Localization of dolichols in phospholipid membranes

An ESR spin label study

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We have used ESR methods employing spin-labeled stearates to investigate the effects of dolichol on the motion of lipid molecules in phospholipid membranes of phosphatidylethanolamine and phosphatidylcholine. The ESR spectra show that the presence of dolichol affects the motion of the spin probes at carbon-16, but not at carbon-5. Similar results are obtained with phospholipid membranes comprising only phosphatidylcholine. It is suggested that dolichol molecules are present mainly in the lipid core region of phospholipid membranes.

Dolichol *Phospholipid membrane* *Spin label* *ESR*

1. INTRODUCTION

Dolichols comprise a family of long-chain polyisoprenoid alcohols that are found in the membranes of most eucaryotic cells [1–3]. Although phosphorylated derivatives of dolichol are essential carriers of activated sugars for the biosynthesis of asparagine-linked oligosaccharide in glycoproteins [4], few functions have yet been ascribed to the pools of free and esterified dolichols which represent the major fraction of the dolichols found in cellular organelles.

In studies with model membrane systems [5,6], the inclusion of dolichol in mixtures of unsaturated phosphatidylethanolamine (PE) and phosphatidylcholine (PC) enhanced the activity of mannosyltransferase II (EC 2.4.1.132). This enzyme, which catalyzes the transfer of mannose from GDP-mannose to oligosaccharide-pyrophosphoryldolichol acceptors, is essentially inactive in PC bilayers with or without dolichol.

^{31}P NMR studies revealed that dolichol promotes a unique phase change in the PE/PC mixtures [6]. Using ESR spin probe methods, we have shown previously that dolichol increases the permeability of PE/PC liposomes to the cationic spin probe TEMPO-choline, whereas the permeability of PC bilayers is not affected by the addition of dolichol [7].

Here, we have employed spin-labeled stearates to probe the localization of dolichol in PE/PC bilayers. This study demonstrates that dolichol has an effect on the motion of the spin probes located at carbon-16, but not at carbon-5, suggesting that the majority of the free dolichols reside near the lipid core region of phospholipid membranes.

2. MATERIALS AND METHODS

PE and PC from soybean were obtained from Avanti Polar Lipids. Dolichol was purchased from Sigma and fatty acid spin probes were products of Aldrich.

Liposomes were prepared by mixing either pure PC or mixtures of PE and PC (1:1, w/w) with dolichol and spin probe at a final ratio of phospholipid/dolichol/probe of 10:1:0.1 (by wt). The samples in chloroform were dried under a

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stream of nitrogen and placed under vacuum overnight. The lipids were hydrated at a final concentration of 10 mg phospholipid/ml by the addition of 10 mM Hepes buffer, pH 7.0, containing 100 mM NaCl and 2 mM EDTA. After 10 min at room temperature the samples were vortex-mixed for 3 min and stored at room temperature for at least 2 h before use. The liposomes so prepared were found to be stable for at least 24 h when stored in ice.

ESR spectra were obtained with a Varian century line spectrometer operating at 9 GHz and equipped with a Varian temperature regulator and a digital thermometer (Fluke 2100 A 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 5 mW.

3. RESULTS AND DISCUSSION

Incorporation of 16-doxylstearate, a spin-labeled stearate with the nitroxide group at carbon-16, into PE/PC liposomes containing dolichol gave rise to the ESR spectra shown in fig.1. The spectra show that the spin probe exhibits a fast anisotropic motion with an isotropic hyperfine splitting constant of 14.5 G which is characteristic of the probe in a hydrophobic environment [8]. The line shapes of the spectra responded to temperature changes (fig.1) indicating that the motion of the probe in the membrane is sensitive to temperature. The spectra for the spin probe in PE/PC membranes without dolichol were similar to those in fig.1 (not shown). Since calculations of rotational correlation times based on the line shapes and linewidths for lipid spin probes undergoing a fast anisotropic motion in phospholipid membranes are not easily interpreted [9], we dediced to use the ratios of peak heights, namely, h_{+1}/h_0 and h_{-1}/h_0 (see fig.1 for definitions), as spectral parameters for measuring dolichol effects on membrane dynamics; the ratios of peak heights decrease as the motion of the probe is restricted.

