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Effects of dolichol on membrane permeability

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Small vesicles containing the tetra-anionic fluorescent probe calcein were prepared by sonication of mixtures of plant phosphatidylethanolamine, plant phosphatidylcholine, and dolichol. Following chromatography, the isolated vesicles were found to retain entrapped calcein over the temperature range of 15 to 40 °C. Utilizing an assay measuring the fluorescence quenching of entrapped calcein by cobalt ions, the presence of dolichol in the membranes was found to promote the permeability of the phospholipid bilayers to the divalent cation. The permeability was shown to be dependent on temperature with an increase in rate of 17-fold between 15 and 35 °C although the plant phospholipids used in these experiments have no known phase transition within this temperature range. The incorporated dolichol was distributed uniformly throughout the vesicle population. Similar vesicles prepared from phosphatidylethanolamine and phosphatidylcholine without added dolichol, from phosphatidylcholine alone, or with phosphatidylcholine and dolichol were far less permeable to the divalent cation under the same assay conditions. These results demonstrate that dolichols have significant effects on the permeability properties of phospholipid bilayers that contain phosphatidylethanolamine.

Introduction

Dolichols comprise a family of polyisoprenoid alcohols usually containing between 15 and 20 isoprene units with the α-isoprene unit being saturated. Phosphate and pyrophosphate esters of dolichol serve as glycosyl carriers in the pathway for biosynthesis of the asparagine-linked oligosaccharide chains of glycoproteins [1–3] where one presumed function of the lipophilic carrier is to aid in the translocation of activated glycosyl units across the membrane of the rough endoplasmic reticulum. In support of this idea, Hasselbeck and Tanner [4,5] reported that dolichol-*P* promoted the translocation of mannose through phospholipid bilayers in the presence of a purified preparation of yeast dolichyl-*P*-mannose synthase (dolichyl-phosphate mannosyltransferase, EC 2.4.1.83). Most of the dolichol found in mammalian tissues and organs, however, is present as either the free alcohol or as fatty acid esters [6,7]. The highest concentrations of the neutral dolichols occur in Golgi and lysosomes, but no functions have yet been ascribed to these dolichol pools. Since some mammalian organs, pituitary and thyroid glands in particular [7,8], contain relatively large amounts of dolichols, it is possible that these compounds have a functional role in cellular membranes.

Abbreviations: PE, plant phosphatidylethanolamine; PC, plant phosphatidylcholine; Dol, dolichol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol; DPH, 1,6-diphenylhexa-1,3,5-triene.

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Previous work in our laboratory has shown that the activities of two liver enzymes involved in the synthesis of dolichyl-linked oligosaccharides, dolichyl-*P*-mannose synthase and mannosyltransferase II (EC 2.4.1.132), were dependent upon reconstitution of the enzymes in a lipid matrix containing high percentages of unsaturated PE* [9–11]. Both enzymes catalyze mannosyltransfer to hydrophobic dolichyl-linked substrates and the activities of the enzymes were significantly modulated by small changes in the lipid environment, including the concentrations of free dolichols. We therefore wanted to further investigate the effects of dolichol on the properties of membranes prepared with unsaturated plant phospholipids in order to provide insight into properties of the lipid environment that are required for the reconstitution of mannosyltransferase activity.

A number of investigations have attempted to establish the orientation and functions of dolichols in model membrane systems [4,5,10–22] prepared with PC, PE, or with mixtures of these phospholipids. The results that were obtained suggested that free and acyl esterified dolichols occupy sites near the hydrophobic center of phospholipid bilayers, whereas, the phosphate moiety of dolichol-*P* is located near the membrane water interface. The addition of dolichols to phospholipid membranes appears to increase the fluidity of the acyl chains of both PE and PC [15,17] although the mobility of spin labeled fatty acids incorporated in phospholipid bilayers is restricted by the presence of dolichols [19,22]. Dolichol and dolichol derivatives have been shown to induce vesicle fusion [17,21] and to have significant effects on the phase properties of PE containing membranes by promoting the formation of the hexagonal II phase [11,17,18]. The incorporation of dolichols in phospholipid bilayers also has effects on the permeability properties of membranes [16,20]. We have previously shown [16] that dolichol promoted the entrapment of the cationic spin labeled molecule (TEMPOcholine) in multilamellar vesicles prepared with highly unsaturated PE and PC. Significantly, in that study dolichol was found to have essentially no effect on the permeability of bilayers composed of only phosphatidylcholine. In the present investigation we have utilized a fluorescence assay to quantify the effects that dolichols have on the permeability properties of sonically prepared vesicles composed of highly unsaturated PE and PC.

