Dolichol induces membrane leakage of liposomes composed of phosphatidylethanolamine and phosphatidylcholine

Ching-San Lai and John S. Schutzbach*

National Biomedical ESR Center, Department of Radiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226 and **University of Alabama in Birmingham, 716 Diabetes Hospital, University Station, Birmingham, AL 35294, USA*

Received 27 December 1983; revised version received 13 February 1984

Dolichol promotes the leakage of membranes in liposomes composed of phosphatidylethanolamine and phosphatidylcholine but not liposomes composed only of phosphatidylcholine. The membrane leakage was assayed by measuring the entrapment of TEMPOcholine, a cationic spin probe, in liposomes using ESR methods. The percent of membrane leakage induced by dolichol was found to be linearly proportional to the concentrations of dolichol. It is proposed that dolichol enhances the formation of non-bilayer configurations in liposomes containing phosphatidylethanolamine, thereby inducing membrane leakage.

Dolichol *Membrane leakage* *Spin label* *ESR*

1. INTRODUCTION

Dolichols, a family of polyisoprenoid lipids, present mainly in lysosomes, Golgi, and endoplasmic reticulum of the cell play important roles in the biosynthesis of glycoproteins [1–3]. Although the exact molecular mechanism is still not known, these long-chain polyprenols may be responsible for transport of sugar units during glycoprotein biosynthesis.

Because of their unusual length (about 100 Å) and poly-*cis* geometry, we thought it of interest to investigate the effects of dolichol molecules on membrane leakage of liposomes composed of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) and those composed of only pure PC.

Membrane leakage was here measured by assaying the entrapment of TEMPOcholine, a cationic spin probe, in liposomes using electron spin resonance (ESR) methods. Briefly, TEMPOcholine in dilute aqueous solution gives rise to an ESR spectrum consisting of 3 sharp lines with an isotropic nitrogen hyperfine constant of about 16.8 G [4]. Under these dilute conditions, the signal amplitude is linearly proportional to the concentrations of TEMPOcholine. This TEMPOcholine ESR signal can be readily destroyed by ascorbate, a spin probe reducing agent. Both TEMPOcholine and ascorbate are membrane impermeable at 4°C. Therefore, if TEMPOcholine is added to liposomes prior to the addition of ascorbate at 4°C, this would allow the destruction of TEMPOcholine remaining outside the liposomes and thus the detection and quantitation of TEMPOcholine entrapped within the liposomes.

2. MATERIALS AND METHODS

PE and PC from soybean were obtained from Avanti Polar Lipids. Dolichol was purchased from Sigma and TEMPOcholine was a product of Molecular Probes.

To prepare liposomes containing dolichol, various amounts of dolichol in CCl_4 were added to pure PC or equal amounts of PE and PC (1:1, w/w) in $CHCl_3$. The samples were dried under a stream of nitrogen and placed under vacuum overnight. The lipids were hydrated by the addition of 1 ml of 10 mM Tris buffer (pH 7.0) containing 100 mM NaCl and 2 mM EDTA and incubated at

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

37°C for 10 min. During this 10 min period, the samples were vortex mixed twice for 10 s. The lipid concentrations were about 10 mg/ml. The liposomes so prepared were found to be stable at 23°C for at least 12 h.

For determining the amount of entrapment of TEMPOcholine, an aliquot (1 μl) of TEMPOcholine solution (0.15 M in H_2O) was added to 100 μl of the liposomes containing various amounts of dolichol. After incubation at 37°C for 10 min, the reaction mixture was chilled to 4°C for 2 min prior to the addition of an aliquot (10 μl) of cold sodium ascorbate (1 M in H_2O). The sample was then transferred into a disposable pasteur pipet which served as an ESR sample tube and was immediately placed into the ESR cavity preset at 4°C.

All ESR spectra were recorded with a Varian Century line spectrometer operating at 9 GHz and equipped with a Varian temperature regulator and a digital thermometer (Fluke 2100A model). The field modulation and field sweep were 100 kHz and 100 G, respectively. The modulation amplitude was 1.0 G. The time constant and scan time were 0.25 s and 4 min, respectively. The microwave power was 10 mW.

