Biochimica et Biophysica Acta, 508 (1978) 185—196

BBA 77974

ACTION OF PHOSPHOLIPASES A_2 ON PHOSPHATIDYLCHOLINE BILAYERS

EFFECTS OF THE PHASE TRANSITION, BILAYER CURVATURE AND STRUCTURAL DEFECTS

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(Received September 5th, 1977)

Summary

We examined the action of porcine pancreatic and bee-venom phospholipase A_2 towards bilayers of phosphatidylcholine as a function of several physical characteristics of the lipid-water interface.

1. Unsonicated liposomes of dimyristoyl phosphatidylcholine are degraded by both phospholipases in the temperature region of the phase transition only (cf. Op den Kamp et al. (1974) Biochim. Biophys. Acta 345, 253—256 and Op den Kamp et al. (1975) Biochim. Biophys. Acta 406, 169—177). With sonicates the temperature range in which hydrolysis occurs is much wider. This discrepancy between liposomes and sonicates cannot be ascribed entirely to differences in available substrate surface.

2. Below the phase-transition temperature the phospholipases degrade dimyristoyl phosphatidylcholine single-bilayer vesicles with a strongly curved surface much more effectively than larger single-bilayer vesicles with a relatively low degree of curvature.

3. Vesicles composed of egg phosphatidylcholine can be degraded by pancreatic phospholipase A_2 at 37°C, provided that the substrate bilayer is strongly curved. The bee-venom enzyme shows a similar, but less pronounced, preference for small substrate vesicles.

4. In a limited temperature region just above the transition temperature of the substrate the action of both phospholipases initially proceeds with a gradually increasing velocity. This stimulation is presumably due to an increase of the transition temperature, effectuated by the products of the phospholipase action.

5. Structural defects in the substrate bilayer, introduced by sonication below the phase-transition temperature (cf. Lawaczeck et al. (1976) Biochim. Biophys. Acta 443, 313—330) facilitate the action of both phospholipases.

The results lead to the general conclusion that structural irregularities in the packing of the substrate molecules facilitate the action of phospholipases A_2 on phosphatidylcholine bilayers. Within the phase transition and with bilayers containing structural defects these irregularities represent boundaries between separate lipid domains. The stimulatory effect of strong bilayer curvature can be ascribed to an overall perturbation of the lipid packing as well as to a change in the phase-transition temperature.

Introduction

Phospholipases are powerful tools in the investigation of the architecture of biological membranes. The asymmetric distribution of membrane phospholipids [4—6] as well as the lipid dependence of membrane-bound enzymes [7—10] can be succesfully studied by selective degradation of different phospholipid pools using various phospholipases. Obviously, in such studies knowledge of the mechanism of action of the lipolytic enzymes used is of crucial importance. Several investigations on model membrane systems have shown that one of the main factors determining whether or not phospholipases will be active is the packing of the substrate molecules in the lipid-water interface. Phospholipids arranged in monolayers are not susceptible to phospholipase action if the surface pressure is increased beyond a critical value, characteristic of a particular phospholipase [11—13]. Using a bilayer substrate Op den Kamp et al. [1,2] demonstrated that pancreatic phospholipase A_2 degrades saturated phosphatidylcholines in the temperature region of the gel to liquid-crystalline phase transition only. The authors suggested that irregularities in the packing of the lipid molecules at the border of fluid and crystalline areas favour insertion of the enzyme into the interface. In a previous study we also observed this characteristic temperature dependence of phospholipase action [14]. Our results, however, differed from those of Op den Kamp et al. [1,2] in two respects. Firstly, we observed a very high activity of the enzyme towards dimyristoyl phosphatidylcholine at temperatures close to, but clearly above the phase-transition temperature. Secondly, in our studies the temperature range in which considerable hydrolysis of the substrate still occurred extended to values below 10°C. These discrepancies can possibly be related to experimental differences. Op den Kamp et al. used unsonicated liposomes or vesicles prepared by the method of Batzri and Korn [15], whereas in our study sonicated substrates were used. Depending on the time of sonication these sonicates contain varying proportions of small single-bilayer vesicles and large probably multilamellar liposomes, heterogeneous in size [16,17].

