Chem. Pharm. Bull. 26(5)1501—1504(1978)

UDC 547.915.5.04 : 547.458.04

Permeability of Liposomal Membranes composed of Unnatural Types of Synthetic Lecithin-Analogues[1]

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(Received October 6, 1977)

Permeability properties of liposomal membranes composed of the synthetic unnatural type phospholipids (IIa—c, III, IV) are compared with those of the corresponding glyceride-type lecithins (Ia, Ib). Certain synthetic analogues (IIa, III and IV) can retain glucose in the liposomes in nearly same order as that in the saturated long-chain lecithin-derived liposomes. Cholesterol fluidizing effect of the lecithin analogues was generally small compared with those of the corresponding lecithins, partly due to bulkiness of trichloromethyl group which may hinder the immobilization of cholesterol along the fatty acid chains.

Interestingly, the liposomes derived from enol-type synthetic lipid (III) can regain the barrier ability at the temperatures above the transition temperature just as natural unsaturated-chain lecithins do.

Keywords——liposome; lecithin; unsaturated fatty acid; unnatural phospholipid; cholesterol effect; permeability; craft point; enol-type phospholipid; lysolecithin; trichloromethyl group

Model systems of phospholipids have provided valuable informations on the important functions of biological membranes like a barrier ability against the permeation of water-soluble materials such as inorganic ions and low molecular organic compounds involving monosaccharides.[3] There have been ample observations[4] that such property of lipid bilayers is markedly dependent on the structural features of lipids such as the nature and chain-length of fatty acid moieties. However, scant informations[5] seem to be available so far on the permeability of multilamellar liposomes derived from synthetic lecithin analogues different from natural phosphoglycerides.

This paper describes the properties of liposomal membranes composed of the following unnatural lipids (IIa—c, III and IV) readily prepared from the vinylene carbonate-carbon tetrachloride telomers[6] in comparison with those of the corresponding glyceride-type lecithins (Ia, Ib). Among the lipids examined, the enol-type of phospholipid (III) has kept a unique place in regard to the temperature-sensitive permeability of the liposome, reflecting an interesting effect of *trans*-enol geometry.

1) Part XI of the series, "Telomers and Oligomers of Vinylene Carbonate." Part X: N. Mitsuo, T. Kunieda, and T. Takizawa, *Chem. Pharm. Bull.* (Tokyo), **26**, 1493 (1978).
2) Location: *Hongo, Bunkyo-ku, Tokyo, 113, Japan.*
3) S.C. Kinsky, P.P.M. Bosen, C.B. Kinsky, L.L.M. van Deenen, and A.F. Rosenthal, *Biochem. Biophys. Acta*, **233**, 815 (1971); R.A. Demel, S.C. Kinsky, C.B. Kinsky, and L.L.M. van Deenen, *ibid.*, **150**, 655 (1968); J. De Gier, J.G. Mandersloot, and L.L.M. van Deenen, *ibid.*, **150**, 666 (1968); D. Papahadjopoulos and H.K. Kimelberg, Phospholipid Vesicles (liposomes) as Models for Biological Membranes: Their Properties and Interaction with cholesterol and Proteins, Pergamon Press, Oxford, 1973, *etc.*
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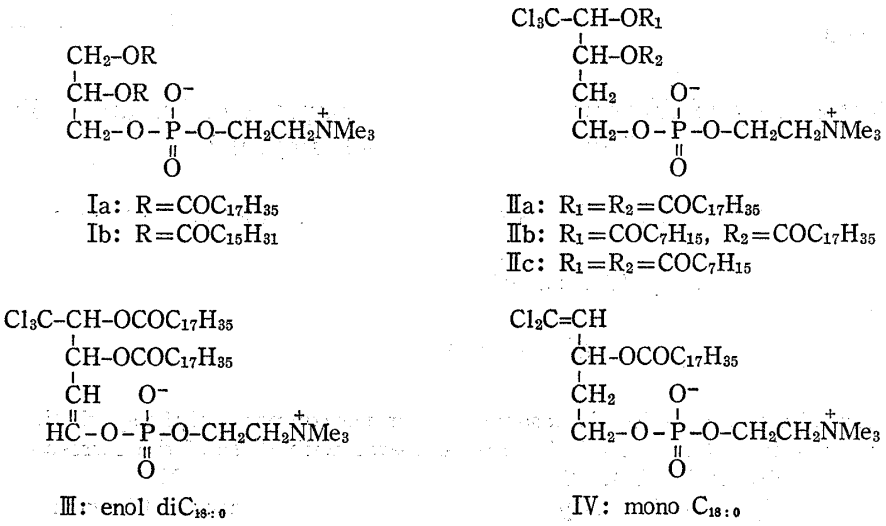