3. RESULTS AND DISCUSSION

Liposomes composed of PE and PC, but no dolichol, were incubated with TEMPOcholine at 37°C for 10 min, followed by the addition of ascorbate at 4°C to reduce TEMPOcholine remaining outside the liposomes. Fig.1, trace 1, shows a composite of two spectra, namely, a small 3-line TEMPOcholine signal plus an ascorbyl radical signal (a doublet) near the central-field peak position of the TEMPOcholine signal. This small TEMPOcholine signal could not be destroyed by ascorbate, suggesting that it represents TEMPOcholine entrapped in liposomes composed of PE and PC in the absence of dolichol. However, the presence of dolichol at a concentration of 2 mg/ml in liposomes composed of PE and PC increased the amount of TEMPOcholine entrapment by a factor of 9, as judged from the increase in the signal amplitude of the low-field peak of TEMPOcholine ESR spectrum (fig.1, trace 2). The results suggest that dolichol induces the leakage of liposomes composed of PE and PC. The addition of 10% Trition X-100 solubilized the liposomic vesicle and released all of the entrapped TEMPOcholine which was then reduced by ascorbate, as evidenced in fig.1, trace 3. This further confirms the suggestion that at 37°C, the presence of dolichol in liposomes composed of PE and PC induces membrane leakage, thereby entrapping TEMPOcholine spin probe. This process was found to be highly temperature dependent: whereas there is profound membrane leakage at 37°C, no detectable leakage occurs at either 23 or 4°C regardless of dolichol contents in the liposomes.

By comparing the signal amplitude of the entrapped spin probe with the signal amplitude of a known concentration of TEMPOcholine solution, the signal amplitude in fig.1, trace 2, corresponds to 0.4 nmol TEMPOcholine trapped in PE/PC membranes containing 2 mg/ml dolichol. To our knowledge, little is known about the encapsulation efficiency for liposomes composed of PE and PC particularly in the presence of dolichol. Assuming 5% of the original volume was captured in the liposomes [5], a complete leakage of the liposomes would contain 7 nmol TEMPOcholine, i.e., 5% of the total TEMPOcholine added. The entrapment of 0.4 nmol TEMPOcholine thus represents about 6% leakage of the liposomes. It is therefore not unreasonable to assume that the leakage induced by dolichol is limited to the first shell of the liposomes. The presence of dolichol in PE/PC membranes did not affect the size distribution of liposomes as determined by electron microscopy techniques (unpublished).

The time course for the entrapment of TEMPOcholine in liposomes based on the signal amplitude of the low-field peak of TEMPOcholine ESR signal for the probe entrapped in PE/PC membranes containing 1 mg/ml dolichol at 37°C is depicted in fig.2 (open circle). The rate of entrapment was linear for the first 5 min and seemed to reach a plateau at about 10 min. Control experiments (fig.2, open triangle) showed that in the absence of dolichol, the amount of TEMPOcholine entrapped was very small even after 10 min incubation. As shown in fig.3, the percent of entrapment of the spin probe in PE/PC membrane vesicles was linearly proportional to the amount of dolichol present.