In the present study we provide evidence that the size of the substrate particles, i.e. the degree of bilayer curvature is an important factor in determining whether or not phospholipases A_2 are able to penetrate the lipid-water interface. In addition, we demonstrate that other irregularities in the substrate bilayer, such as the structural defects described by Lawaczeck et al. [3], also enhance the susceptibility of the phospholipids to phospholipase action.

Materials and Methods

Lipids and phospholipases. Dimyristoyl phosphatidylcholine (1,2-ditetradecyl-*sn*-glycero-3-phosphorylcholine) and egg-yolk phosphatidylcholine were obtained from Sigma. The lipids were labelled with ^{14}C by a demethylation-remethylation procedure as described by Stoffel [18], with several minor modifications. The radioactive products were purified by preparative thin-layer chromatography. Neither the radioactive nor the initial unlabelled phosphatidylcholines contained any detectable impurities as determined by thin-layer chromatography on silica gel HF (Merck) with chloroform/methanol/7 M ammonia (67 : 33 : 5, by vol.) as developing solvent and visualization of the lipids with iodine staining and/or illumination with ultraviolet light. The radioactive phosphatidylcholines were diluted with unlabelled lipid to a suitable specific radioactivity.

Pure phospholipase A_2 (EC 3.1.1.4) from porcine pancreas was purchased from Boehringer. The preparation was dialyzed against distilled water and subsequently lyophilized. Pure phospholipase A_2 from bee-venom was obtained from Sigma. Phospholipase A_2 from *Naja naja* (Sigma) was partially purified according to the method of Cremona and Kearney [19]. The enzymes were dissolved in dilute Tris · HCl buffer (pH 7.5) at a concentration of 0.5 mg/ml.

Preparation of lipid dispersions. Unsonicated liposomes: Chloroform solutions of phosphatidylcholine were taken to dryness in vacuo. Residual lipid was dispersed in 0.05 or 0.1 M Tris · HCl (pH 7.5) by shaking with some glass beads at approx. 35° C.

Sonicates: Liposome preparations were sonicated during 3 min in 15-s periods (unless stated otherwise) at a temperature above the phase-transition temperature of the lipid. A Branson sonifier with a titanium probe was used throughout (power setting 3-4, approx. 70 W). With egg phosphatidylcholine the preparation was cooled in ice-water and nitrogen was flushed over the surface of the suspension during sonication. Preparations sonicated during more than 10 min were freed from titanium particles by centrifugation. Multilamellar liposomes were separated from single-bilayer vesicles by gel filtration on Sepharose 4B [16]. Usually 2 ml of a sonicate containing 40 μmol of lipid was applied to a 50 × 1.5 cm column. If necessary, fractions were concentrated by means of ultrafiltration (Amicon cell, model 12, with a PM-10 or XM-100 filter).

Injection vesicles: Single-bilayer injection vesicles were prepared by the method of Batzri and Korn [15] as modified by Kremer et al. [20]. This modified procedure provides the possibility to vary the diameter of the vesicles. Size and size distribution were determined by light-scattering measurements. The preparations used were monodisperse, i.e. the vesicles were homogeneous in size, according to the criterions applied by Kremer et al. [20].

Sonicated bilayers with structural defects: For the purpose of introducing structural defects into the bilayer dimyristoyl phosphatidylcholine was sonicated in 0.05 M Tris · HCl (pH 7.5), containing 5.0 mM $CaCl_2$, at a temperature well below the transition temperature [3]. An ice-salt mixture was used to cool the suspension during sonication. The structural defects were "annealed" by incubating the "unannealed" preparation during 30 min at 45° C [3].

Incubations. Incubations were carried out in 0.05 or 0.1 M Tris · HCl (pH 7.5) usually at a lipid concentration of 1.0 mM in a volume of 1.0 ml. $CaCl_2$ was added to a final concentration of 2.5 mM. After preincubation at the desired temperature the reaction was initiated by the addition of phospholipase. The reaction was terminated usually after 10 min by addition of EDTA to a final concentration of 20 mM and 6 ml chloroform/methanol (1 : 2, v/v). Controls, incubated in the absence of phospholipase, did not show any detectable lipid degradation.