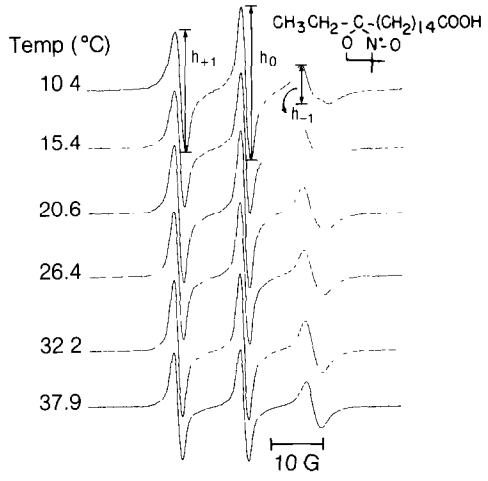

Fig.1. ESR spectra of 16-doxylstearate in liposomes composed of PE and PC containing dolichol. Liposomes were prepared as described in section 2. The sample was equilibrated at a given temperature as indicated for 10 min prior to ESR measurement.

The effects of dolichol on the ratios h_{+1}/h_0 and h_{-1}/h_0 for 16-doxylstearate in PE/PC membranes are shown in fig.2. Between 10 and 40°C, the presence of dolichol in the membrane reduced both h_{+1}/h_0 and h_{-1}/h_0, indicating that dolichol exerts an effect on the motion of the probe in the membrane. Since the nitroxide group of 16-doxylstearate is located near the lipid core region of phospholipid membranes, it is probable that the probe detects the presence of dolichol at that

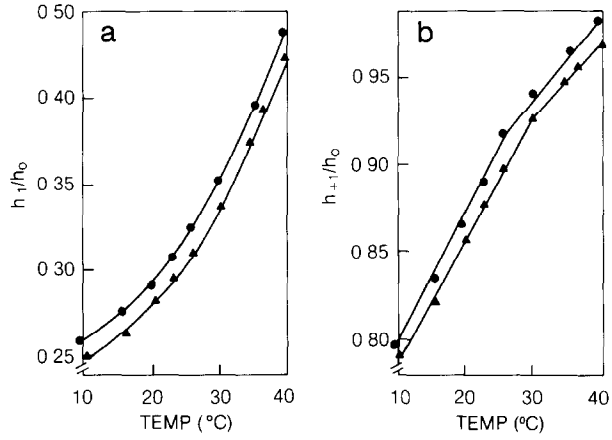

Fig.2. Temperature dependence of the spectral parameters of 16-doxylstearate in liposomes composed of PE and PC and containing 1 mg/ml dolichol. Liposomes were prepared as described in section 2. h_{+1}, h_0 and h_{-1} are peak-to-peak heights of the first derivative ESR lines of the low-, mid- and high-field components, respectively. (●—●) No dolichol, (▲—▲) 1 mg/ml dolichol.

region of the membrane. The reason for observed nonlinearities in the plots of h_{+1}/h_0 and h_{-1}/h_0 vs temperature (fig.2) is not yet known; no major structural transitions can be ascribed to those breaks between 20 and 40°C, inasmuch as both main phase transitions and bilayer-hexagonal transitions of PE/PC phospholipid membranes used in this study occur below 0°C.

To determine whether the motion of the spin probes near the polar head group region of the membrane is also affected by dolichol, we incorporated 5-doxylstearate (fig.3) into PE/PC liposomes. The spectra in fig.3 indicate that the nitroxide group of 5-doxylstearate is located near the polar head group region, thereby probing the molecular motion near that region of the membrane. The maximum splitting values in gauss, $2T_{\parallel}$, connected by two dashed lines in fig.3, are known to be a sensitive spectral parameter for detecting the motion of 5-doxylstearate in the membrane [8]. As shown in fig.4, the $2T_{\parallel}$ values decreased with increasing temperature. However, between 10 and 40°C, the presence of dolichol has no detectable effect on the $2T_{\parallel}$ values (fig.4). It is suggested that the motion of 5-doxylstearate in which the nitroxide reporter group is located near the polar head group is not affected by the presence of dolichol in the membrane. We have

