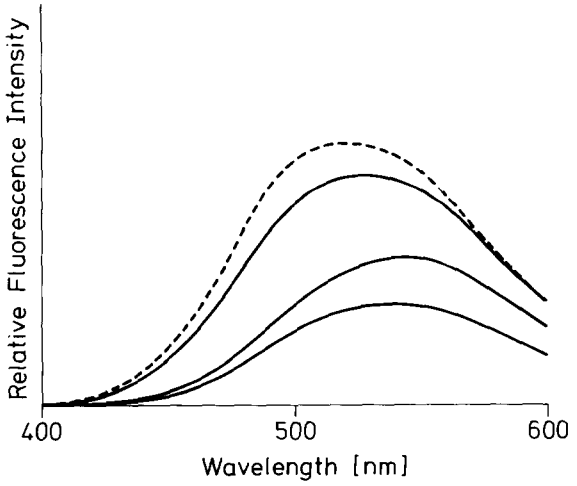



Fig. 6. Fluorescence emission spectra (from bottom) for dansyl-HPE (10 $\mu$g) in water, dansyl-HPE (10 $\mu$g) + TTGPMe (150 $\mu$g) in water, dansyl-HPE (10 $\mu$g) + TTGPMe (150 $\mu$g) + pro-PLA (80 $\mu$g) in water and in deuterated water. Excitation at 345 nm. Other conditions as in Fig. 1. PLA, phospholipase $A_2$.

(i) Pro-phospholipase $A_2$ does not exhibit interfacial catalysis. As shown in Fig. 6, binding of pro-phospholipase $A_2$ to vesicles containing dansyl-HPE shows an increase in the fluorescence intensity of the dansyl chromophore. However, the efficiency of energy transfer from Trp-3 to the dansyl chromophore in this case is $< 25\%$, the dansyl emission intensity is lower, and no significant blue shift of the dansyl fluorescence emission is observed indicating that this chromophore is in a considerably more polar environment in the pro-phospholipase $A_2$/TTGPMe complex than it is in the phospholipase $A_2$/TTGPMe complex. The fluorescence intensity of the dansyl chromophore in the pro-phospholipase $A_2$/TTGPMe complex also remains higher in deuterated water. It may also be noted that the accessibility of Trp-3 of pro-phospholipase $A_2$ to acrylamide in the free form and the bound form is essentially identical to that for phospholipase $A_2$ in the corresponding states (Fig. 4).

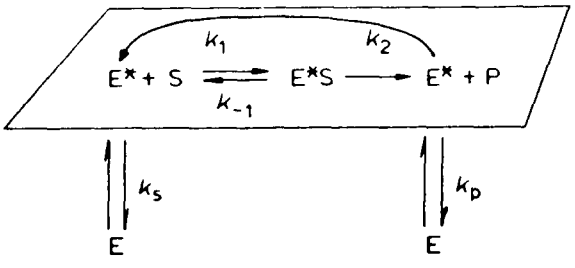
(j) The kinetics of binding of phospholipase $A_2$ to TTGPMe vesicles is virtually identical whether it is monitored as an increase of the Trp-3 emission at 333 nm or as an increase of the dansyl emission at 500 nm in TTGPMe vesicles containing dansyl-HPE.

## Discussion

The binding of phospholipase $A_2$ to the surface of TTGPMe bilayers as shown by the resonance energy transfer is of high affinity. The effects described here are not due to specific binding of the probe to phospholipase $A_2$ but are a general property of the interactions of this protein with the interface. Based on the shape of the titration curves [2,3] and kinetic data [1,2,4] the apparent dissociation constant for the bound enzyme is estimated to be $< 10^{-13}$ M. Further, the observations reported here demonstrate that both the tryptophan-3 residue of phospholipase $A_2$ as well as the lipid-attached dansyl chromophore are inaccessible to the bulk solvent when phospholipase $A_2$ is bound to bilayers of TTGPMe. We have interpreted this result as a desolvation of the protein–lipid microinterface which can be visualized as one of the causes which contributes to the essentially irreversible binding of phospholipase

$A_2$ to TTGPMe, the $E \rightarrow E^*$ step in the Scheme I which we have previously used to account for the kinetics of catalysis by phospholipase $A_2$ and its interfacial activation [1–4].

Since inaccessibility of the binding interface to bulk solvent has not been reported before, some comments about the possible role of interfacial desolvation on the catalytic function and interfacial activation of phospholipase $A_2$ in the scooting mode are in order. The high affinity binding of phospholipase $A_2$ to the substrate bilayer surface occurs without any significant change in the gross organization of the bilayer or leakage of vesicle-trapped components (Jain and Maliwal, to be published). This implies that interaction of ionic groups of the lipid bilayer and protein at the binding microinterface does not cause a major organizational or morphological change of the membrane. However, desolvation of the protein-bilayer microinterface could appreciably facilitate dislodging of a monomer substrate lipid molecule from the bilayer to the active site of the enzyme, i.e. the $E^* + S \rightarrow E^*S$ step. This dislodging of the monomer is postulated to be the rate-limiting step in the catalytic turnover by the bound enzyme in the scooting mode [4]. Since binding of pro-phospholipase $A_2$ occurs without desolvation, its catalytic turnover cycle would have to include a desolvation step followed by the catalytic cycle and resolvation, i.e. the $E \rightarrow E^*$ step. This is the case of catalysis in the hopping mode. Thus, the turnover number would be appreciably lower for the pro-phospholipase $A_2$-catalyzed reaction than for the phospholipase $A_2$-catalyzed one. In fact, based on the pre-steady state kinetics of the binding, the rate of desolvation and the relaxation constant for the catalytic turnover of pro-phospholipase $A_2$ are within a factor of three (Jain and De Haas, in preparation).