Chart 1. Long-chained Lecithins and Synthetic Analogues

Materials and Methods

Unnatural phospholipids (lecithin analogues) (IIa—c, III, IV) used for the present liposome study were prepared in our laboratory as described in the preceding paper,[7] and distearoyl- and dipalmitoyllecithins were kindly supplied by Prof. S. Nojima of this faculty. According to the literature's method,[8] liposomes were prepared from the lecithins and the analogues in glucose solutions with or without cholesterol above the transition temperatures. The amount of marker glucose trapped in the liposomes and the diffusion through the liposomal membranes were assayed enzymatically in the virtually same way as described in the literatures.[4,8] In the cases of leakage experiments carried out above 42°, the reaction mixture was treated with the enzyme system to determine the diffused amounts of glucose after it was quenched rapidly to room temperature (20°),[4] at which the glucose-release was observed to be very slow.

Results and Discussion

Glucose Trapping Capability of the Liposomes

Liposomes of the saturated lecithins (Ia, Ib) and the synthetic analogues (IIa—c, III, IV) were prepared without or with cholesterol in molar ratios to phospholipid of 1:1 and 1.5:1 above the transition temperatures, and the glucose retained inside the liposomes was assayed after dialysis at room temperature or 0°. Certain liposomes thus obtained showed an appreciable ability to retain glucose as summarized in Table I.

Table I. Amount of Trapped Glucose in Liposomes (mol/mol of Phospholipid)

Mol ratio Lecithin:Cholesterol	Liposomes derived from						
	Ia[a] (Tc 58°)	Ib[a] (Tc 41°)	IIa[b] (Tc 49°)	III[b] (Tc 49°)	IV[c] (Tc r.t)	IIb[c] (Tc r.t)	IIc[c] (Tc r.t)
1:0	2.3	4.5	5.2	2.9	6.1	0.9	0.1
1:1	4.3 (4.2)[d]	— (6.3)[d]	6.8 (2.8)[d]	5.8 (0.9)[d]	2.6	0.9	0.1
1:1.5	—	—	—	—	1.7	1.4	0.5

Liposomes of mixtures of lecithin (analogs) and dicetyl phosphate (1:0.1 molar ratio) were prepared with or without cholesterol at 68°(*a*), 60°(*b*), 37°(*c*) and room temperature(*d*). After dialysis against isotonic solution containing 0.075 M KCl and 0.075 M NaCl for 2 hr at room temperature (20°) (*a*, *b*, *d*) and 0°(*c*), the amount of retained glucose was enzymatically assayed.
r.t=20°

7) N. Mitsuo, T. Kunieda, and T. Takizawa, *Chem. Pharm. Bull.* (Tokyo), **26**, 1493 (1978).
8) S.C. Kinsky, J.A. Haxby, D.A. Zopf, C.R. Alving, and C.B. Kinsky, *Biochemistry*, **8**, 4149 (1969).