Lipid analyses. Analyses were carried out as described before [14] with the exception that usually the amounts of lysophosphatidylcholine and phosphatidylcholine were determined by radioactivity measurements [21]. In some experiments phosphate was assayed to determine the extent of lipid hydrolysis [14].

Results

Phospholipase action on dimyristoyl phosphatidylcholine. Effect of sonication

Unsonicated and sonicated dimyristoyl phosphatidylcholine dispersions were subjected to the action of pancreatic and bee-venom phospholipase A_2. The results are shown in Fig. 1. With unsonicated substrate the temperature range in which degradation occurs is rather narrow (lower curves Fig. 1). With the bee-venom phospholipase this temperature range consistently extended to lower values than with the pancreatic enzyme. Towards sonicates the phospholipases display a different behaviour (upper curves Fig. 1). At the lower temperatures sonicates are degraded by the pancreatic phospholipase to a substantial degree, in contrast to unsonicated substrate. The bee-venom enzyme also shows an increased activity at the lower temperatures, but, in addition, a very pronounced rise in the higher temperature region as well.

The behaviour of phospholipase A_2 from *N. naja* was found to be very much similar to that of the bee-venom enzyme in this respect (results not shown).

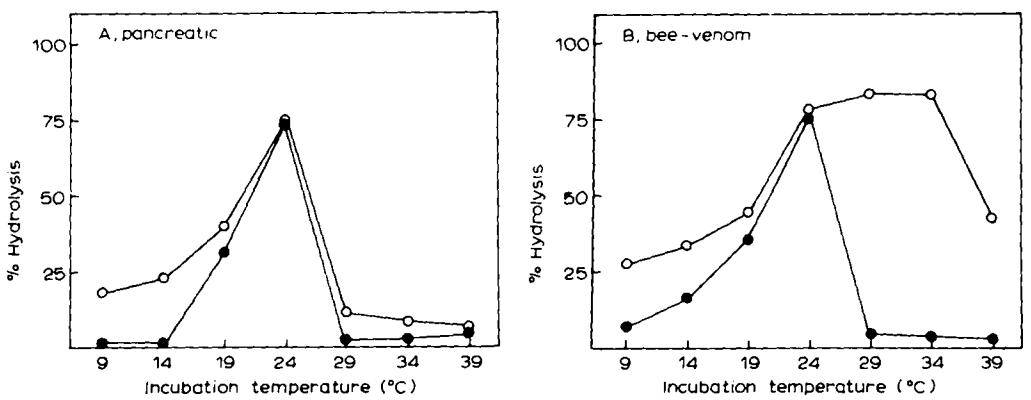

Fig. 1. Degradation of dimyristoyl phosphatidylcholine liposomes (●) and sonicates (○) by pancreatic (A) and bee-venom (B) phospholipase A_2 at different temperatures. Incubations were carried out in 0.1 M Tris · HCl (pH 7.5) during 10 min in a final volume of 1.0 ml. Concentrations: lipid, 1.0 mM; $CaCl_2$, 2.5 mM; pancreatic phospholipase, 3.5 and 1.5 μg/ml for liposomes and sonicates, respectively; bee-venom phospholipase, 1.0 μg/ml.

Time course of phospholipase action at various temperatures

The results presented in Fig. 1 concerning the activity of pancreatic phospholipase towards sonicated substrate are in agreement with our previously published data [14], with an exception for the enzyme activity at 29°C. It should be emphasized, however, that at temperatures just above the transition temperature we found considerable variation in the degree of lipid hydrolysis depending on small differences in phospholipase concentration. We tentatively concluded that initially the reaction rate is low, but subsequently increases possibly as a result of a stimulatory effect of the reaction products. In order to substantiate this interpretation we studied the time course of phospholipase action on sonicated dimyristoyl phosphatidylcholine at one particular phospholipase concentration. The results, presented in Fig. 2, indicate that above the transition temperature the reaction velocities do increase indeed during the first few minutes of the reaction. The stimulatory effect diminished with increasing temperature. With the pancreatic phospholipase the effect has even vanished at 34°C (Fig. 2A), while similarly, with the venom enzyme the effect is less pronounced at 34°C than at 29°C (Fig. 2B). Consequently, the high activities of the phospholipases at temperatures just above the transition temperature, as presented in Fig. 1B and previously [14], are likely to represent secondary effects.