Methods and Materials

Materials. Plant PC and plant PE were obtained from Avanti Polar Lipids, Inc. and were stored at −20 °C under an atmosphere of nitrogen. The percent fatty acid composition of a typical preparation of PE (Lot No. PPE-67) was as follows: myristate, 0.08; palmitate, 22.45; stearate, 2.3; oleate, 8.24; linoleate 60.48; and linolenate 6.07. The bilayer-hexogonal II phase transition for similar plant PE has been reported as low as −30 °C [23] although data presented by Yeagle and Sen [24] suggest that the bilayer-hexagonal phase transition temperature is about 15 °C. Dolichol, 2,4-bis-[*N*,*N*′-di(carboxymethyl)aminomethyl]fluorescein (calcein), and fluorescein isothiocyanate-dextran (FITC-Dextran) with an average mol. wt. of 9000 were purchased from Sigma. Radiolabeled di[1-^{14}C]palmitoylPE (110 mCi/mmol) and [*2-palmitoyl*-9,10-^{3}H]dipalmitoylPC (60 Ci/mmol) were from New England Nuclear. [^{3}H]Dolichol (155 mCi/mol) was a gift from Dr. R.K. Keller, University of South Florida. All other materials were of the highest purity available from commercial sources.

Preparation of vesicles. All of the vesicles used in these experiments were prepared with equal mixtures of PE and PC (w/w) unless otherwise noted and the amount of dolichol incorporated is expressed as weight percent. Mixtures of phospholipids (10 mg total phospholipid) and dolichol in the indicated amounts were mixed in 16 × 150 mm test tubes and the organic solvents were removed under a stream of dry nitrogen gas. Residual solvents were removed under continuous vacuum for 15 h. The phospholipids were hydrated in 1 ml of 20 mM Hepes buffer (pH 7.0) containing 0.1 M NaCl, 2 mM EDTA, and either 0.5 or 40 mM calcein. For some experiments, FITC-Dextran at 4.5 mg/ml was incorporated in the buffer in place of calcein. After 30 min at room temperature, the phospholipids were suspended by thorough stirring on a vortex mixer for 1.5 min, and the phospholipid dispersions were

transferred to 15 ml conical plastic tubes. Small vesicles were prepared by probe sonication using a well-tuned Heat Systems Model W-225 Sonifier equipped with a model C2 converter and standard tapered microtip with the output control set between 3 and 4. The phospholipids were cooled in an ice bath and were sonified for 15 min with the duty cycle at 50% to control heating of the phospholipid suspension. Sonication under these conditions did not cause breakdown of the phospholipid as judged by thin-layer chromatography. The vesicle preparations were centrifuged at $34000 \times g$ for 30 min and the supernatant fraction was collected. Trapped volumes for typical vesicle preparations were 0.84 l/mol for PE/PC vesicles and 0.93 l/mol for PC/PC/dolichol vesicles as determined by the procedure of Oku et al. [25] or by measuring the percent of trapped calcein following column chromatography as described below.

Vesicles containing entrapped calcein were separated from free calcein on columns of Sephadex G-50 (1.5×13 cm) at 4°C. The columns were eluted with 20 mM Hepes buffer (pH 7.0) containing 0.1 M NaCl and 2 mM EDTA, and 0.7 ml fractions were collected. Vesicles eluting in the void volume were stored at ice bath temperatures and were used within 1 to 8 h. Sonified vesicles containing entrapped FITC-Dextran were isolated following chromatography on columns of Sephadex CL-6B (1.5×27 cm).

Assays for membrane permeability. Fluorescence measurements were performed with continuous stirring in quartz cuvettes with a 1 cm path-length using either an SLM 4800 spectrofluorometer interfaced with an HP9815-A calculator and 9862-A plotter, or in a Gilford Fluoro IV interfaced with a printer/plotter. Constant temperatures were maintained with the use of a circulating water bath. For all experiments the excitation and emission wavelengths were 490 and 520 nm, respectively. Excitation and emission slit widths were set at either 8 nm or 5 nm. Relative fluorescence untis were usually plotted from either 0 to 10 or from 0 to 100 and in all experiments where repeated measurements were performed, the photomultiplier voltage was held constant.

Unless otherwise noted, a typical experiment consisted of adding 0.01 ml of the peak fraction of calcein-loaded liposomes (20 to 50 nmol lipid phosphate) to 3 ml of Hepes buffer, allowing 3 min for temperature equilibration with stirring, followed by initiation of data acquisition. With the SLM 4800, data points were sampled every 5 s for a total of 500 s and were stored on marked tape files for plotting and linear regression analysis. Each data point was the average of 10 separate measurements accumulated using the digital averaging features of the instrument. Typically, data was collected for 50 s in order to establish a baseline, followed by addition of either Co^{2+}, Triton X-100, or other divalent cations. Data collection continued for a total of 500 s. The data points were then plotted and the rate of change in relative fluorescence as a function of time ($\Delta F/\Delta t$) was determined by linear regression.

