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THE PROPERTIES OF MEMBRANES FORMED FROM CYCLOPENTANOID ANALOGUES OF PHOSPHATIDYLCHOLINE *

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We have examined the thermal characteristics and barrier properties of vesicles formed from six analogues of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). These analogues differ from DPPC in that the glycerol backbone has been replaced by each of the diastereoisomeric cyclopentane-1,2,3-triols. All of these compounds have main gel to liquid-crystal phase transition temperatures within 5 Kelvin of DPPC and four possess comparable enthalpies and entropies of transition. For two of the analogous, however, the values of the enthalpy and entropy of transition are more than double that of DPPC. The permeability characteristics and organization (as measured by diphenylhexatriene fluorescence depolarization) of vesicles formed from these two compounds suggest that their large transition enthalpy and entropy result from either a reorganization of the polar head group region during the transition or interdigitation of the acyl chains of opposing monolayers.

Aqueous dispersions of phospholipids have proven to be invaluable model systems for studying the functional and dynamic properties of extended phospholipid arrays such as bilayer membranes. The properties of such mixtures are a function of, among other factors, the structure of the phospholipid species. In this study, we have utilized a unique set of six analogues of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) [1] to form phospholipid/water dispersions. These analogues differ from DPPC in that the glycerol backbone has been replaced by each of the diastereoisomeric cyclopentane-1,2,3-triols resulting in substantial covalent restriction of intramolecular rotation about the backbone (Fig. 1).

Recent work has shown that the study of cyclopentano analogues of glycerol phosphate [2] and of neutral triacylglycerol analogues [3] and cyclopentano-phosphatidic acid analogues *** gives data significant for the identification of the 'active' rotameric state of the glycerol backbone of these biomolecules. In addition, preliminary examination of the six cyclopentano phosphatidylcholines has shown that only one of them, the all-*trans* (1,3/2-1P) isomer, is susceptible to en-

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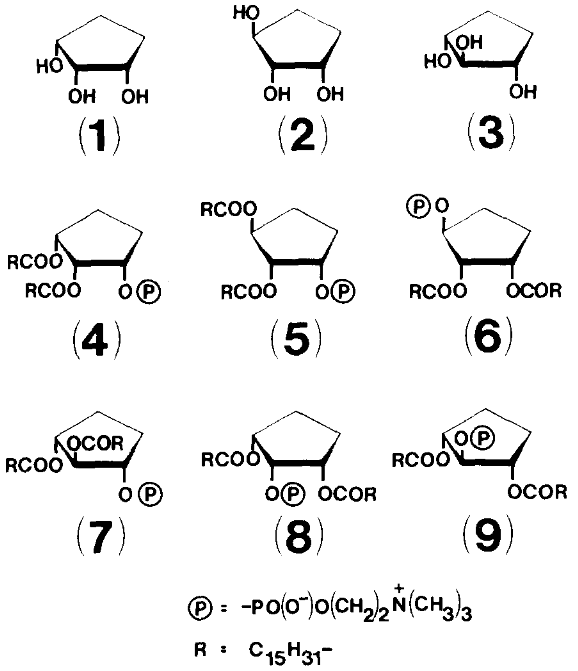

Fig. 1. Configuration of cyclopentane-1,2,3-triols. 1, (1,2,3/0)-cyclopentane-1,2,3-triol; 2, DL-(1,2/3)-cyclopentane-1,2,3-triol; 3, (1,3/2)-cyclopentane-1,2,3-triol. Configuration of cyclopentane phosphatidylcholines 4, 1,2,3/0-1P; 5, 1,2/3-1P; 6, 1,2/3-3P; 7, 1,3/2-1P; 8, 1,2,3/0-2P, 1,3/2-2P.

zymatic hydrolysis by phospholipase A_2 (*Crotalus adamanteus* and *Apis* sp. [4]).

In this communication, we report the thermal characteristics and barrier properties of vesicles formed from the cyclopentano phosphatidylcholines (Fig. 1, 4–9).