The stimulation phenomenon is not confined to sonicated substrate. The bee-venom phospholipase, in apparent contrast to the pancreatic enzyme, displayed high activities towards unsonicated dimyristoyl phosphatidylcholine in a limited temperature region just above the transition temperature, provided that the amount of enzyme added was increased by a factor of about 2 in comparison with the amount mentioned in the legend to Fig. 1 (results not shown). This indicates that in the temperature region concerned the initial activity of the bee-venom enzyme is higher than that of the pancreatic one.

Below the transition temperature (14°C) there is no indication whatsoever of the stimulatory effect of the reaction products (Fig. 2).

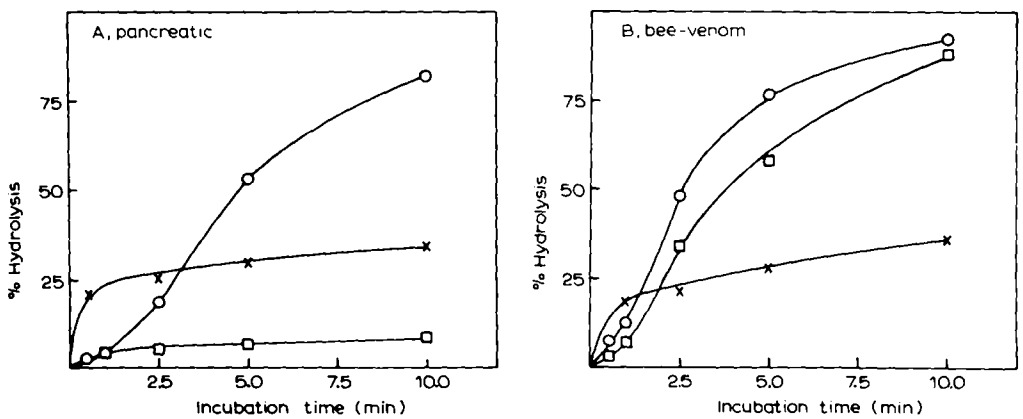

Fig. 2. Time course of the degradation of a dimyristoyl phosphatidylcholine sonicate at 14°C (X), 29°C (○) and 34°C (□) by pancreatic (A) and bee-venom (B) phospholipase A_2. Incubations were carried out in 0.05 M Tris · HCl (pH 7.5) in a volume of 1.0 ml. Concentrations: lipid, 1.0 mM; $CaCl_2$, 2.5 mM; pancreatic phospholipase, 5.0 μg/ml; bee-venom phospholipase, 2.5 μg/ml.

Effects of substrate-particle size and bilayer curvature on phospholipase action

Gel filtration of a phosphatidylcholine sonicate on Sepharose 4B yields two fractions [16]. In the void volume of the column large multilamellar liposomes are recovered and in the second fraction small single-bilayer vesicles. We treated both fractions as well as the initial dimyristoyl phosphatidylcholine sonicate, each at a lipid concentration of 1.0 mM, with the two phospholipases, which were added in concentrations as mentioned in the legend to Fig. 2. The incubations were carried out at 14°C, since in this temperature region the degree of hydrolysis is not influenced by a secondary stimulatory effect of the reaction products (Fig. 2). It appeared that the smaller particles in a sonicate are degraded much more effectively than the larger ones. With each of the phospholipases after 10 min of incubation the extent of lipid hydrolysis in single-bilayer vesicles and multilamellar liposomes was approx. 50 and 10%, respectively. These two values did not vary extensively with the sonication time. The degree of hydrolysis of the initial sonicate and, accordingly, the relative amount of lipid recovered in the second Sepharose fraction increased with the sonication time.