Other methods. The average diameter and heterogeneity of sonified vesicle preparations was assessed by chromatography on columns of Sephacryl S-1000 according to the procedures of Reynolds et al. [26,27] and Reers et al. [28]. Vesicles containing 5% dolichol and tracer quantities of [^{14}C]PE (0.2 μCi) and [^{3}H]dolichol (0.13 μCi) were prepared as described above. The sonified vesicles were diluted 1 : 2 with Hepes buffer and 0.5 ml samples were applied to 0.9×56 cm columns of Sephadex S-1000 that had been equilibrated with the same buffer at 4°C. Fraction size was 0.6 ml. The column was calibrated with the use of polystyrene beads of defined sizes (Polysciences) at room temperature in buffer containing 0.1% (w/v) sodium dodecyl sulfate.

Vesicles were also examined by negative stain electron microscopy. The vesicles were prepared as described above, diluted 10 fold with buffer, and applied to carbon coated formvar grids. Excess buffer was removed by blotting and the vesicles were stained with either 1% phosphotungstate (pH 7.0) or uranyl acetate.

Phospholipid phosphorus was determined by the procedure of Bartlett [29] or of Duck-Chong [30]. The purity of phospholipids and dolichol was assessed by thin-layer chromatography carried out on silica gel plates using the following solvents: (a) chloroform/methanol/water (65 : 35 : 4, v/v). (b) chloroform/methanol/ammonium hydroxide (65 : 25 : 5, v/v), and (c) ethylacetate/benzene (5 : 95, v/v).

Results

Preparation of vesicles

Vesicles were prepared by probe sonication of mixtures of plant PE and plant PC, with and without dolichol. Phospholipid dispersions containing only PE and PC were readily clarified by sonication, whereas, mixtures containing 5% to 10% dolichol were more difficult to disperse, and it was found necessary to maintain ice bath temperatures during sonication. This temperature range is still above the gel to liquid-crystalline phase transition temperatures for the plant phospholipids used in these experiments and above the melting point of pure dolichol [31]. The sonically prepared vesicles were subjected to centrifugation at $34000 \times g$ for 30 min to remove titanium particles and phospholipid aggregates. When radiolabeled PC or PE was incorporated in the vesicle preparations as a tracer, the recovery of isotope in the supernatants following centrifugation of PE/PC vesicles ranged from 85 to 95% and for PE/PC/dolichol vesicles ranged from 54 to 93%. The vesicular nature of the sonified phospholipids was assessed by the following procedures.

Entrapment of calcein

Vesicles prepared by sonication of phospholipids in the presence of 0.5 mM calcein were centrifuged at $34000 \times g$ for 30 min and were applied to a column of Sephadex G-50 (Fig. 1). Phospholipid eluted in the void volume of the column. The presence of encapsulated calcein was