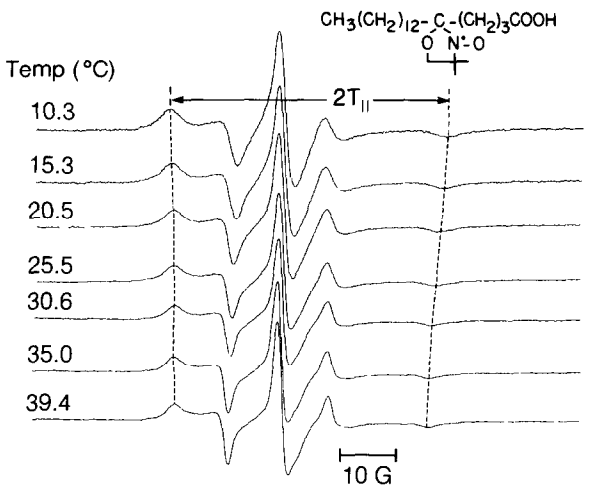

Fig.3. ESR spectra of 5-doxylstearate in liposomes composed of PE and PC. Liposomes were prepared as described in section 2. The sample was equilibrated at a given temperature as indicated for 10 min prior to ESR measurement.

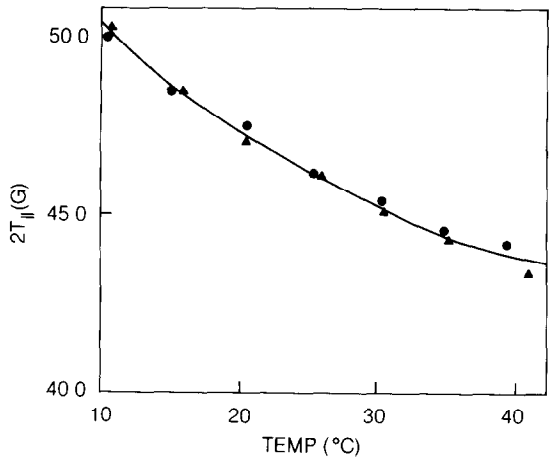

Fig.4. Temperature dependence of the spectral parameter $2T_{\parallel}$ of the ESR spectra of 5-doxylstearate in liposomes composed of PE and PC. Liposomes were prepared as described in section 2. The values of $2T_{\parallel}$ are obtained by the measurement of the maximal separation (in G) as marked by the two dashed lines in fig.3. (●—●) No dolichol, (▲—▲) 1 mg/ml dolichol.

carried out similar experiments using phospholipid membranes composed only of PC. The results are essentially similar to those for PE/PC membranes.

Dolichol has been shown to induce unique phase changes in phospholipid bilayers composed of either mixtures of plant PE and PC [6] or of dielaidoyl-PE [11] and to promote increased permeability in the PE/PC membranes [7]. In comparison, dolichol does not appear to affect greatly either the phase properties or the permeability of bilayers containing PC as the only phospholipid [6,10]. The mechanism for the effects of dolichol on PE/PC membranes is currently unknown but may be related to the insertion of the long polyisoprene chain in the membrane and to a specific association with PE [11]. The relatively high concentrations of free dolichols found in some tissues [1–3] suggest that the polyisoprenoid compounds may play a significant structural or functional role in biological membranes similar to those noted with the model systems.

Our results indicate that when dolichol is incorporated into membranes composed of unsaturated species of PE and PC, the dolichol pool is located mainly near the lipid core region of the phospholipid bilayer. This conclusion is based on the observations that dolichol has an effect on the

motion of the nitroxide reporter group of 16-doxylstearate, but not on the motion of 5-doxylstearate, when the probes are incorporated into the PE/PC membranes. Previous studies employing spin-labeled dolichols in PC bilayers [10] or utilizing ^{31}P-NMR [11] to examine the effects of dolichol on the phase properties of dielaidoyl-PE have also concluded that free dolichols reside at the center of the phospholipid bilayers. Whereas Valtersson et al. [11] reported that dolichol increases the fluidity of PE containing membranes, our investigation suggests that dolichol decreases the flexibility of the acyl chain in the 16-doxylstearate probe employed in our work. This variance may be attributed to the use of different experimental techniques as well as to differences in the PE phospholipids used in the studies.

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