The cyclopentanoid analogues of DPPC were synthesized according to the procedures described earlier [1]. DPPC was obtained from Sigma Chemical Co., St. Louis, MO. All the compounds gave a single spot in TLC analysis on silicic acid using $CHCl_3/CH_3OH/H_2O$ (65 : 25 : 4, v/v) and $CHCl_3$/acetone/CH_3OH/acetic acid/water (6 : 8 : 2 : 2 : 1, v/v). Vesicles to be used for efflux measurements were prepared by drying down an appropriate aliquot of a stock chloroform solution of a given lipid and dispersing the dried lipid in 50 mM NaCl/5 mM Tris-HCl/^{22}NaCl (3 μCi/ml), pH 7.5, above the lipid transition temperature. The vesicles were dialyzed overnight at 5°C against buffer lacking the isotope to remove untrapped tracer and then incubated at different temperatures in stoppered glass tubes to measure efflux rates [5].

Differential scanning calorimetry (DSC) was performed on a Perkin-Elmer DSC-2 calorimeter. Typically, 25 mg of the lipid were dispersed in 250 μl Tris-buffered saline. The sample was vortexed at 50°C until a uniform dispersion was obtained. A 65 μl aliquot of the sample was transferred to a 75 μl stainless steel pan and the pan then sealed. The heat absorption was calculated by comparison of the area under the endotherm with that of an indium standard. A scan rate of 2.5 K · min^{-1} was employed, the enthalpies being calculated from the average of at least three scans. The molar enthalpy of melting was calculated on the basis of the measured heat absorption and the number of moles of lipid in the pan, based upon a phosphorus analysis [6] of the contents of the pan, which was opened at the end of the experiment.

The fluorescence depolarization of approx. 10^{-6} M 1,6-diphenyl-1,3,5-hexatriene (0.1% in the lipid) was conducted on an SLM Instruments fluorometer, as previously described [7]. The temperature of the mixture was controlled by a water jacket connected to a programmable heating/cooling bath (Neslab RTE-5). The temperature was monitored (±0.1 K) with a Bailey electronic thermometer (model BAT-8). Typical heating and cooling rates were 0.5 K · min^{-1}.

The main transition temperatures (gel to liquid-crystal) for each of the cyclopentano phos-

TABLE I

THERMAL PROPERTIES OF DPPC AND CYCLIC ANALOGUES OF DPPC

Lipid	T_c (K)	ΔH (kcal·mol^{-1})	ΔS (cal·mol·K^{-1})
DPPC	314	8.6	27
1,2,3/0-1P	311	10	32.1
1,2,/3-3P	318	18	56.6
1,3/2-2P	315	9.9	31.4
1,2/3-1P	309	8.9	28.3
1,2,3/0-2P	317	7.8	24.6
1,3/2-1P	319	19	59.6

T_c refers to the main gel to liquid-crystal transition temperature. ΔS was calculated from the relationship $\Delta S = \Delta H/T_c$.