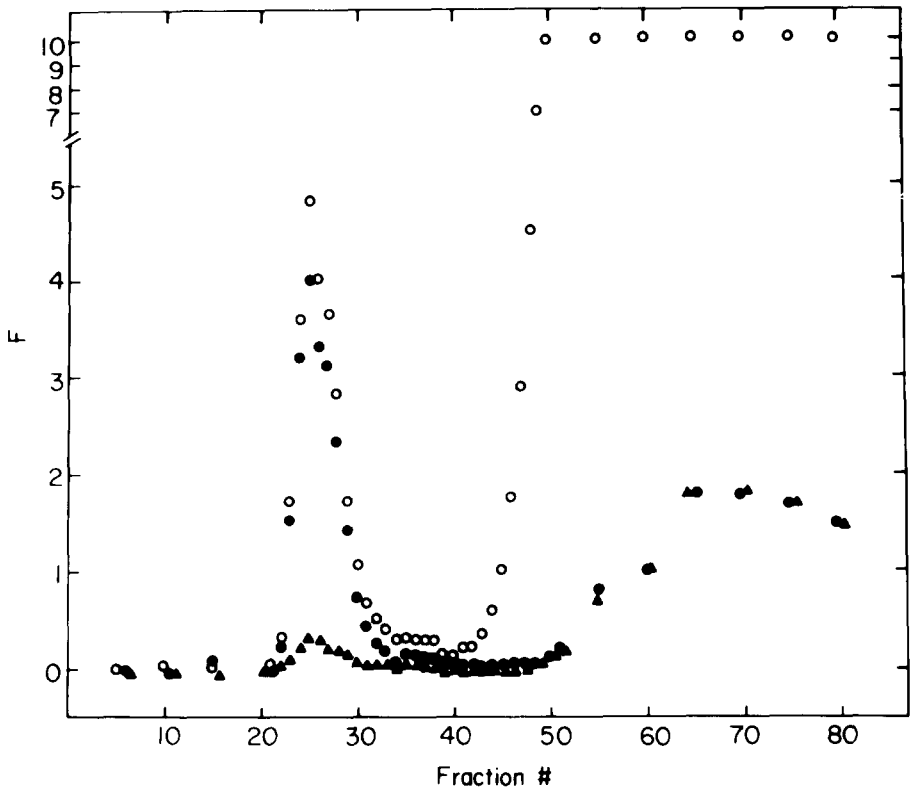

Fig. 1. Elution of calcein loaded vesicles from a Sephadex G-50 column. Sonified PE/PC/10% dolichol vesicles were prepared in the presence of 0.5 mM calcein, applied to a column (1.0×54 cm) of Sephadex G-50, and eluted with Hepes buffer at 4°C. Fraction size was 0.65 ml. The relative fluorescence of a 0.01 ml aliquot of each fraction in 3 ml of Hepes buffer was measured at 15°C using excitation and emission wavelengths of 490 and 520 nm. The following were measured in sequence in the same cuvette with continuous stirring: total fluorescence (○), fluorescence after addition of 0.01 ml of 1 M Co^{2+} (●), and fluorescence after addition of 0.02 ml of 10% Triton X-100 to solubilize the liposomes (▲).

assessed by measuring total fluorescence, followed by measuring fluorescence after the sequential addition of Co^{2+} and Triton X-100 to each fraction. Co^{2+} has been shown to completely quench the fluorescent signal due to calcein [32]. Calcein that was eluted in the vesicle containing fractions (void volume) was found to be resistant to fluorescence quenching by Co^{2+} ions at 15 °C, unless Triton X-100 was added to solubilize the phospholipids. The vesicular structure of the sonified phospholipids was confirmed by electron microscopy which revealed that sonified PE/PC/dolichol vesicles were heterogeneous in size and that some vesicles appeared to contain smaller entrapped vesicles (results not shown).

Assays for membrane permeability

Calcein and other fluorescent dyes have been used as probes of membrane permeability or leakiness [33–35], with assays usually involving measurements of the increase in fluorescence intensity upon release of the dye from self-quenching concentrations within the phospholipid vesicles. A similar methodology was tested in the present investigation. Vesicles were prepared from mixtures of PE/PC and PE/PC/10% dolichol in the presence of 40 mM calcein. Following centrifugation and column chromatography on Sephadex G-50, these vesicles were found to be impermeable, or non-leaky to the tetra-anionic fluorophore when incubated at 35 °C (Fig. 2). Solubilization of the membranes by the addition of detergent resulted in a large increase in fluorescence due to dilution of calcein below self-quenching concentrations. Although calcium ions have been reported to cause the aggregation and release of contents from vesicles composed of PE and cholesteryl hemisuccinate [36], the addition of either Ca^{2+} or Mg^{2+} had no effect on the release of calcein from PE/PC/dolichol vesicles. When Co^{2+} was added to PE/PC/dolichol vesicles, however, a slow rate of quenching of the residual fluorescence was noted suggesting that either the vesicles were permeable to the divalent cation at 35 °C or else the addition of cobalt ions resulted in disruption of the vesicular structure. Addition of detergent to solubilize the membranes resulted in immediate and complete quenching of residual fluorescence. Essentially identical rates of fluorescence quenching were obtained if manganese ions were added at the same concentrations in place of Co^{2+} (data not shown).