Two possible explanations for the higher rate of degradation of small vesicles should be considered. Firstly, single-bilayer vesicles expose a larger surface area than multilayered liposomes at the same lipid concentration, i.e. they provide a higher effective substrate concentration. Secondly, because of their strongly curved surface, vesicles possess a "strained" configuration [17]. This distortion of the lipid packing could conceivably facilitate penetration of phospholipases. In order to discriminate between these possibilities we followed two experimental approaches. Firstly, we varied the substrate concentration for both liposomes and vesicles. If the discrepancy between these two kinds of substrate particles is entirely the result of a difference in exposed surface area the maximal reaction velocities at saturating substrate concentration will be the same

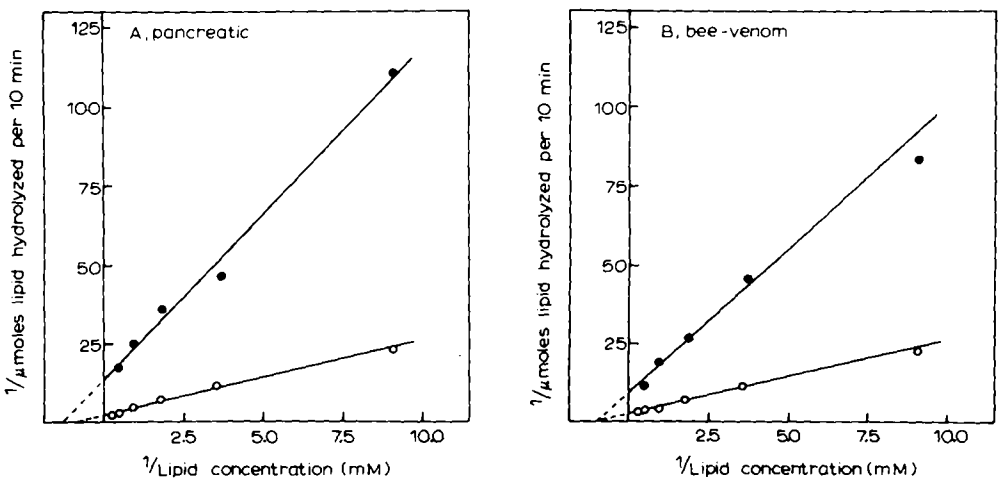

Fig. 3. Lineweaver-Burk plots of the action of pancreatic (A) and bee-venom (B) phospholipase A_2 on dimyristoyl phosphatidylcholine liposomes (•) and vesicles (○). Liposomes and vesicles were prepared by sonication (during 3 min) and gel filtration on Sepharose 4B. The vesicle preparation was concentrated by ultrafiltration. Incubations were carried out at 14°C during 10 min. Further experimental conditions were as Fig. 2, except for the lipid concentration, which was varied.

for both lipid preparations. Secondly, single-bilayer vesicle preparations varying in degree of bilayer curvature were subjected to the action of the phospholipases. The results of the concentration dependence study are shown in Fig. 3. By presenting the data as Lineweaver-Burk plots we do not intend to suggest that the classical theory of enzyme kinetics can indiscriminately be applied to the complex events taking place at lipid-water interfaces. However, plotting the results in this manner allows extrapolation to infinite substrate concentrations. Thus a comparison can be made between the values of the apparent maximal velocities for the two kinds of substrate particles used. Although these values are strictly confined to the experimental conditions concerned it is obvious that much higher velocities are attained with small vesicles than with large liposomes. For the pancreatic phospholipase the apparent V values for liposomes and vesicles are 0.08 and 0.51 (μmol of lipid hydrolyzed per 10 min), respectively. For the bee-venom enzyme these values are 0.11 and 0.39, respectively. This indicates that the difference in rate of hydrolysis between liposomes and vesicles cannot be ascribed to a difference in effective substrate concentration, at least not entirely. The second experimental approach proves in a more direct way that bilayer curvature is a major factor in determining the susceptibility of substrates to phospholipase action. In Table I it is shown that the rate of hydrolysis of single-bilayer vesicles markedly increases with the degree of surface curvature. It should be realized that in small vesicles with a diameter of approx. 30 nm the number of lipid molecules in the outer monolayer exceeds that in the inner one by a factor of approx. 2 [16]. Consequently, at the same concentration small vesicles expose more lipid molecules to phospholipase action than larger ones. However, this difference is insufficient to account for the difference in phospholipase activity towards small and large vesicles (Table I).

