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HYDROLYSIS OF PHOSPHATIDYLCHOLINE LIPOSOMES BY PANCREATIC PHOSPHOLIPASE A_2 AT THE TRANSITION TEMPERATURE

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Summary

Dilauroylphosphatidylcholine, dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine dispersed as liposomes in water are hydrolysed by phospholipase A_2 from pig pancreas only near the transition temperature of these lipids. Both above and below the transition temperature of the lipids the rate of hydrolysis in these model membranes is negligible. By contrast, hydrolysis of these liposomes with bee venom phospholipase A_2 occurs below, at and above the transition temperature.

The concept of lateral compressibility of lipids at the transition temperature is firmly substantiated by these observations.

The transition of lipids in a membrane from the lipid crystalline state to the gel phase and vice versa has a great influence on membrane-oriented processes. Haest et al. [1] and Papahadjopoulos et al. [2] demonstrated that the passive leak of cations from lecithin liposomes is markedly increased in the vicinity of the transition temperature. McConnell and coworkers [3, 4] elaborated on these findings and suggested that the increased transport is due to enhanced lateral compressibility of the bilayers when liquid and solid phases coexist. The findings are supposed to have biological implications and comparable results have been obtained by studying ion release [1] and sugar uptake [3] in a fatty acid auxotroph of *Escherichia coli* grown on different unsaturated fatty acids.

In this report we describe the effect of temperature on the degradation of lecithin in liposomes by phospholipase A_2. In general, this enzyme, when

isolated from pig pancreas, is not able to penetrate lecithin liposomes and to perform any hydrolysis [5]. However, in this paper we will show that when the reaction is carried out at the temperature of the gel—liquid crystalline transition, hydrolysis by this enzyme is easily accomplished.

Hand shaken liposomes were prepared from pure phosphatidylcholine, synthesized in our laboratory according to the procedures described by Slotboom and Bonsen [12]. 1.7 μmoles of each phospholipid was dispersed in 1 ml 0.1 M Tris buffer, pH 7.2, containing 1 mM $CaCl_2$. 250 μl of this liposome suspension were incubated with 5 μg of highly purified pig pancreatic phospholipase A_2 at different temperatures for 15 min. The reaction was terminated by the addition of 0.5 ml 0.2 M EDTA and the reaction products were extracted according to Goerke et al. [6]. In a control experiment the incubation was carried out in the absence of phospholipase A_2 at 37°C and 5 μg of this enzyme were added after the addition of EDTA. Lipid extracts were chromatographed on silica H plates with chloroform—methanol—water (65:35:5, by vol.) as developing system. Compounds were visualized with iodine staining or charring with 20% sulfuric acid. Similar experiments were