Liposomes composed only of pure PC were also

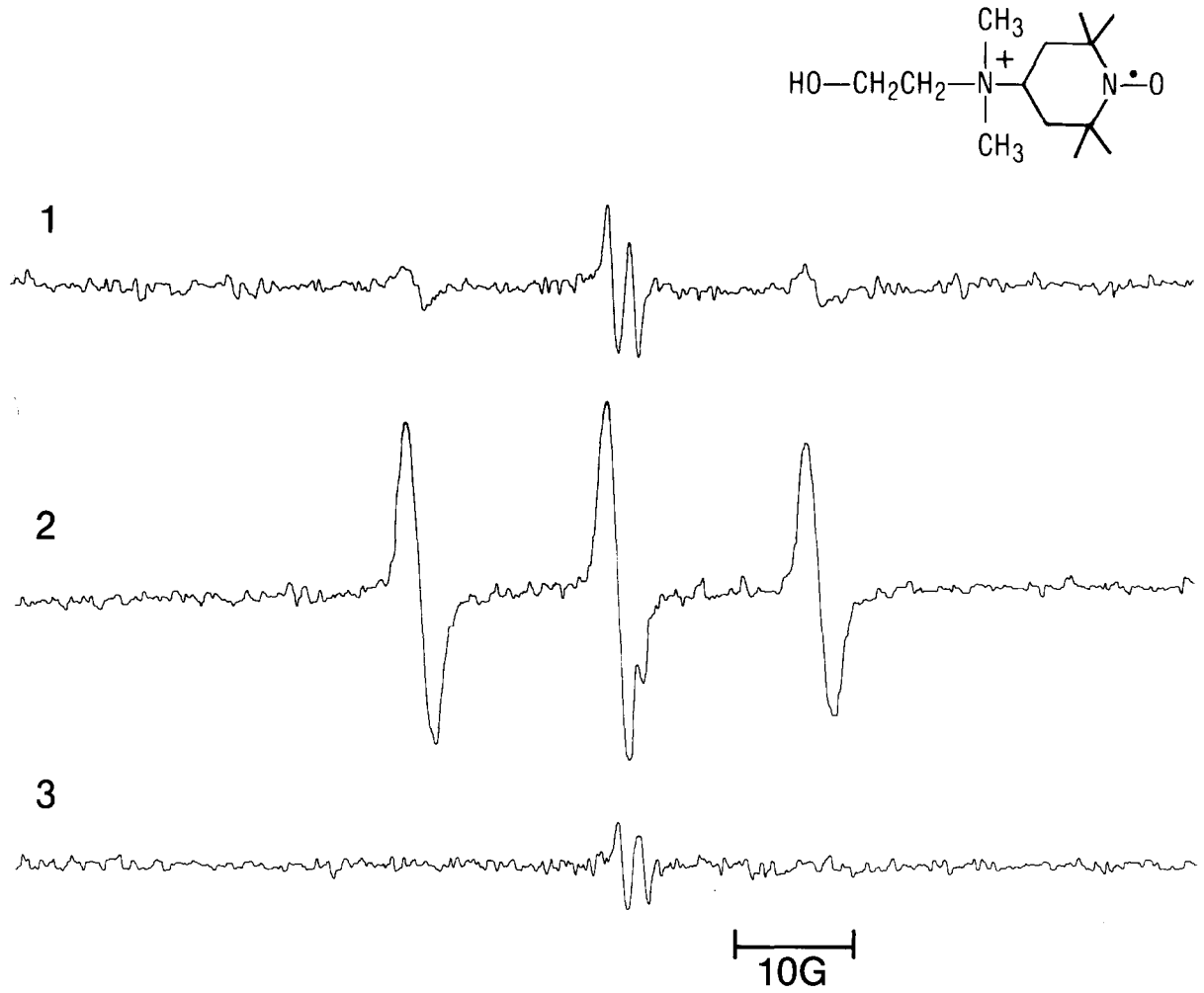

Fig.1. Effect of dolichol on the ESR spectrum of TEMPOcholine entrapped in the liposomes composed of PE and PC. Liposomes composed of PE and PC (1:1, w/w) containing (1) no dolichol or (2) 2 mg/ml dolichol prepared in 10 mM Tris buffer (pH 7.0) with 100 mM NaCl and 2 mM EDTA were incubated with 1.4×10^{-3} M TEMPOcholine at 37°C for 10 min. The liposomes were cooled to 4°C and after the addition of cold ascorbate (90 mM), the ESR spectra were recorded at 4°C. (3) Same as (2) except for the addition of 10% Triton X-100.

found to entrap TEMPOcholine at 37°C in the absence of dolichol, suggesting that the vesicle is leaky at 37°C. The addition of dolichol at a concentration of 0.2 mg/ml to PC membrane vesicles reduces the membrane leakage by a factor of 4 (not shown). Therefore, whereas the presence of dolichol in PE/PC membranes induces membrane leakage, the presence of dolichol in pure PC membranes inhibits membrane leakage. The different effects of dolichol on membrane leakage between liposomes composed only of pure PC and of PE/PC membrane vesicles suggest that the interactions of dolichol with these two membranes occurs via largely different mechanisms. PC membranes with a main phase transition temperature of about −7 to −15°C are highly fluid at 37°C [6]. It is conceivable that the presence of dolichol, a long-chain polyisoprenoid, may decrease the membrane fluidity and thus reduce the membrane leakage in pure PC membrane vesicles although the direct evidence to support this claim is still lacking.

