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Influence of dolichyl phosphate on permeability and stability of bilayer lipid membranes

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The ionic permeability coefficients, ionic transference numbers, activation energy of ion transport and breakdown voltage of bilayer lipid membranes made from dioleoylphosphatidylcholine or its mixtures with dolichyl 12-phosphate have been studied. The electrical measurements showed that dolichyl phosphate in phospholipid bilayers decreases membrane permeability, changes membrane ionic selectivity and increases membrane stability. These results are discussed in light of the aggregation behavior and the intramolecular clustering of a dolichyl phosphate molecule in phospholipid membranes. From our data we suggest that the hydrophilic part of dolichyl phosphate molecules regulates their behavior in membranes.

Introduction

Dolichyl phosphate functions as a hydrophobic carrier of glycosyl units across membranes during glycosylation reactions in eucaryotic cells [1–4]. Although the level of dolichyl phosphate in tissues is lower than that of dolichol, studies of subcellular distribution of dolichyl phosphate showed its accumulation in rough endoplasmic reticulum membranes [4]. The rat liver rough endoplasmic membranes contain twice as much dolichyl phosphate as dolichol, even though only 7% of rat liver dolichol was found to be phosphorylated [4]. In comparison with corresponding organs of the rat, chicken, rabbit and pig, human tissues contain unusually high levels of dolichol and dolichyl phosphate [5]. The highest dolichyl phosphate level, on a weight basis, was found in the human pituitary gland (283 μg per g wet weight), reaching the level 4.5% (w/w) of total phospholipids [5].

The behavior of dolichyl phosphate in liposome membranes has recently been intensively studied. Dolichyl phosphate influences the thermotropic mesomorphism of phospholipid molecules [7,8], decreases the membrane motional freedom in the liquid-crystalline state and increases the rate of fusion between phospholipid vesicles [7,8]. The EPR study of spin-labeled dolichyl phosphate [9] showed a slow transbilayer movement and a monomolecular dispersion of dolichyl phosphate in phosphatidylcholine vesicles. NMR studies of the headgroup ^{2}H-labeled dolichyl phosphate [9] suggest some unusual conformation of the long poly-*cis* prenyl chains.

In this paper the effect of dolichyl phosphate on the permeability and stability of a model membrane system – bilayer lipid membranes (BLMs) is

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reported [10,11]. The results are discussed in light of the aggregation behavior and the intramolecular clustering of a dolichyl phosphate molecule.

Materials and Methods

Chemicals. DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) was purchased from Sigma. Dol-12-*P* (dolichyl 12-phosphate) was a generous gift of Prof. T. Chojnacki (Polish Academy of Science, Warsaw, Poland). *n*-Decane and butanol were purchased from Aldrich and Fisher, respectively.

Membrane formation. Experiments were performed by using both macrovesicular and planar bilayer lipid membranes. Macrovesicular bilayer lipid membranes were formed according to the technique of Schagina et al. [12] on a Teflon capillary tube in unbuffered (pH 6) aqueous solution of 0.1 mol/dm^3 and 0.2 mol/dm^3 NaCl (inside and outside of the membrane, respectively). For other details see [13]. Planar bilayer lipid membranes were formed using the microsyringe technique [10,14]. The area of the macrovesicular bilayer lipid membranes was about 50 mm^2, the area of the planar bilayer lipid membranes was about 1 mm^2. DOPC or DOPC/Dol-12-*P* mixtures used for membrane formation were dissolved in *n*-decane/butanol (3 : 1, v/v) to obtain a concentration of 20 mg of lipid per ml of solvent.

Electrical measurements. The electric circuit (Fig. 1) consisted of two saturated silver chloride electrodes placed in the bathing solution on both sides of the membrane, an electrometer (Keithley 610C), a d.c. millivolt voltage source, an EC/225 voltammetric analyzer (IBM) and an I-6 low level capacitance meter (ICE/Electronics). The membrane resistance was measured by using the d.c. method [10] with one electrode connected to the millivolt voltage source while the other was connected to the electrometer. To obtain the values of the breakdown voltage, the applied voltage from the voltammetric analyzer was increased by a scan rate of 10 mV · s^{-1}. The membrane rupture was reflected by a rapid increase of current. The temperature was controlled by water circulation from an external bath.