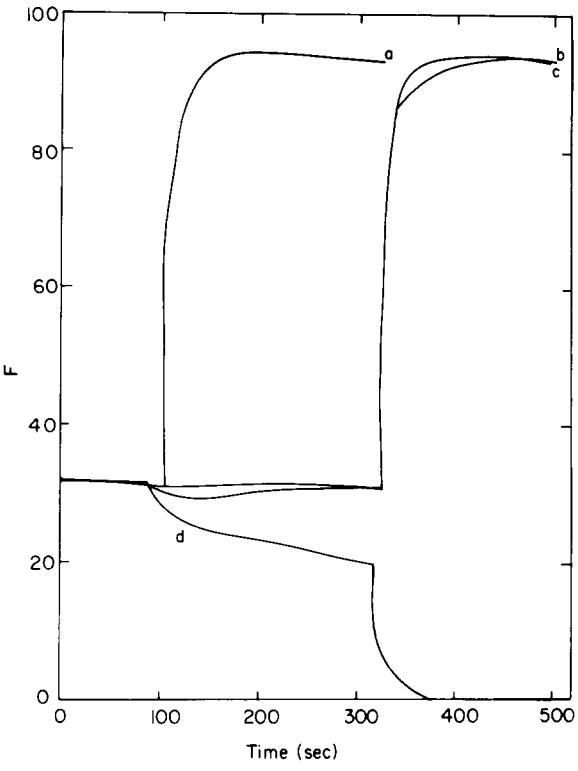

Fig. 2. Retention of calcein in PE/PC/dolichol vesicles. Self-quenching concentrations of calcein (40 mM) were entrapped in sonified PE/PC/10% dolichol vesicles and total fluorescence at 35 °C was determined as a function of time. Curve a, recovery of fluorescence upon solubilization of membranes by the addition of 0.02 ml of 10% Triton X-100 at 100 s. Curves b and c, fluorescence following the addition of Ca^{2+}, or Mg^{2+} (7 mM final concentration) at 100 s, and after the subsequent addition of detergent at 325 s. Curve d, quenching of residual fluorescence following addition of Co^{2+} (3 mM final concentration) at 90 s, and after the subsequent addition of detergent at 320 s, resulting in complete quenching.

Effect of incubation on vesicles

The results presented above demonstrate that it was possible to prepare small vesicles containing an entrapped fluorescent probe and that the vesicles were stable for at least 8 h following column chromatography. The addition of Mg^{2+} or Ca^{2+} to vesicles containing self-quenching concentrations of calcein did not induce leakage of the probe and thus did not induce destabilization

of the membrane bilayer or cause vesicle collapse under the conditions of the assay, thus suggesting that quenching of the calcein fluorescence by cobalt or manganese ions was due to selective permeability of the membranes to the divalent cations. Although the incorporation of dolichols in PE/PC membranes has been shown to induce the formation of the hexagonal phase at high lipid concentrations [11,17,18], the formation of the hexagonal phase would not be compatible with a sealed vesicle containing a trapped probe. Several experiments suggest, however, that under the conditions employed for the assays used in these studies, i.e. the use of highly unsaturated plant phospholipids, low lipid concentrations in the assays, and incorporating 10 weight % or less of dolichol, intact vesicles remained following incubation with 3 mM cobalt or manganese ions.

In order to assess the effects of incubation under the assay conditions, vesicle size was determined before and after incubation and the distribution of dolichol in the vesicle populations was also measured. Vesicles composed of PE, PC and 5% dolichol containing tracer quantities of ^{14}C-labeled PC and ^{3}H-labeled dolichol were prepared as before, and a sample of the vesicles was analyzed by gel chromatography on Sephacryl S-1000 (Fig. 3). A second sample of the vesicle preparation was incubated at 35°C for 15 min prior to application to the column and a third sample was incubated at 35°C in the presence of 3 mM cobalt chloride. Incubation at 35°C always resulted in a slight increase in apparent average vesicle size but the presence of cobalt ions during the incubation had no additional effect on vesicle size. In the same experiment, the [^{14}C]phospholipid/[^{3}H]dolichol ratio was found to be constant through the peak tubes showing that labeled dolichol co-eluted with phospholipid. Thus the long chain polyisoprenol was uniformly dispersed throughout the vesicle population. The recovery of lipid in the vesicle fraction following column chromatography was as high as 85% in most experiments indicating that a significant percentage of the vesicle population remained intact during the assays for membrane permeability.

To further demonstrate that intact vesicles remained following incubation in the presence of cobalt ions, sonified vesicles (PE/PC/5% dolichol) containing entrapped FITC-Dextran (average mol. wt. 9000) were prepared and isolated following column chromatography. Samples of the vesicles were incubated at 35°C in the presence of 10 mM cobalt ions for 15 min. Most of the FITC-Dextran (86%) was retained in the vesicle fraction as judged by column chromatography when compared with control vesicles incubated on ice in the absence of divalent cation. These results provide additional evidence that cobalt ions did not induce reorganization of the vesicular structure of PE/PC/dolichol liposomes prepared with plant phospholipids. Since we have had difficulty in preparing stable sonified vesicle preparations from an equimolar mixture of dioleolylPE and dioleolylPC containing 5 or 10% dolichol (results not shown), it is possible that the heterogeneous acyl chain composition of the plant phospholipids more readily incorporates the long dolichol chains, thus resulting in more stable vesicle preparations.

