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PHASE TRANSITION BEHAVIOUR OF ARTIFICIAL LIPOSOMES COMPOSED OF PHOSPHATIDYLCHOLINES ACYLATED WITH CYCLOPROPANE FATTY ACIDS

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Summary

Phase transitions of liposomes composed of synthetic phosphatidylcholines acylated with the cyclopropane fatty acids, lactobacillic and dihydrosterculic acid, were studied by differential scanning calorimetry. Transition temperatures were approx. 16°C higher than for phosphatidylcholines acylated with the corresponding unsaturated fatty acids, *cis*-vaccenic and oleic acid. Though our transition temperatures were all several degrees lower than those determined by Silvius and McElhaney ((1979) Chem. Phys. Lipids 25, 125—134), the increase produced by replacement of the double bond with a cyclopropane ring was the same. We propose that this replacement, through its effect on membrane fluidity, may serve to regulate the activity of membrane-associated processes such as transport.

Introduction

The idea that cyclopropane fatty acids confer an advantage to organisms living under adverse conditions has been proposed to explain their presence in bacteria and higher organisms [1,2]. However, though it has been clearly established that the physicochemical properties of natural and artificial membranes are a function of the relative proportions of the phospholipid fatty acyl chains [3—6], the role of cyclopropane fatty acids within this scheme has remained obscure. Several groups [7—9] have shown that the molecular packing of phospholipids acylated with either cyclopropane or unsaturated fatty acids is similar, but the melting temperature of free cyclopropane fatty acids are higher, and the substitution of a cyclopropane ring for a double bond may, therefore, result in a decrease in the fluidity of the membrane phospholipid.

To test this possibility, we determined the transition temperatures of liposomes composed of synthetic phosphatidylcholines acylated with unsaturated fatty acids or their cyclopropane fatty acid analogs. Shortly before this paper was submitted, the results of a similar study by Silvius and McElhaney [10] appeared. Our results confirm their finding that replacement of a *cis* double bond by a cyclopropane ring in the acyl chains of phosphatidylcholines raises the transition temperature by about 16°C.

Methods

Cyclopropane fatty acids were synthesized from palmitoleic (16 : 1, c^9) *, oleic (18 : 1, c^9) and *cis*-vaccenic acid (18 : 1, c^{11}) by using the method of Simmons and Smith [11]. The resultant methyl esters were purified by argentation silica-gel column chromatography [12] and analyzed by either argentation thin-layer chromatography (TLC) or by gas-liquid chromatography (GLC) on butanediol succinate [13]. The cyclopropane fatty acid methyl esters ranged in purity from 93 to 96%. After saponification according to the method of Kates [14], crystallization of the fatty acids at −10°C from petroleum ether did not result in further purification. The overall yield, based on the amount of unsaturated fatty acid subjected to the reaction employed by Simmons and Smith [11], was 25—30%.

Phosphatidylcholines acylated with unsaturated or cyclopropane fatty acyl esters were synthesized, by using the method of Warner and Benson [15], from the free fatty acids and the $CdCl_2$ adduct of *sn*-glycero-3-phosphorylcholine (prepared from commercial egg yolk lecithin according to the method of Chadha [16]). The synthetic phosphatidylcholines were purified by using the method of Pugh and Kates [17]. Purified samples ran as single components on analytical TLC. GLC analysis of the methyl esters prepared from the purified phosphatidylcholines showed that those synthesized from the cyclopropane fatty acids contained 93—96% of the correct *cis*-isomer, while the product from *cis*-vaccenate was 99% pure (Table I). After deacylation [14], the water-soluble product was confirmed to be glycerophosphorylcholine by paper chromatography [18].

Membrane phase transitions of artificial liposome preparations were measured by differential scanning calorimetry [3] with a Perkin-Elmer DSC-2. Up to 10 mg lipid were stirred with at least 2 vol. of solvent (50% ethylene glycol in water) in aluminum pans, which were sealed after incubation at 37°C for 30 min.

Results and Discussion

Analysis of liposome preparations by differential scanning calorimetry yielded transition temperatures of −7°C for phosphatidylcholines acylated with

* Fatty acids are denoted by their carbon number followed (after a colon) by the number of double bonds or 'cp' for cyclopropane, the configuration (*c* = *cis*, *t* = *trans*) and position (superscript) of the double bond or ring.