The results presented so far indicate that dimyristoyl phosphatidylcholine vesicles can be degraded by the phospholipases below the phase-transition temperature, provided that the substrate surface is relatively strongly curved. It

TABLE I

ACTION OF PANCREACTIC AND BEE-VENOM PHOSPHOLIPASE A_2 ON LARGE AND SMALL UNILAMELLAR DIMYRISTOYL PHOSPHATIDYLCHOLINE VESICLES

Dimyristoyl phosphatidylcholine vesicles were prepared by the ethanol injection procedure as described by Kremer et al. [20]. Lipid concentrations in ethanol were 35 and 14 μmol/ml for large and small vesicles, respectively. The small-vesicle preparation was concentrated by ultrafiltration. The size of the vesicles was determined by light scattering measurements [20]. Part of the small-vesicle preparation was sonicated during 30 min above the phase-transition temperature. In this preparation the vesicle diameter range was probably 25—35 nm. Incubations were carried out at 14°C during 10 min. For further experimental details see legend to Fig. 2.

Vesicle diameter (nm)	Hydrolysis (%)	
	Pancreatic phospholipase	Bee-venom phospholipase
100	14	17
58	46	42
"sonicated"	67	68

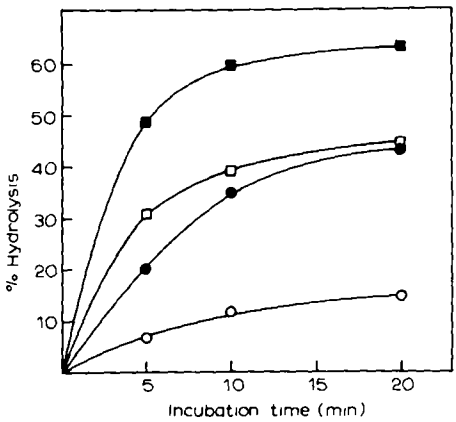

Fig. 4. Action of pancreatic and bee-venom phospholipase A_2 on small and large single-bilayer egg phosphatidylcholine vesicles at 37°C. Vesicles were prepared at 25°C by the method of Kremer et al. (ref. 20, see also legend to Table I). Initial lipid concentration in ethanol, 35 mM (vesicle diameter, approx. 135 nm). Part of the preparation was sonicated during 30 min in order to reduce the vesicle size to approx. 30 nm [16]. Incubations were carried out in 0.05 M Tris · HCl (pH 7.5) at 37°C. Concentrations: lipid, 1.0 mM; $CaCl_2$, 2.5 mM; pancreatic phospholipase, 20 μg/ml (circles); bee-venom phospholipase, 10 μg/ml (squares). Open symbols, unsonicated; closed symbols, sonicated.

could be argued that bilayer curvature as such is not the primary cause of the effect on phospholipase activity, but rather affects the lipid phase transition, thus secondarily influencing phospholipase action. Therefore, we subjected large and small single-bilayer vesicles composed of egg phosphatidylcholine to attack by the phospholipases at 37°C. With this phospholipid at this temperature there is a wide gap between phase-transition temperature (−10°C, ref. 22) and temperature of incubation. Therefore, phospholipase activity is not likely to be affected by a possible influence of bilayer curvature upon the transition temperature. Moreover, if bilayer curvature has any effect on the transition temperature, it will lower it [23–25], thus even influencing phospholipase action unfavourably. The results, presented in Fig. 4, indicate that egg phosphatidylcholine can indeed be degraded by the pancreatic phospholipase, provided that the radius of curvature of the substrate particles is sufficiently low. Although the bee-venom phospholipase also shows a preference for strongly curved bilayers, this enzyme apparently can attack particles with a larger radius of curvature as well. It should be noted, that the time course of the phospholipase action indicates that there is no product activation.