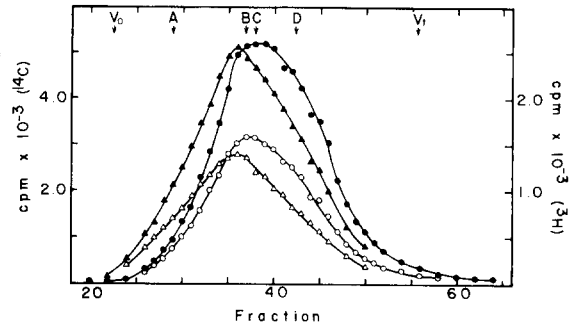

Fig. 3. Analysis of phospholipid vesicles on columns of Sephadex S-1000. Sonified vesicles were prepared from phospholipid mixtures containing PE/PC/5% dolichol and tracer quantities of [^{14}C]PE and [^{3}H]dolichol. The vesicles were applied to a column of Sephadex S-1000 and carbon-14 (solid symbols) and tritium (open symbols) counts in each fraction were determined. Vesicles were incubated at either 4°C (circles), or at 37°C (triangles) for 15 min prior to application to the column. Vesicles incubated at 37°C for 15 min in the presence of 3 mM Co^{2+} gave an identical elution profile to vesicles incubated in the absence of divalent cation and the data is not shown for clarity. The column void volume (V_0) was determined with the use of 0.5 and 0.95 μm diameter polystyrene beads and the column was calibrated with 0.19 (A), 0.1 (B), and 0.08 (C) μ beads. The total volume (V_t) was determined with mannose. The arrow at (D) represents the peak fraction of vesicles prepared from PE and PC (1:1) in the absence of dolichol. Recovery of total lipid ranged from 54 to 85%.

Membrane permeability to cobalt ion

In order to further assess the permeability of PE/PC/dolichol membranes, vesicles were prepared by probe sonication in the presence of 0.5 mM calcein, the vesicles were separated from free calcein on columns of Sephadex G-50, and the rate of fluorescence quenching by cobalt ions was determined (Fig. 4). Although the rate of fluorescence quenching was dependent on Co^{2+} concentration, the cation concentration used in the standard assay (3 mM) was kept rate limiting so that quenching rates could be accurately quantified: Addition of Co^{2+} to PE/PC/dolichol vesicles containing entrapped calcein resulted in a rapid rate of fluorescence quenching at 35 °C (Fig. 4). Since free calcein did not leak from the vesicles under the incubation conditions as was shown above, these results suggested that the PE/PC/dolichol membranes were permeable to the divalent cation. When the rate of fluorescence quenching was determined as a function of temperature, a sharp increase in rate was noted between 15 and 35 °C (Fig. 5). The increased rate of membrane permeability to Co^{2+} occurred only in PE/PC membranes that contained dolichol. Membranes composed of equal amount of PE and PC, without dolichol, did not show a comparable increase in the rate of cation permeability as the temperature was raised from 15 to 35 °C. Whereas, the rate of fluorescence quenching was 17-fold higher at 35 °C than at 15 °C for PE/PC/dolichol membranes, the rate increased only 2- to 3-fold for bilayers composed only of PE and PC. Significantly, PE/PC vesicles were no more permeable to Co^{2+} than

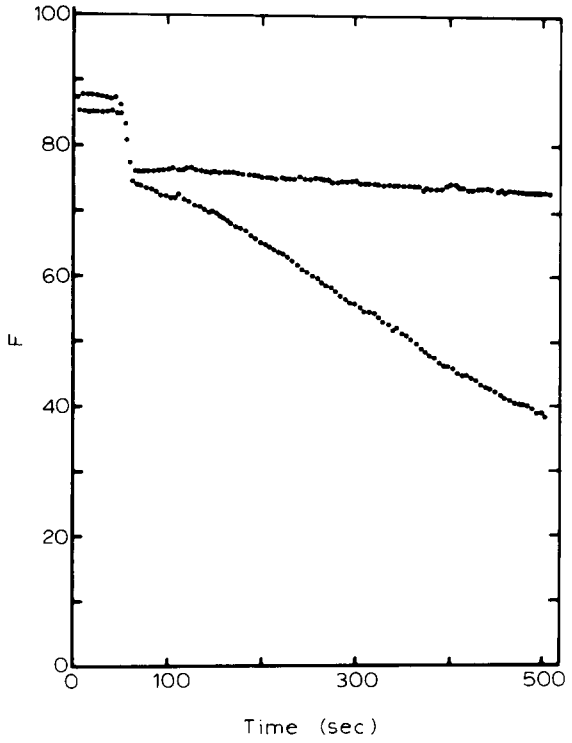

Fig. 4. Representative assays of membrane permeability to cobalt ions. PE/PC/10% dolichol vesicles containing 0.5 mM entrapped calcein were prepared and the rate of fluorescence quenching by cobalt ions was determined. Each data point represents the average calcein fluorescence measured during a 5 s interval. At 50 s, 0.01 ml of 1 M $CoCl_2$ was added (3 mM final concentration) and the change in fluorescence at 15 °C (top curve) and at 35 °C (bottom curve) was measured.