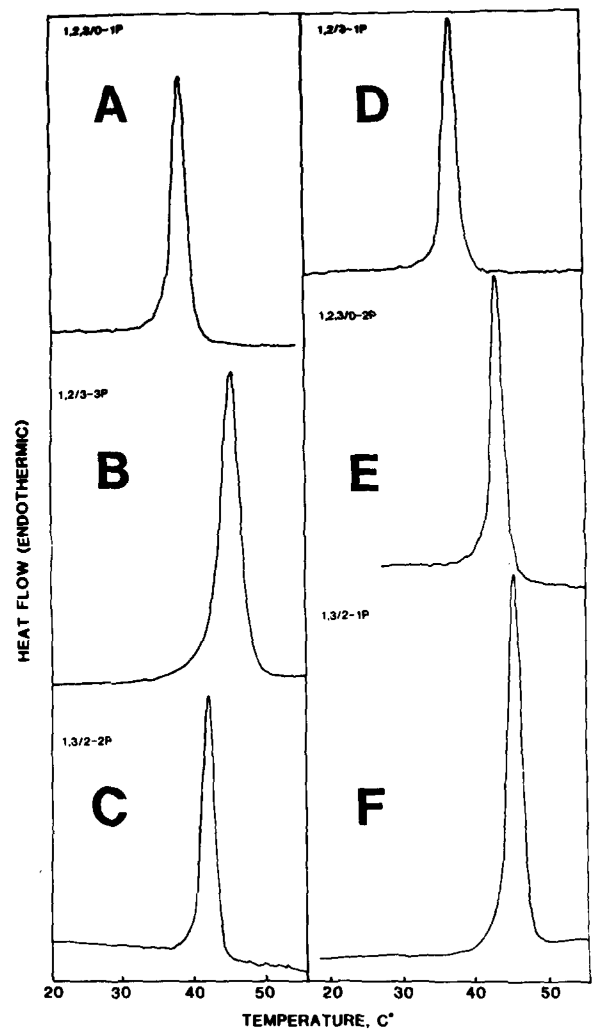

Fig. 2. DSC heating curves of cyclopentano phosphatidylcholines. Each sample was scanned at 2.5 K·min^{-1} and a sensitivity of 2 mcal·s^{-1}. Typically, 25 mg of phospholipid were dispersed in 250 μl Tris-buffered saline. A, 1,2,3/0-1P; B, 1,2/3-3P; C, 1,3/2-2P; D, 1,2/3-1P; E, 1,2,3/0-2P; F, 1,3/2-1P.

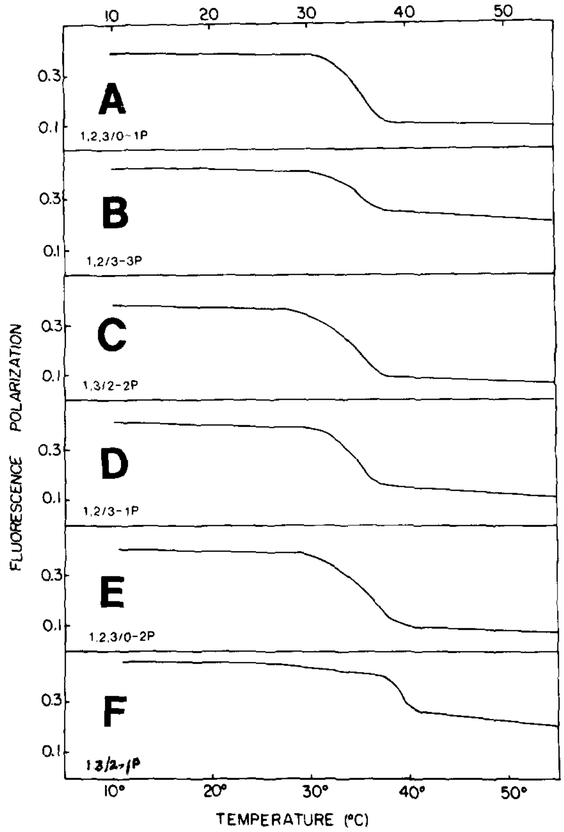

Fig. 3. The thermal dependence of the steady-state fluorescence polarization of diphenylhexatriene in various lipid dispersions (excitation wavelength 360 nm; emission wavelength 420 nm). A, 1,2,3/0-1P; B, 1,2/3-3P; C, 1,3/2-2P; D, 1,2/3-1P; E, 1,2,3/0-2P; F, 1,3/2-1P.

phatidylcholine isomers, together with the corresponding experimental thermodynamic data, are presented in Table I. The transition temperatures lie in the range of 36–45°C and so span the value for DPPC (41°C). In no experiments using the cyclopentano phosphatidylcholines was any indication of pretransition phenomena noted. The DSC traces are shown in Fig. 2. The values for the enthalpy and entropy of transition of four of the analogues (the all-*cis* isomers, 1,2,3/0-1P and 1,2,3/0-2P; the *cis-trans* isomer, 1,2/3-IP; the all-*trans* isomer, 1,3/2-2P) are close to that of DPPC but two analogues have values more than double that of DPPC.

Vesicles formed from the two isomers possessing 'high enthalpy' also reveal characteristics different from DPPC with respect to their degree of organization, as indicated by diphenylhexatriene fluorescence depolarization studies. These characteristics are illustrated in Fig. 3, and indicate that the membrane aggregates formed by the all-*trans* isomer (1,3/2-IP) and the *cis-trans* isomer (1,2/3-3P) are much more anisotropic and organized in structure above their transition temperature than are the other cyclopentano phosphatidylcholines.