Influence of structural defects in the substrate bilayer

If phosphatidylcholine dispersions are sonicated below the transition temperature so-called structural defects are introduced into the lipid bilayer [3]. These defects can be eliminated ("annealed") by incubating the sonicate above the transition temperature. Subsequent incubation below this temperature does not result in reintroduction of the defects, unless a second sonication is carried out. We subjected unannealed and annealed dimyristoyl phosphatidylcholine preparations to the action of the two phospholipases at 14°C. It appeared that the presence of structural defects in the substrate bilayer considerably enhances the susceptibility of the lipid molecules to the phospholipases

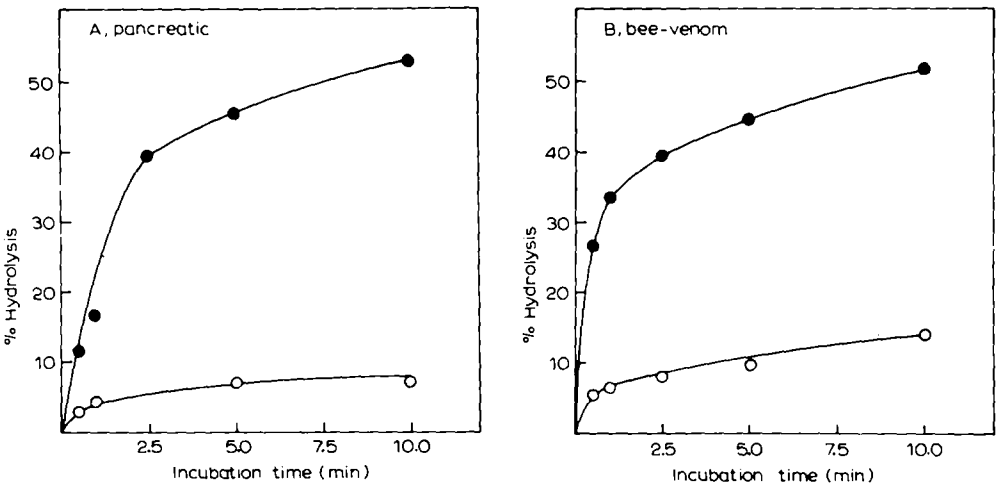

Fig. 5 Action of pancreatic (A) and bee-venom (B) phospholipase A_2 on unannealed (•) and annealed (○) dimyristoyl phosphatidylcholine bilayers. Unannealed dispersions were prepared by sonication during 30 s at a temperature well below the transition temperature (see also Materials and Methods). Half of the sonicate was annealed by incubation at 45°C during 30 min. Incubations were carried out at 14°C in 0.05 M Tris · HCl (pH 7.5), containing 5.0 mM $CaCl_2$, at a lipid concentration of 1.0 mM. Final volume, 1.0 ml. Phospholipase concentrations: pancreatic, 10 μg/ml; bee-venom, 5.0 μg/ml.

(Fig. 5). Both enzymes degraded approx. 50% of the lipid in the unannealed preparation within 10 min, whereas with the annealed preparation only about 10% hydrolysis of the lipid was observed. In order to minimize the effect of bilayer curvature the sonication time was reduced to 30 s in this experiment. This relatively short sonication time explains the low degree of hydrolysis of the annealed preparation, as compared to the values presented in Figs. 1 and 2.

Discussion

Effect of the reaction products on phospholipase action

The results presented in Fig. 2 demonstrate a stimulatory effect of the reaction products on phospholipase activity. It is interesting to note that well above (Fig. 4) as well as below (Fig. 2) the phase-transition temperature of the substrate this effect is lacking. Apparently, the influence of the products is confined to a limited temperature region just above the transition temperature. As phospholipase activities are very high within the phase-transition temperature range, this observation could suggest that the reaction products act to increase the transition temperature, which would be in agreement with the results of several recent investigations [23,26,27]. In these studies an increase of the transition temperature of dimyristoyl or dipalmitoyl phosphatidylcholine was observed due to the presence of myristic acid [23,26,27] or lysophosphatidylcholine [23] in the bilayer structure.