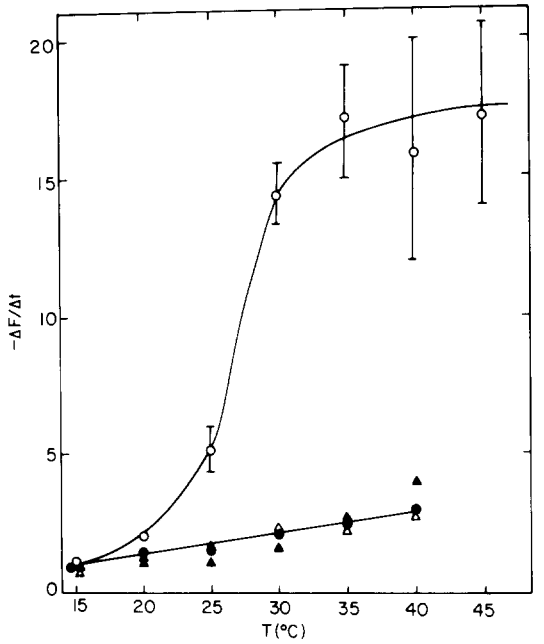

Fig. 5. Membrane permeability as a function of temperature. Calcein was entrapped in sonified vesicles of different phospholipid composition with and without dolichol, and fluorescence was measured as described (see Fig. 4). The rate of fluorescence quenching ($\Delta F/\Delta t$) following the addition of Co^{2+} was determined over 5 Cdeg. intervals from 15 °C to 40 °C. Each data point represents the average of two or more separate determinations. Where shown, the bars represent the standard error of the mean of at least three assays. The compositions of the vesicles were: PE/PC/10% dolichol (○), PE/PC, 1:1 (●), PC/10% dolichol, (▲), and PC (△).

control vesicles composed only of PC. Dolichol, when incorporated into PC bilayers at the same concentration (10 weight %), had no additional effect on the permeability of PC bilayers.

When the effect of polyisoprenol concentration on membrane permeability in sonified vesicles was determined (Fig. 6), 5% dolichol was found to increase the quenching rate of calcein by a factor of ten and 1% dolichol by a factor of three. In the latter experiment, the ratio of total phospholipid to dolichol was 100:1 which is equivalent to the dolichol concentrations found in some tissues. The effect of the ratio of PE to PC in the bilayers was also assessed at a constant dolichol concentration of 10%. In other studies, high concentrations of PE have been shown to destabilize the bilayer structure of membranes [37] although equimolar mixtures of PE and PC are expected to form stable bilayers. Dolichol, however, can destabilize the bilayer structure of PE/PC membranes [11,17] and it was of interest to determine whether the permeability of membranes containing larger amounts of PC, i.e. more stable bilayers, was also affected by dolichol. Vesicles containing PC, dolichol and 0%, 20%, 33% and 50% PE were prepared, and the rates of fluorescence quenching at 35°C were determined using the standard assay. Taking the rate of fluorescence quenching in PC/10% dolichol membranes at 15°C as 1.00, the rate measured at 35°C for PC/dolichol vesicles was 2 and with 20%, 33%, and 50% PE the rates were 9.2, 10.4 and 14, respectively. Thus the presence of dolichol promoted significant leakage, even in bilayers containing only 20% PE. As noted above, however, dolichol did not increase the rate of fluorescence quenching in the absence of PE.

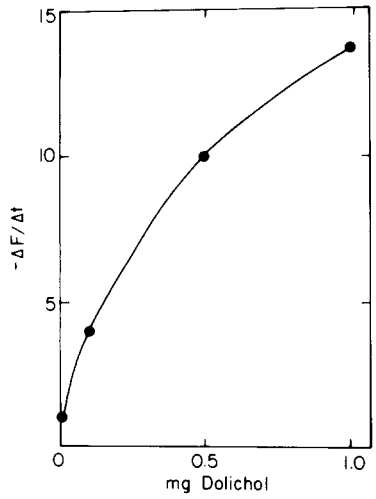

Fig. 6. Membrane permeability as a function of dolichol concentration. Calcein was entrapped in sonified phospholipid vesicles containing 5 mg PE, 5 mg PC, and the indicated amount of dolichol. The rate of fluorescence quenching ($\Delta F/\Delta t$) at 35°C was determined following the addition of cobalt ions.