Fig. 4 illustrates the changes in ^{22}Na effluxes as

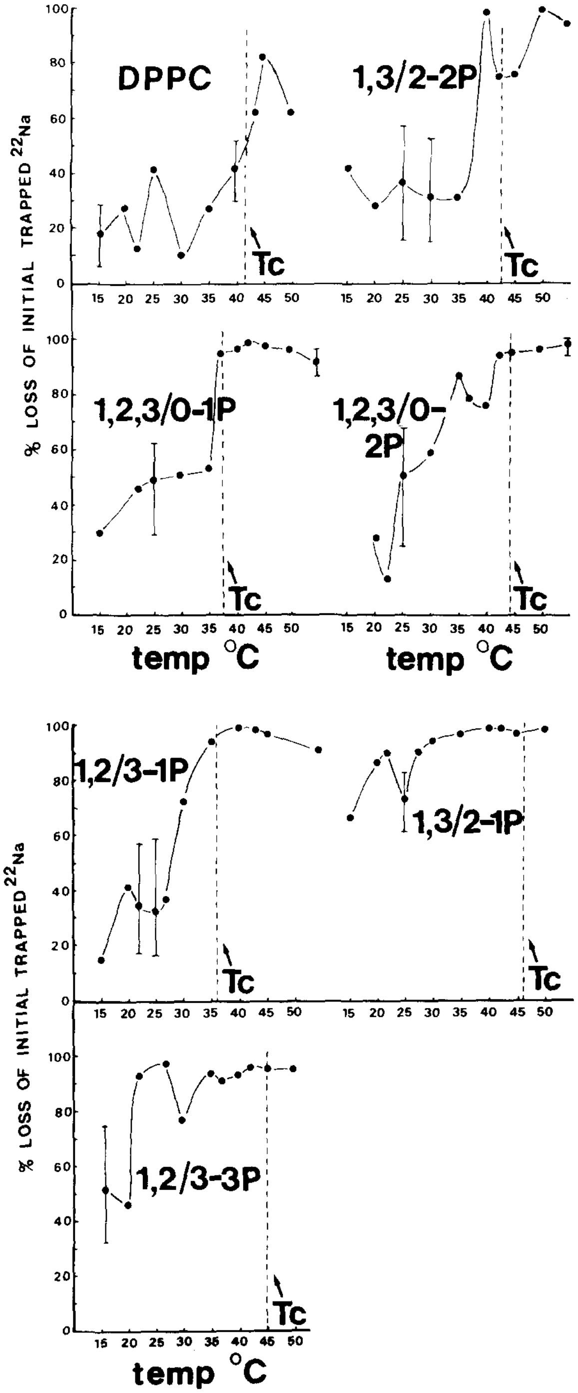

Fig. 4. ^{22}Na effluxes in different vesicles at different temperatures. Vesicles composed of the lipids indicated in the Figure were swollen in 50 mM NaCl/5 mM Tris-HCl/^{22}NaCl, pH 7.5. The ordinate refers to the percentage of initial trapped ^{22}Na lost over 180 min (3 h). Experimental points for each temperature represent one, or in some cases, two separate experiments each performed in triplicate. The results illustrated are the means with error bars indicating the range of values obtained. To simplify the figure, error bars have been given for only some points. The dashed vertical lines indicate the phase transition temperatures of the lipids in question.

a function of temperature from vesicles formed either from DPPC or from each one of the six analogues. In order to allow for comparison with previously reported studies [5,8], we have plotted the three-hour cumulative effluxes in this figure. Vesicles prepared from DPPC display a distinct permeability maximum at a temperature near to its main phase transition. In contrast, vesicles prepared from the six analogues do not show such sharp permeability maxima although there are differences among these lipids. The four conformers with thermal properties similar to those of DPPC do generally show a decrease in ^{22}Na efflux below their respective transition temperatures while the two 'high enthalpy' compounds display high ^{22}Na efflux rates even at temperatures well below their main gel to liquid-crystal phase transitions.