The membrane permeability coefficients for Na^+ and Cl^- ions and the ionic transference numbers were calculated as shown in Refs. 10, 13.

Results

Experiments were performed in which the conductance of macrovesicular bilayer lipid membranes studied was measured as a function of temperature in the range of 25–41° C. Typical trends are reported in Fig. 2. An increase of

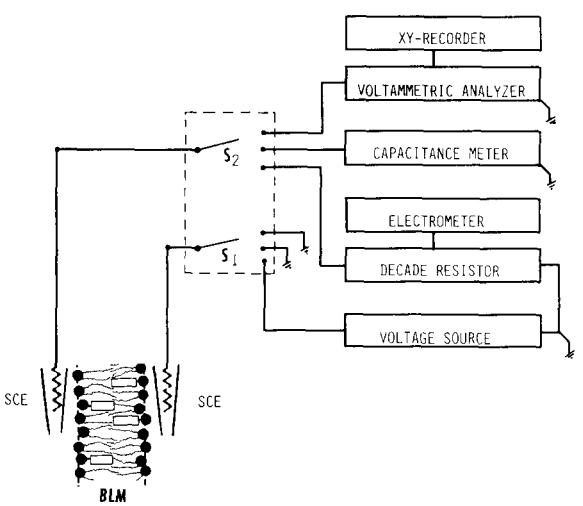

Fig. 1. The experimental set-up used for electrical measurements of bilayer lipid membranes. BLM, bilayer lipid membrane; RE, reference electrode.

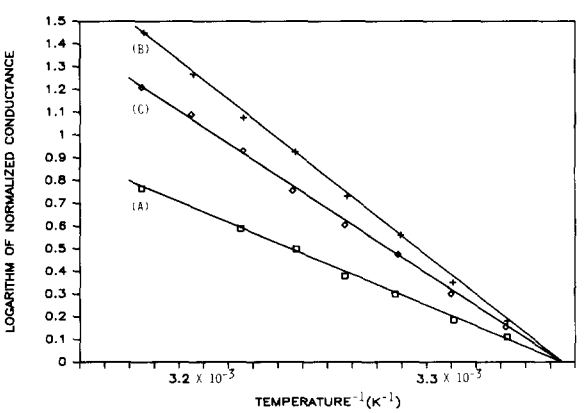

Fig. 2. Arrhenius plots of normalized conductance of macrovesicular bilayer lipid membranes made from (A) DOPC; (B) Dol-12-*P*/DOPC, mole ratio 1 : 100; (C) Dol-12-*P*/DOPC, mole ratio 1 : 10. Logarithm of normalized conductance was calculated as: $\ln [(G/C)/(G_0/C_0)]$; G, C represent membrane conductance and capacitance, respectively; G_0 and C_0 are the conductance and capacitance, respectively, at 25° C.

TABLE I

PROPERTIES OF BILAYER LIPID MEMBRANES MADE FROM DOPC, DOPC/Dol-12-*P*, MOLE RATIO 0.01 AND DOPC/Dol-12-*P*, MOLE RATIO 0.1

Experiments were performed at 25±1°C. The temperature range for the determination of activation energy was 25–41°C. The figures present means (±S.D.) for 5–7 different bilayers.