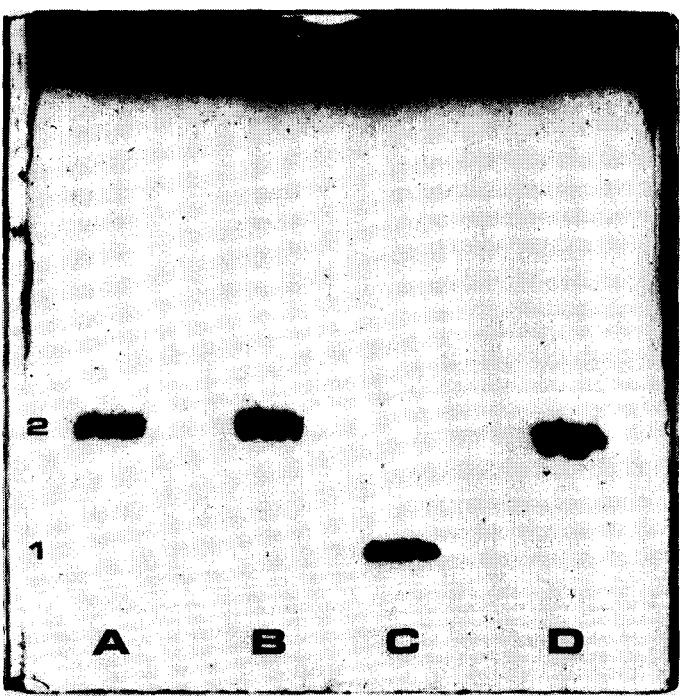

Fig. 1. Thin-layer chromatogram of dimyristoylphosphatidylcholine after treatment with phospholipase A_2 from pig pancreas. Samples of 0.4 μmole of dimyristoylphosphatidylcholine in the form of liposomes in 100 mM Tris—HCl buffer, pH 7.2, containing 1 mM $CaCl_2$, were incubated for 15 min. The chromatogram shows the results of a control experiment without phospholipase (A) and incubations at 15°C (B), 23°C (C) and 37°C (D) in the presence of 5 μg phospholipase A_2. The compounds are: lysophosphatidylcholine (1), phosphatidylcholine (2).

carried out with purified bee venom phospholipase A_2. When indicated the remaining lecithin and the formed lysolecithin were scraped off the silica-gel plate and determined quantitatively by assaying for phosphorus [6].

Fig. 1 clearly demonstrates that under the experimental conditions employed, hydrolysis of the dimyristoylphosphatidylcholine is negligible at 15°C and 37°C but complete at 23°C, which is the transition temperature of this lipid [7]. Similar experiments carried out at several temperatures below 23°C (0°C, 10°C, 18°C) as well as above (30°C, 41°C, 47°C) showed little, if any, hydrolysis of the lecithin.

Dilauroylphosphatidylcholine which has a transition temperature of 0°C [8], was hydrolysed completely at this temperature but not at 10°C, 20°C and even higher temperatures. Also dipalmitoylphosphatidylcholine was degraded only in the vicinity of its transition temperature, 41°C [7, 8]. Experiments carried out at 10, 23, 30 and 47°C clearly showed that the lecithin in liposomes at these temperatures was inaccessible to the phospholipase A_2 from pig pancreas.

In a series of control experiments we tested the activity of purified bee venom phospholipase A_2 since it is known that this enzyme preferentially hydrolyses phospholipids which are present in closely packed interfaces [9]. Table I demonstrates that hydrolysis of dimyristoylphosphatidylcholine with this enzyme occurs both below, near and above the transition temperature, in contrast with the pattern obtained with the phospholipase A_2 from pig pancreas which has a preference for more widely spaced lipid molecules in the interface [9].

TABLE I

HYDROLYSIS OF DIMYRISTOYLPHOSPHATIDYLCHOLINE WITH PHOSPHOLIPASE A_2 FROM PIG PANCREAS AND FROM BEE VENOM

0.4 μmole of lecithin in the form of liposomes were incubated with 5 μg of pig pancreatic phospholipase A_2 or bee venom phospholipase A_2, respectively, during 15 min at the indicated temperatures. Data are expressed as the percentage of total P which is recovered in the lysolecithin fractions.

Temp. (°C)	% lysolecithin formed with	
	Pig pancreatic phospholipase A_2	Bee venom phospholipase A_2
15	< 5	15
23	100	55
37	< 5	> 95

Summarizing the results, it is obvious that pig pancreatic phospholipase A_2 hydrolyses lecithins, when present in liposomes, only near the temperature of the gel to liquid crystalline transition, whereas below and above this transition temperature hydrolysis is hardly detectable. Taking into consideration the well-documented properties of this enzyme with respect to the quality of the interface in which the substrate is present [9—11], these results can be interpreted most satisfactorily according to the concept of lateral compressibility as proposed by Linden et al. [3]. At the temperature of the phase transition, part

of the lipid molecules are in the liquid crystalline phase and these molecules are in equilibrium with molecules in the gel phase. At the border of these domains of frozen molecules the action of the enzyme is facilitated because the space and possibly also the energy which are required for the formation of the enzyme—substrate complex are supplied by the transition of the paraffin chains from the liquid to the solid state. Below as well as above the transition temperature, the packing of the lipids in the artificial bilayers is tight, thereby preventing the penetration of the enzyme.

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