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The amounts of glucose trapped in the liposomes prepared from IIa (di-$C_{18:0}$), III(enol di-$C_{18:0}$) and IV (mono $C_{18:0}$) with or without cholesterol were in nearly same order as those in corresponding lecithin-derived liposomes[4] and not so critically affected by the structural modification like the additional introduction of lipophilic trichloromethyl and methylene groups. In contrast with natural lysolecithin and ethylene glycol type lecithin[9] which existed in the micells under the conditions, monochain lecithin analogue (IV) formed the liposomes which could trap considerable amounts of glucose, presumably due to the dichlorovinyl group effective enough to play a role in favorable alteration of the ratio of hydrophobic to hydrophilic portions. Among the lipids examined, the least amounts of marker could be trapped in the lamellar layers derived from dishort ($C_{8:0}$) chain lipid analogue (IIc), as might be expected, and short-long chain lipid (IIb) was intermediate.

Based on less amounts of glucose trapped in the liposomes prepared from the lecithin analogues (IIa and III) with equimolar cholesterol at room temperature (Table I), cholesterol-fluidizing effect[10] seems not to be so significant with the present cases, in contrast to that commonly observed with natural type lecithins.[4] This is probably due to steric factor of bulky trichloromethyl group which may hinder an approach of cholesterol hydroxy group to the ester carbonyl function, and hence, prevent the immobilization of cholesterol nucleus along the lipid hydrocarbon chain to form stable bilayers.

Temperature Dependence of Permeability

Glucose-containing liposomes prepared from synthetic lipid analogues (IIa, III and IV) without or with cholesterol (in the ratios of 1: 1 and 1.5: 1) were incubated at various temperatures for 10 min and the released glucose was determined.

The permeability curves thus obtained were sensitive to temperature and the liposomes prepared from synthetic lipids IIa and III without cholesterol showed maximum peaks at