	DOPC	DOPC/Dol-12-*P*	
		0.01	0.1
Normalized resistance ($\Omega \cdot cm^2$)	$(1.9 \pm 0.47) \cdot 10^7$	$(2.0 \pm 0.28) \cdot 10^8$	$(7.6 \pm 1.5) \cdot 10^7$
Ratio of ionic transference numbers (t_{Na^+}/t_{Cl^-})	1.1± 0.10	1.7± 0.13	2.5± 0.15
Permeability coefficient for Na^+ ions ($cm \cdot s^{-1}$)	$(5.2 \pm 1.7) \cdot 10^{-11}$	$(7.1 \pm 1.5) \cdot 10^{-12}$	$(1.9 \pm 0.48) \cdot 10^{-11}$
Permeability coefficient for Cl^- ions ($cm \cdot s^{-1}$)	$(4.8 \pm 1.6) \cdot 10^{-11}$	$(2.4 \pm 0.5) \cdot 10^{-12}$	$(6.4 \pm 1.6) \cdot 10^{-12}$
Activation energy [a] ($kJ \cdot mol^{-1}$)	48.6± 4.1	90.0± 2.2	76.3± 1.4
Breakdown voltage (mV)	193 ±19	307 ±14	249 ±17

[a] Of macrovesicular BLMs, all other properties refer to planar BLMs.

normalized conductance is observed with increasing rate, depending on the percentage of dolichyl phosphate in the membrane. The Arrhenius plots are linear without any change of the slope.

Table I collects data on normalized resistance, the ratio of ionic transference numbers (t_{Na^+}/t_{Cl^-}), permeability coefficients for Na^+ and Cl^- ions and breakdown voltage of the planar bilayer lipid membranes. The values of the activation energies were derived from the Arrhenius plots by the least-squares fitting. It appears from Table I that the values of permeability coefficients for bilayers prepared with the Dol-12-*P*/DOPC mixture, mole ratio 0.01, is over 10-fold smaller in comparison with bilayers prepared of DOPC. The increase in the value of activation energy for ion migration across the membrane and in the value of breakdown voltage is 85% and 59%, respectively. As shown in Table I the changes in membrane properties for bilayers prepared with the Dol-12-*P*/DOPC mixture (mole ratio 0.1) are smaller but still substantial. The ratio of ionic transference numbers (t_{Na^+}/t_{Cl^-}) increases up to 2.5, with increasing concentration of Dol-12-*P* in DOPC membranes.

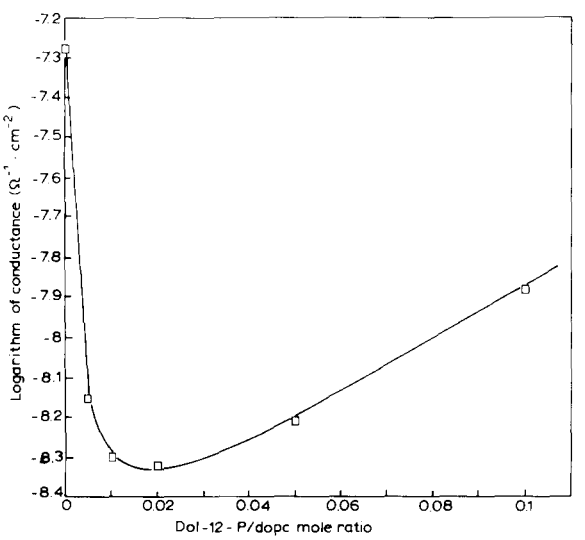

Fig. 3. Ionic conductance verses the Dol-12-*P*/DOPC mole ratio. Each point represents the mean value (±S.D.) obtained from five to seven different macrovesicular bilayer lipid membranes. Experiments were performed at 25±1°C.

The ionic conductance of macrovesicular bilayer lipid membranes formed from various mixtures of Dol-12-*P* and DOPC is reported on a semilogarithmic scale in Fig. 3. A minimum of membrane conductance occurs at Dol-*P*/DOPC mole ratio of 0.01 : 0.02.

Discussion

In order to get an insight into a possible molecular mechanism of dolichyl phosphate-mediated glycosyl transfer across membranes we studied permeability and stability of bilayer lipid membranes prepared from DOPC and Dol-12-*P*. Since the main phase transition temperature of DOPC bilayers is below −20°C [15], the DOPC membrane was at liquid-crystalline state in our experimental conditions.