Fluorescence polarization

The effect of dolichol on the steady state fluorescence polarization of DPH in membranes composed of PE and PC was determined as a function of the dolichol concentration and temperature (Table I). The fluorescence polarization of DPH in membranes composed of PE and PC was significantly reduced as a function of increasing dolichol concentration at 15, 25, and 35°C. The addition of dolichol to PC membranes, however, did not result in any significant effect on the polarization of DPH and the results are not shown. DPH is sensitive to the environment of both the polar head groups and the acyl chain composition of phospholipid bilayers [39], but the results suggest that dolichol increased the apparent fluidity of membranes composed of PE and PC mixtures. This increased fluidity may account, at least in part, for the greater permeability of the PE/PC/dolichol membranes to divalent cations. The same effect may also account for the in-

TABLE I

EFFECT OF DOLICHOL ON THE STEADY-STATE FLUORESCENCE POLARIZATION (P) OF DPH IN MEMBRANES COMPOSED OF A 1:1 MIXTURE OF PE AND PC

Steady-state polarization was determined as previously described [38] at a lipid concentration of 1 mg/ml and a DPH concentration of 1 μM. All assays were carried out in triplicate and polarization values are presented as means ± S.D.

Dolichol (wt.%)	Polarization (P) at temperature (°C)		
	15	25	35
0	0.182 ± 0.012	0.163 ± 0.012	0.152 ± 0.014
1	0.159 ± 0.004	0.139 ± 0.011	0.125 ± 0.015
5	0.136 ± 0.013	0.124 ± 0.010	0.116 ± 0.010
10	0.119 ± 0.020	0.110 ± 0.016	0.101 ± 0.015

creased activity of mannosyltransferases in PE/PC membranes that contain similar concentrations of dolichol.

Discussion

Two of the mannosyltransferases involved in the synthesis of dolichyl-linked oligosaccharides have been shown to be optimally active when reconstituted with lipid mixtures containing highly unsaturated PE and both enzymes are sensitive to the addition of other lipophilic components including free dolichols [9–11]. Since structural properties of the membrane appear to be important for the synthesis, and possibly for the translocation, of dolichyl-linked oligosaccharide chains, the present investigation was carried out to obtain information as to the effects of dolichol on the properties of PE containing membranes.

Although dolichol has been shown to destabilize the bilayer structure of membranes composed of PE and PC when examined at high lipid concentrations [11,17,18], the present results show that it is possible to prepare small sonified vesicles from mixtures of highly unsaturated PE and PC containing up to 10% dolichol. These vesicles are stable, retain entrapped calcein for at least 8 h when stored at ice bath temperatures, and maintain their vesicular structure when incubated for 15 min at 37°C in the presence of divalent cations. One effect of added dolichol was to increase the permeability of PE/PC bilayers to divalent cations. Dolichol was also found to decrease the fluorescence polarization of DPH in PE/PC membranes and this result can be interpreted to suggest that the increased permeability to divalent cations might be related to an increase in the apparent fluidity of the acyl chains in PE/PC membranes.

It is important to note, however, that the dolichol enhanced membrane permeability and fluidization occurred only in membranes containing PE. Dolichol did not affect either the cation permeability or the fluorescence polarization of DPH in membranes composed of PC as the sole phospholipid. Since the effect of dolichol was noted only in the presence of PE, it is possible that the propensity of PE to form hexagonal II phases in isolation is involved in the mechanism by which the polyisoprenoid compound exerts its effect on membrane permeability. A report [17] demonstrating that dolichol may selectively interact with PE, rather than with PC, in mixed phospholipid bilayers supports this possibility. Since dolichols have been shown to destabilize bilayer structure at high lipid concentrations, it was necessary to confirm that the fluorescence quenching of calcein by cobalt ions was not the result of vesicle collapse and loss of contents. The size and stability of dolichol containing vesicles were found to be essentially unaffected by addition of metal ions. Divalent cations had little effect on vesicle size as judged by gel filtration chromatography and the results demonstrate that dolichol did not segregate according to vesicle size but rather remained distributed throughout the vesicle population, even after incubation at 35°C and in the presence of cobalt ions. As additional evidence, the high molecular weight fluorescent probe FITC-Dextran, was retained following incubation with Co^{2+} at 37°C confirming the presence of vesicular phospholipid.

The effect of dolichol on membrane permeability, and the apparent specific requirement for unsaturated PE, can be correlated with similar requirement for the activities of dolichyl-*P*-mannose synthase and mannosyltransferase II. Although the molecular architecture that is involved in these effects is not yet known, a unique membrane structure possibly involving transiently formed inverted lipid phases may be required for the proper orientation of enzyme and substrate in a lipid matrix. The same structure may enhance membrane permeability and also facilitate the translocation of oligosaccharides across membranes of the rough endoplasmic reticulum.

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