Scheme I. Scheme of interfacial catalysis.

Two of the possible functional of desolvation of the microinterface between the bilayer and the phospholipase $A_2$ are considered below. First, as implied in the model for catalysis in the scooting mode [4], the substrate molecules in the desolvated region are more likely to leave the bilayer at the microinterface and would, as a consequence, be more accessible to the catalytic site of the enzyme for the formation of the interfacial Michaelis-Menten complex. In the binding of pro-phospholipase $A_2$ to the surface of the charged lipid bilayer, desolvation of the binding microinterface does not occur, thus reducing the probability of the formation of the Michaelis-Menten complex, increasing the rate of intervesicle exchange, and thus contributing to the lack of interfacial activation in this case. Second, desolvation could provide a basis for the apparently high binding energy of phospholipase $A_2$ to the charged bilayer surface. The macroscopic binding equilibrium is between only two distinguishable macrostates: the free enzyme E, and the bound enzyme, $E^*$. Based on kinetic studies [1–3] the apparent dissociation constant for $E^*$ is about $10^{-13}$ M. Such a large binding energy probably arises from a confluence of many factors. The bound enzyme at the desolvated interface is less likely to leave the surface of the bilayer because this would require a resolvation step which could contribute to a loss of entropy. The entropy-driven binding of phospholipase $A_2$ is also presumably aided by ionic interactions at multiple sites (see Ref. 2). Moreover, the enzyme in the interface is more likely to successively exchange the bound phospholipid molecules with the excess phospholipid molecules in the bilayer, thus creating a series of discrete but isoenergetic states. If the exchange of $E^*$ between these microstates occurs over a distance that is shorter than the root mean square distance for leaving the interface ($E^* \rightarrow E$), it is conceivable that $E^*$ would be energetically stabilized. This postulate provides a mechanism for preventing the thermal dissipation of the energy of the bound state.

Finally, a lipid-protein interaction promoted by ionic interactions and followed by desolvation of the microinterface has far-reaching consequences

regarding which we can only speculate. For example, hydrophobic anchoring of proteins to the bilayer interface [16] could provide the putative defect sites not only for high affinity binding of a protein to the bilayer, but also for removal of phospholipid monomers by PLA and phospholipid exchange proteins, for post-translocational translocation of proteins, for accelerated transbilayer movement of lipids, and for fusion induced by proteins.

## Acknowledgements

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## References

1 Jain, M.K., Rogers, J., Jahagirdar, D.V., Marecek, J.F. and Ramirez, F. (1986) Biochim. Biophys. Acta 860, 435–447
2 Jain, M.K., Maliwal, B.P., DeHaas, G.H. and Slotboom, A.J. (1986) Biochim. Biophys. Acta 860, 448–461
3 Jain, M.K., Rogers, J., Marecek, J.F., Ramirez, F. and Eibl, H. (1986) Biochim. Biophys. Acta 860, 462–474
4 Jain, M.K., DeHaas, G.H., Marecek, J.F. and Ramirez, F. (1986) Biochim. Biophys. Acta 860, 475–483
5 Stryer, L. (1966) J. Am. Chem. Soc. 88, 5708–5712
6 Förster, T. and Rokos, K. (1967) Chem. Phys. Lett. 1, 279–280
7 Vaz, W.L.C. and Schoellmann, G. (1976) Biochim. Biophys. Acta 439, 206–218
8 Ricci, R.W. (1970) Photochem. Photobiol. 12, 67–75
9 Vaz, W.L.C., Kaufmann, K. and Nicksch, A. (1977) Anal. Biochem. 83, 385–393
10 Ghiggino, K.P., Lee, A.G., Meech, S.R., O'Connor, D.V. and Philips, D. (1981) Biochemistry 20, 5381–5389
11 Jain, M.K., Jahagirdar, D.V., Van Linde, M., Roelofsen, B. and Eibl, H. (1985) Biochim. Biophys. Acta 818, 356–364
12 Jain, M.K., Egmond, M.R., Verheij, H.M., Apitz-Castro, R.J., Dijkman, R. and DeHaas, G.H. (1982) Biochim. Biophys. Acta 688; 341–348
13 Hille, J.D.R., Egmond, M.R., Dijkman, R., Van Dort, M.G., Jirgensons, B. and DeHaas, G.H. (1983) Biochemistry 22, 5347–5353
14 Jain, M.K., Crecely, R., Hille, J.D.R., DeHaas, G.H. and Grunner, S. (1985) Biochim. Biophys. Acta 813, 68–76
15 Jain, M.K. and Maliwal, B.P. (1985) Biochim. Biophys. Acta 814, 135–140
16 Jain, M.K. and Zakim, D. (1987) Biochim. Biophys. Acta 906, 33–68