Dolichyl phosphate molecules consist of a long, unsaturated, mainly poly-*cis* configuration isoprenoid chain with a phosphate group bonded to the saturated α-isoprene residue. The *cis* geometry enables the chain to be more compact and fold into a shorter length than poly-*trans* isoprenoids [12]. The studies of McCloskey and Troy [9] and Valtersson et al. [7,8] indicate that dolichyl phosphate molecules are oriented in the membrane with their phosphate headgroups at the hydrophilic interface.

Our investigations show that dolichyl phosphate substantially increases the energy barrier for ion migration through membranes, giving rise to the decrease of ion permeability. Dolichyl phosphate also increases the value of the breakdown voltage of the membranes, which reflects the stabilization effect of dolichyl phosphate on the phosphatidylcholine bilayer. The activation energy was found to be essentially independent of temperature over the range studied. Such a phenomenon indicates that the influence of temperature on the aggregation behavior of dolichyl phosphate and phosphatidylcholine molecules in the membrane is negligible.

The observed effect of dolichyl phosphate on the permeability and stability of DOPC bilayers can result from different values of hydrophilic-lipophilic balance of dolichyl phosphate and DOPC molecules, calculated as the ratio of the headgroups area to the mean value of the cross sectional area of the chain region. The hydrophilic-lipophilic balance of unsaturated phosphatidylcholine can be estimated as about 1.2 [16] giving rise to the formation of gaps in the interior of the phosphatidylcholine bilayer.

Troy and co-workers [9] noted the aggregation of neutral spin-labeled polyisoprenoids in liquid-crystalline phospholipid membranes. Because of the flexibility and the length of the polyprenyl chain of dolichyl phosphate molecules and their monomolecular dispersion in membranes, it seems probable that an intramolecular clustering of isoprenyl residues within a dolichyl phosphate molecule occurs. In this model, the ω-end of a dolichyl phosphate molecule can be surrounded as well by phosphatidylcholine hydrocarbon chains as by isoprenyl residues of its native dolichyl phosphate molecule. Such an intramolecular clustering results in a hydrophilic-lipophilic balance much less than 1. In this case, the dolichyl phosphate molecules embedded into a phospholipid bilayer decrease the amount of gaps in the membrane interior making it less permeable and more stable. It could be speculated that the intramolecular clustering can, in appropriate conditions, lead to a conversion of the dolichyl phosphate molecule into a lipophilic ion. This possibility will have to be tested in the future.

Contrary to the behavior of Dol-*P*, polyisoprenols increase membrane permeability, decrease the activation energy of ionic transmembrane migration, decrease membrane stability and do not change membrane selectivity [7,13,17–20]. The aggregation of neutral polyisoprenoids in phospholipid membranes was observed even at relative concentrations less than 0.005 [9]. These aggregates can modulate the permeability and stability of polyisoprenol-phospholipid membranes.

As shown in Fig. 3, the membrane's ionic conductance increases above the Dol-*P*/DOPC mole ratio of 0.02, although it is smaller than the conductance of DOPC bilayers. Spin-labeled dolichyl phosphate exists monomolecularly dispersed within phospholipid membranes at a relative concentration of 0.03 or less [9]. Therefore, the increase in conductivity of the bilayer lipid membrane could result from aggregation of Dol-*P* molecules at higher concentrations.

As shown by Ohki [21], phosphatidic acid, having a phosphate group as its polar part similar to Dol-*P* molecules, increases the ratio of ionic transference numbers (t_{Na^+}/t_{Cl^-}) of phospholipid bilayers at simlar relative concentrations as that of Dol-*P* in our study. Hence, the increase in t_{Na^+}/t_{Cl^-} ratio can result from a negative surface charge of Dol-*P*/DOPC bilayers. This is indicative of a net negative charge of the Dol-*P* headgroup at the membrane/aqueous solution interface. The repulsion electric forces between negatively charged head groups can therefore explain the phenomenon of the monomolecular dispersion of Dol-*P* in phospholipid membranes.

In conclusion, from these results we suggest that the hydrophilic part of Dol-*P* regulates the behavior of these molecules in membranes, possibly due to its influence on aggregation and conformation of polyprenyl chains.

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