The dynamic interaction of dolichol with PE/PC membranes is still poorly understood. PE constitutes 10–40 mol% of the membrane

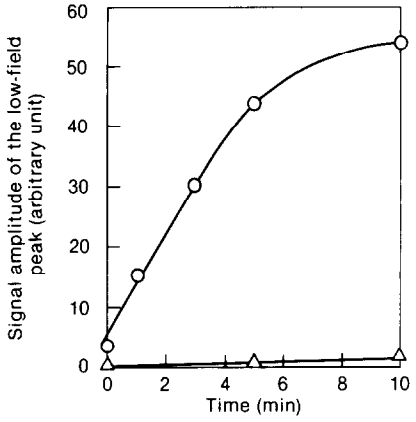

Fig.2. Time dependency of the entrapment of TEMPOcholine in PE/PC membranes containing dolichol. The PE/PC membranes (1 : 1, w/w) containing 1 mg/ml dolichol (○) or no dolichol (△) prepared in 10 mM Tris buffer (pH 7.0) with 100 mM NaCl and 2 mM EDTA were incubated with TEMPOcholine at 37°C for various lengths of time as indicated. After the addition of cold ascorbate (90 mM), the ESR spectra were recorded at 4°C.

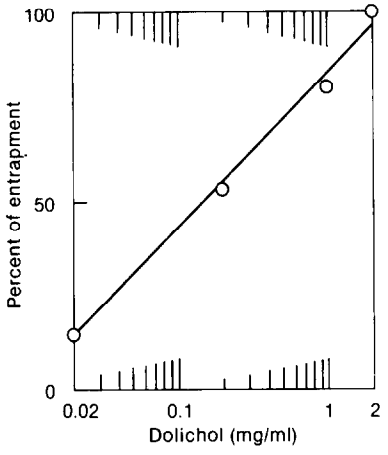

Fig.3. The effect of dolichol concentrations on the percent of entrapment of TEMPOcholine inside PE/PC membranes. PE/PC membranes (1 : 1, w/w) containing various amounts of dolichol from 0 to 2 mg/ml were prepared in 10 mM Tris buffer (pH 7.0) with 100 mM NaCl and 2 mM EDTA and incubated with TEMPOcholine at 37°C for 10 min. After the addition of cold ascorbate (90 mM) the ESR spectra were recorded at 4°C. 100% represents the maximum amount of spin probe entrapped in vesicles containing 2 mg/ml of dolichol.

phospholipid in most biological membranes of higher organisms [5]. It tends to adopt non-bilayer phases, particularly the hexagonal (H_{II}) phase in hydrated dispersions as demonstrated by ^{2}H NMR spectroscopy [7] and by ^{31}P NMR spectroscopy in conjunction with freeze-fracture electron microscopy [8,9]. The presence of the hexagonal (H_{II}) phase in the membrane may promote the formation of transmembrane ion channels where phospholipids form an aqueous pore through the membrane [10]. We hypothesize that the presence of dolichol, a polyisoprenoid lipid of length 100 Å, in PE-containing membranes may enhance the formation of transmembrane ion channels, thereby inducing membrane leakage. This mechanism would be consistent with a functional role of dolichol in the transport of polar carbohydrate units through the extremely hydrophobic nature of the lipid core [1,2].

ACKNOWLEDGEMENTS

This work was supported in part by the National Institutes of Health grant RR-01008, and the National Cancer Institute grant CA-16777.

REFERENCES

[1] Stuck, D.K. and Lennarz, W.T. (1980) in: The Biochemistry of Glycoproteins and Proteoglycans (Lennarz, W.T. ed) pp.35–83, Plenum, New York.
[2] Hubbard, S.C. and Ivatt, R.J. (1981) Annu. Rev. Biochem. 50, 555–583.
[3] Wong, J.K., Decker, G.L. and Lennarz, W.J. (1982) J. Biol. Chem. 257, 6614–6618.
[4] Kornberg, R.D. and McConnell, H.M. (1971) Biochemistry 10, 1111–1120.
[5] Jain, M.K. and Wagner, R.C. (1980) in: Introduction to Biological Membranes, pp.36, John Wiley and Sons, New York.
[6] Ladbrooke, B.D. and Chapman, D. (1969) Chem. Phys. Lipids 3, 304–319.
[7] Tilcock, C.P.S., Bally, M.B., Farren, S.B. and Cullis, P.R. (1982) Biochemistry 21, 4596–4601.
[8] Cullis, P.R. and DeKruijff, B. (1979) Biochim. Biophys. Acta 559, 399–420.
[9] Hui, S.W., Stewart, T.P., Yeagle, P.L. and Albert, A.D. (1981) Arch. Biochem. Biophys. 207, 227–240.
[10] Cullis, P.R., De Kruijff, B., Hope, M.J., Nayar, R. and Schmid, S.L. (1980) Can. J. Biochem. 58, 1091–1100.