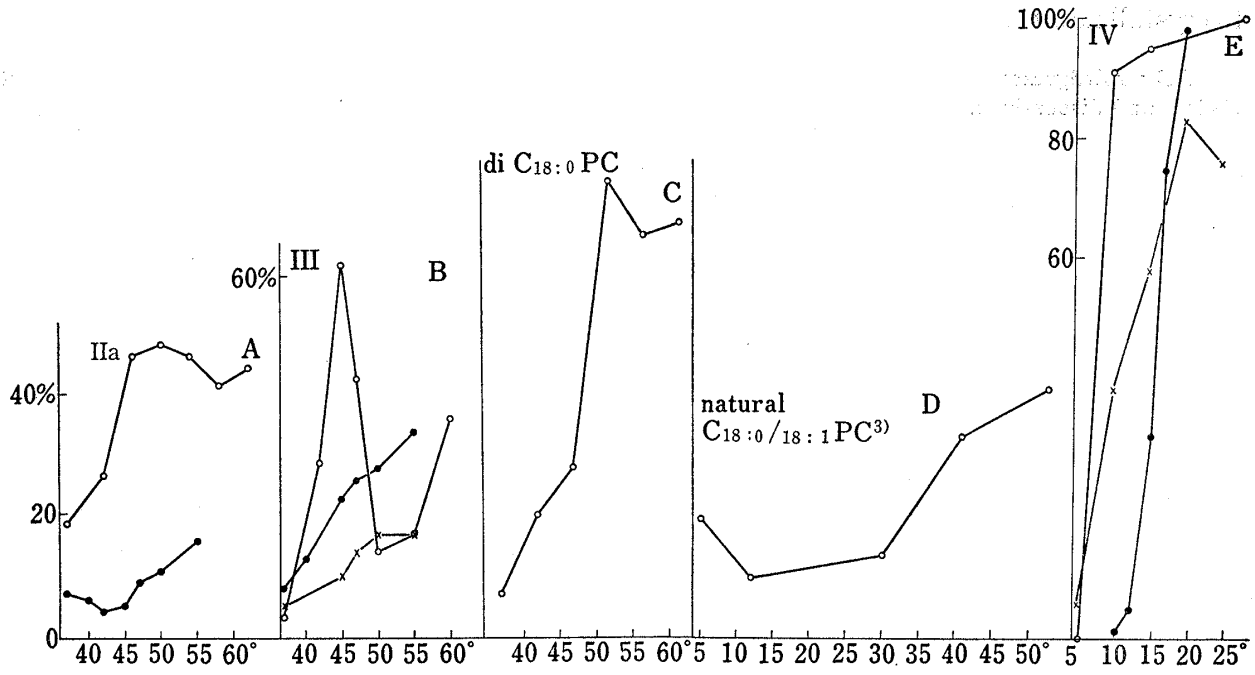

Fig. 1. Temperature Dependence of Glucose Permeability through Liposomes prepared with Synthetic and Natural Phospholipid

—○—: without cholesterol, —●—: with 1 eq cholesterol, —×—: with 1.5 eq cholesterol.

9) E. Baer, *J. Am. Chem. Soc.*, **75**, 5533 (1953).
10) B.D. Ladbrooke and D. Chapman, *Chem. Phys. Lipids*, **3**, 304 (1969).

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50° and 45°, respectively, (Fig. 1A,B), of which the former was rather similar to that of natural lecithin liposome[4] (Fig. IC). It is quite characteristic that the liposomes derived from III could regain the barrier ability at the temperatures above 45° significantly. Such behavior has been commonly observed with the liposomes from the lecithins with unsaturated fatty acid residues,[11] though the saturated-chain lecithins show no such appreciable ability. Insufficient barrier ability was observed with the lecithin analogue-liposomes prepared even with more than equimolar amount of cholestreol, in contrast to the corresponding saturated lecithin liposomes.[4] The liposomes derived from monochain analogue IV without cholesterol released almost 100% of trapped glucose within 10 min in the incubation at 10° (Fig. 1e). That may suggest that the transition temperature of IV is about 10° which is intermediate between those of dilauloyl ($C_{12:0}$) (Tc 0°)[12] and dimyristoyl ($C_{14:0}$) (Tc 23°)[12] lecithins.

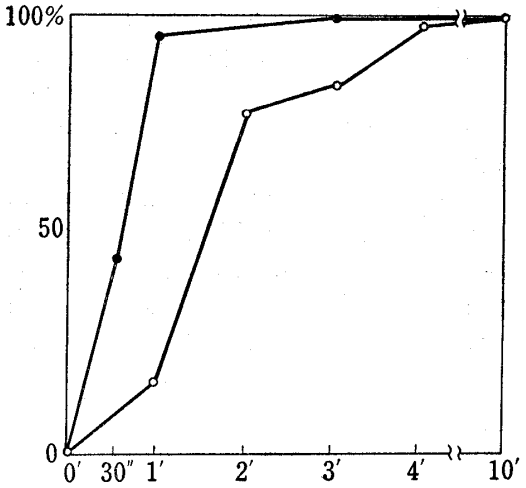

Fig. 2. Time Course of Diffusion of Glucose from Liposomes of Synthetic Phospholipids (IIa and III)

—●—: IIa at 50°,
—○—: III at 45°.

Diffusion of glucose from liposomes derived from IIa (at 50°) and enol lecithin III (at 45°) at transition temperatures were determined as function of minutes (Fig. 2). The former liposomes released almost all of trapped glucose within 1 min, while the latter required four minutes for complete diffusion. Thus, liposomes from the enol lecithin analogues (III) was less leaky at the transition temperature, suggesting that liquid-crystalline structure of III might be similar to crystalline structure and the phase separation was not so outstanding.

Acknowledgement We are grateful to Professor S. Nojima and Dr. K. Inoue of this faculty for helpful advices and discussions.

11) R.A. Demel, S.C. Kinsky, C.B. Kinsky, and L.L.M. van Deenen, *Biochem. Biophys. Acta*, **150**, 655 (1968).
12) G.B. Ansel, J.N. Hawthorne, and R.M.C. Dawson "Form and Function of Phospholipids," Elsevier Scientific Publishing, Amsterdam, 1973.

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