To verify that these cyclic analogues form intact vesicles we examined both the osmotic sensitivity and morphology of these structures by negative stain electron microscopy [9]. Osmotic experiments were performed with vesicles containing 10 mol% dicetyl phosphate. Dicetyl phosphate gives a negative charge to the vesicles, increasing their internal volume and thereby making the measurement of volume alterations technically easier. Changes in volume were monitored by measuring the absorbance at 450 nm. All six compounds gave vesicles which displayed a linear relationship between the extent of shrinkage and the reciprocal of the transmembrane NaCl concentration [10]. These studies were performed above the transition temperature since the times for equilibration were prolonged when the lipids were in the gel state. Morphologically, each of the compounds formed closed vesicles, although, interestingly, the vesicles appeared to be single-walled. Multilamellar liposomes were difficult to find.

These experimental observations demonstrate that by replacing the glycerol backbone in DPPC with cyclopentane triols and then manipulating the relative positions of the head group and acyl chains on the ring one can obtain lipid analogues with unusual physical and thermodynamic properties. Two of the conformers display extremely large values of enthalpy and entropy of transition (Table I) which would correspond to a glycerol-based phosphatidylcholine having acyl chains of twenty-six carbons in length *. In addition, these two lipids form vesicles which are quite permeable to ^{22}Na both above and below the transition temperature yet maintain a more organized and anisotropic membrane structure than DPPC or the other conformers (as measured by DPH fluorescence depolarization) in the liquid-crystal state.

At present, we do not have sufficient experimental data to describe the actual packing arrangement of these two unusual cyclopentano phosphatidylcholines but we offer the following speculations. Firstly, we believe the present results are inconsistent with a model in which the large values of enthalpy and entropy of transition result from disruption of chain/chain interactions, as generally envisaged in a lipid bilayer. If these two cyclic analogues were behaving as if functionally their acyl chains were quite long, one would have expected vesicles formed from them to display a reduced rather than an increased rate of ^{22}Na efflux, since glycerol-based phosphatidylcholines show an inverse relationship between chain length and ^{22}Na efflux rate [8]. Furthermore, the observation that the two 'high enthalpy' conformers form membranes with a more organized structure above the transition temperature than do either the other four analogues or DPPC is not to be expected if an extensive disruption of chain/chain interactions occurring during the transition is the factor responsible for their unusual thermal properties.

We suggest that these two conformers either undergo a reorganization of their polar head group regions during the transition or that they form bilayers with the acyl chains of opposing monolayers interdigitated. Changes in polar head group structure can significantly alter bilayer permeability [8] and, similarily, one would anticipate that the reduced thickness of an interdigitated bilayer would also result in an increase in the ^{22}Na efflux rate. We believe future experiments will allow us to decide between these possibilities.

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References

1 Hancock, A.J. Lister, M.D. and Sable, H.Z. (1982) J. Lipid Res. 23, 183–189

2 Weissman, J.D., Hancock, A.J., MacQuarrie, R., Lee-Abrahams, I.S. and Sable, H.Z. (1982) J. Biol. Chem. 257, 3618–3622

3 Dorset, D.L. and Hancock, A.J. (1977) Z. Naturforsch. 32C, 573–580

4 Lister, M.D. and Hancock, A.J. (1981) 17th Midwest Regional Meeting American Chemical Society, Columbia, MO Abstr. No. 207

5 Singer, M. (1982) Chem. Phys. Lipids 31, 145–159

6 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468

7 Mantulin, W.W., Massey, J.B., Gotto, A.M., Jr. and Pownall, H.J. (1981) J. Biol. Chem. 256, 10815–10819

8 Singer, M. (1981) Chem. Phys. Lipids 28, 253–267

9 Egerdie, B. and Singer, M. (1982) Chem. Phys. Lipids 31, 75–85

10 Blok, M.C., Van Deenen, L.L.M. and De Gier, J. (1976) Biochim. Biophys. Acta 433, 1–12

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* If one plots enthalpy versus chain length for known phosphatidylcholines, a value of 18 kcal·mol^{-1} extrapolates to a chain length of twenty-six carbons.