TABLE I

FATTY ACID COMPOSITIONS OF SYNTHETIC PHOSPHATIDYLCHOLINES

Fatty acid	Main fatty ester			
	cis-Vaccenate	Dihydrosterculate	Lactobacillate	*cis*-9,10-Methylene hexadecanoate
16 : 1, c^9		0.3		1.2
17 cp, t^9				4.8
17 cp, c^9				94.0
18 : 0	1.0			
18 : 1, c^{11}	99.0		2.7	
19 cp, t^{11}			0.7	
19 cp, c^{11}			95.8	
18 : 1, c^9		1.1		
19 cp, t^9		4.2		
19 cp, c^9		92.8		
Unknown		1.6		

the 19-carbon cyclopropane fatty acids, lactobacillic or dihydrosterculic acid, and −23°C for di(*cis*-vaccenoyl) phosphatidylcholine (Fig. 1). Introduction of the cyclopropane ring thus increased the melting temperature, as expected, though not to as great a degree as saturation (Table II). Phase transitions for phosphatidylcholines acylated with *cis*-9,10-methylene hexadecanoic or palmitoleic acid were not observed at temperature in excess of −40°C, the lower limit of the temperature control of the calorimeter. Liposomes prepared from commercial dipalmitoyl phosphatidylcholine (Sigma) gave a transition at 41°C, in agreement with literature values [3,19,20].

In terms of the magnitude of the change (15—16°C) produced in the transition temperature by replacement of the double bond with a cyclopropane ring, our results are in excellent agreement with those of Silvius and McElhaney [10]. However, their actual transition temperatures were 3.5—6.5°C higher than ours. In the case of dioleoyl phosphatidylcholine, which we did not study, their value of −15.7°C was approx. 6°C higher than values previously reported [3,20]. The reason for the discrepancies is not clear, but may be related to the type of analysis, as most previous studies, and ours, have employed differential

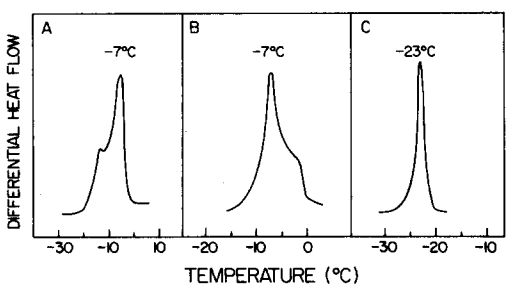

Fig. 1. Differential scanning calorimetry scans of artificial liposome transitions, all obtained with 50% ethylene glycol as solvent. Liposomes were prepared from phosphatidylcholines acylated with (A) lactobacillic (19 cp, c^{11}), (B) dihydrosterculic (19 cp, c^9) or (C) *cis*-vaccenic acid (18 : 1, c^{11}).

TABLE II

TRANSITION TEMPERATURES (T_c) OF PHOSPHATIDYLCHOLINES ACYLATED WITH VARIOUS FATTY ACIDS

Fatty acid	T_c (°C)
Saturated	
12 : 0	0 *
14 : 0	23 *
16 : 0	41 *
18 : 0	58 *
Unsaturated	
16 : 1, c^9 (palmitoleic)	<—40
18 : 1, c^9 (oleic)	—22 **
18 : 1, c^{11} (*cis*-vaccenic)	—23
Cyclopropane	
17 cp, c^9	<—40
19 cp, c^9 (dihydrosterculic)	—7
19 cp, c^{11} (lactobacillic)	—7

* Values reported by Ladbrooke and Chapman [3] and Phillips et al. [20].
** Value reported by Phillips et al. [20,21].

scanning calorimetry, whereas Silvius and McElhaney used differential thermal analysis.

The significance of the double-bond to cyclopropane-ring conversion during the growth of many bacteria remains controversial. Because the fluidity of the membrane has been shown to influence the activity of a wide variety of membrane processes [22], we believe that methylation of unsaturated fatty acids could play a useful regulatory role, increasing the viscosity of the membrane and, therefore, attenuating the activity of membrane functions under certain environmental conditions. This mechanism of decreasing membrane fluidity would be particularly advantageous under growth-limiting conditions, where synthesis of new phospholipid, even containing only saturated fatty acids, would be insufficient to alter significantly the composition of the membrane. These are precisely the conditions under which cyclopropane fatty acids are most abundant.

A number of other possible roles for cyclopropane fatty acids have been suggested in the literature. These include protecting membrane lipids from oxidation [23], reducing their tendency to undergo lamellar-to-hexagonal phase transitions [10], increasing resistance to lactic acid [24] and eliminating active methyl groups [25]. The definitive experiments remain to be carried out.

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