Pancreatic phospholipase vs. venom phospholipases

Unsonicated dimyristoyl phosphatidylcholine is hydrolyzed by the pancreatic phospholipase in the temperature region of the phase transition only. Soni-

cated preparations are degraded in a wider temperature range reaching relatively low values. This effect of sonication enlightens the difference, referred to in the introduction, between our previous findings [14] and those of Op den Kamp et al. [1,2]. With the sonicates our present measurements are in agreement with our previously published data, which were also obtained with sonicated substrate. With unsonicated substrate the results essentially confirm the findings of Op den Kamp et al. obtained with either unsonicated preparations [1] or injection vesicles [2]. The results in Figs. 3 and 4 and in Table I indicate that the effect of sonication can be ascribed mainly to an increased degree of bilayer curvature. Taking this into consideration we assume that the vesicles used by Op den Kamp et al. [2], which were not characterized, must have been relatively large.

Several lines of evidence in our experiments indicate that the pancreatic phospholipase is influenced more explicitly by a phase transition in (Fig. 1) or strong curvature of (Figs. 3 and 4) the substrate bilayer than the venom phospholipases, indicating that these latter enzymes more readily attack closely packed interfaces than the pancreatic enzyme. This is in good agreement with the results of monolayer studies [11,12]. Yet, there appears to exist a greater similarity between the venom and pancreatic phospholipases than suggested by Op den Kamp et al. [1], who reported a high activity of the bee-venom enzyme towards unsonicated dimyristoyl phosphatidylcholine at 37°C. They concluded that this phospholipase is not influenced by a phase transition in the substrate bilayer and that the enzyme even preferentially attacks closely packed interfaces. The ratio of the amounts of enzyme and substrate they used lead us to believe that the activity observed at 37°C was a secondary effect of the reaction products. In our view the venom and the pancreatic phospholipases are basically similar in the way they are influenced by a phase transition in the substrate bilayer (Fig. 1).

Requirements of phospholipases with respect to substrate surface

Irregularities in the packing of the substrate molecules strongly enhance the action of phospholipases on lipid bilayers (refs. 1 and 2, this paper). Such irregularities are not only present at the gel to liquid-crystalline phase transition, but also in strongly curved bilayers and in bilayers containing structural defects. Knowledge of interrelationships between these three kinds of distortions in lipid packing could provide more insight into the specific requirements of phospholipases with respect to the properties of the lipid-water interface.

There is still some controversy as to whether the phase transition is influenced by bilayer curvature. De Kruyff et al. [28] reported that prolonged sonication does not significantly affect the phase transition. More recent evidence, however, indicates a lowering and broadening of the transition temperature range upon sonication [23—25]. The high phospholipase activities towards small vesicles below the transition temperature may thus in part be the result of an effect of bilayer curvature on the phase transition. This does not imply, however, that the phase transition is necessarily involved whenever a structural irregularity enhances phospholipase action. Such a generalization would be contradicted by the influence upon phospholipase action of structural defects in unannealed substrate bilayers (Fig. 5). Lawaczeck et al. [3]

propose that in such unannealed bilayers population defects exist between the inner and outer monolayer resulting in discrete defects or dislocations between tightly packed crystalline domains. Since unannealed bilayers, in contrast to annealed ones, are effectively degraded by the phospholipases, the enzymes presumably penetrate the lipid-water interface at the dislocations only. Apparently, the coexistence of separate crystalline lipid domains is as favourable a condition for phospholipase action as the coexistence of fluid and crystalline domains within the phase transition. One might speculate that fluid-fluid immiscibility [29] could facilitate phospholipase action as well.

The coexistence of separate lipid domains is a favourable condition, but not a prerequisite for phospholipase action. An overall perturbation, like bilayer curvature, can be effective as well, irrespective of its possible effect upon the phase transition. Our results indicate that bilayer curvature can facilitate phospholipase action merely due to an increased average distance between individual molecules (Fig. 4).

Recent studies indicate that bilayer curvature may induce segregation of lipids into separate domains [30]. In biological membranes regions of high curvature exist (e.g. erythrocyte membranes, mitochondrial cristae membranes). Phospholipase action on such strongly curved membranes could very well be enhanced by local lipid-domain formation or by curvature effects as such.

Acknowledgements

We wish to thank Dr. J.M.H. Kremer from the Van 't Hoff laboratorium, University of Utrecht, for preparing and characterizing the injection vesicles as well as for providing us with a copy of ref. 20 prior to publication.

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