Modulation of Dolichyl-Phosphomannose Synthase Activity by Changes in the Lipid Environment of the Enzyme†

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ABSTRACT: Rat liver dolichyl-phosphomannose synthase (GDP mannose-dolicholphosphate mannosyltransferase; EC 2.4.1.83) was previously shown to catalyze optimal rates of mannosyl transfer to dolichyl-P when the polyprenol acceptor was incorporated into a phosphatidylethanolamine (PE) matrix that has a tendency to adopt a nonbilayer (hexagonal H_{II}) phase [Jensen, J. W., & Schutzbach, J. S. (1985) *Eur. J. Biochem. 153*, 41–48]. The present investigations now further define the properties of the lipid environment that are essential for mannosyltransferase activity. Monogalactosyl diglyceride (MGDG), a glycoglycerolipid that prefers a nonbilayer-phase organization in isolation, was shown to provide a suitable lipid matrix for synthase activity. By comparison, the enzyme was not activated by digalactosyl diglyceride (DGDG), which forms stable bilayer structures upon hydration. Enzyme activity in MGDG/DGDG mixtures decreased as the proportion of DGDG in the dispersion was increased. Although bilayer-forming phospholipids supported low rates of mannosyl transfer, enzyme activity was stimulated by the addition of MGDG to either phosphatidylcholine (PC) or PE/PC (1:1) membranes. The incorporation of agents known to destabilize bilayer structures including dolichols, ubiquinone, dodecane, and cholesterol into PE/PC (1:1) membranes also increased the rate of mannosyl transfer. Enzyme activity in PC membranes was stimulated by the presence of gramicidin and also by greatly increased concentrations of the substrate, dolichyl-P. The results demonstrate that the enzyme does not have a requirement for PE and suggest that the physical state of the lipid matrix is an important determinant for reconstitution of the synthase and polyprenol phosphate substrate in a productive complex. The formation of an enzyme/lipid complex was demonstrated by sucrose density gradient centrifugation and could be correlated with the lipid requirements for enzyme activity.

The biosynthesis of asparagine-linked oligosaccharides of glycoproteins is initiated by the assembly of a dolichylpyrophosphoryloligosaccharide intermediate ($Glc_3Man_9GlcNAc_2$-P-P-dolichyl) on membranes of the rough endoplasmic reticulum (Struck & Lennarz, 1980; Hubbard & Ivatt, 1981; Snider, 1984). The four mannosyl-transfer reactions that occur during the latter stages of oligosaccharide–lipid formation require dolichyl-P-mannose as a glycosyl donor. Dolichylphosphomannose synthase (GDPmannose-dolicholphosphate mannosyltransferase; EC 2.4.1.83) catalyzes the synthesis of dolichyl-P-mannose from GDP-mannose present in the cytosol and membrane-associated dol-P.[1] A stable, detergent-free preparation of this enzyme has been isolated from rat liver microsomes (Jensen & Schutzbach, 1985). The partially purified synthase did not catalyze mannosyl transfer to the polyprenol acceptor in the presence of nonionic detergents (Jensen & Schutzbach, 1985, 1986), but mannosyl transfer was markedly stimulated when the hydrophobic acceptor was incorporated into a lipid matrix containing unsaturated species of PE (Jensen & Schutzbach, 1985, 1986). Some other glycosyltransferases, which utilize hydrophobic substrates, have also been reported to require PE (Jensen & Schutzbach, 1982, 1984; Rothfield & Romeo, 1971), but most membrane-bound enzymes do not require specific lipids for reconstitution of activity.

We have suggested that the ability of PE to activate the synthase is related to either specific structural features of this lipid or to its unusual macroscopic organization (Jensen & Schutzbach, 1987). While most polar lipids adopt a bilayer or micellar phase upon hydration, PE prefers a nonbilayer hexagonal (H_{II}) organization (De Kruijff et al., 1985). The macroscopic structure adopted by various lipid classes has often been rationalized by using the concept of "dynamic molecular shape" (Cullis et al., 1986). According to this construct, lipids with a dynamic cylindrical shape such as PC prefer to organize in the bilayer phase; lipids having the shape of an inverted cone such as lysolipids organize in the micellar phase; and conically shaped lipids such as PE preferentially adopt the hexagonal phase (H_{II}). Although it is not possible for a biological membrane to contain any extensive domain of nonlamellar structure and retain its semipermeable character, lipid shape factors appear to strongly influence the lipid composition of biological membranes (Wieslander et al., 1986).

In order to gain further insight into how the lipid matrix modulates the activity of dolichyl-phosphomannose synthase, we have examined the effects of altering the lipid matrix composition on enzyme activity and on the ability of the enzyme to physically interact with specific lipid matrices. The results suggest that the physical state of the lipid/substrate complex largely determines whether the enzyme can form a productive complex with its hydrophobic substrate.

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[1] Abbreviations: DGDG, digalactosyl diglyceride; DLPC, dilauroylphosphatidylcholine; dol-P, dolichyl monophosphate; DOPC, dioleoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; EDTA, ethylenediaminetetraacetic acid; MGDG, monogalactosyl diglyceride; PC, plant phosphatidylcholine; PE, plant phosphatidylethanolamine; Tris, tris(hydroxymethyl)aminomethane.

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Materials and Methods

Materials. GDP-[^{3}H]mannose (10.9 Ci/mmol) was obtained from New England Nuclear, and the specific activity was diluted to 0.69 Ci/mmol with unlabeled GDP-mannose. C-95 dolichyl phosphate was a generous gift of T. Chojnacki, Warsaw, Poland. Phospholipids were purchased from Avanti Polar Lipids (Birmingham, AL) and were stored under nitrogen at −20 °C. All phospholipids were from plant sources unless otherwise indicated. Cholesterol, ubiquinone 50, MGDG, and DGDG were obtained from Sigma Chemical Co. Nonidet P-40 was a product of LKB-Produkter AB and was further purified before use (Chang & Bock, 1980). Enzyme preparations used in this study were purified from rat liver microsomes through the DEAE-cellulose step as previously described (Jensen & Schutzbach, 1985). After detergent removal, the enzyme was stored frozen in the presence of 10% glycerol. The preparations had specific activities that ranged from 8 to 13 nmol of mannose transferred/(min·mg protein). All other chemicals and standard compounds were purchased from commercial sources.

Analytical Methods. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as the standard. Phosphate was analyzed by the method of Duck-Chong (1979), and lipid purity was assessed by using thin-layer chromatography.

Chromatography. Thin-layer chromatography was performed by using precoated silica gel G plates (Analtech Inc.) in chloroform/methanol/water (65:25:4) and chloroform/methanol/15 M ammonium hydroxide/water (65:35:4:4). Plates were developed with iodine vapor or with a molybdenum spray reagent (Dittmer & Lester, 1964).

Enzyme Assays. The activity of dolichyl-P-mannose synthase was determined by using a previously described procedure (Jensen & Schutzbach, 1985). All assays were performed in duplicate, and lipid compositions are presented as weight/weight ratios. The rate of mannosyl transfer in the presence of 20 μg of PE and 0.5 μg of dol-P (1.75 μM) was used as a standard reference to compare the effects of other lipid environments on mannosyltransferase activity. The ability of a compound to modulate the mannosyl-transfer rate was assessed by adding increasing quantities of the agent to dispersions (20 μg) of either PC or PE/PC (1:1) containing 0.5 μg of dol-P. The rate of mannosyl transfer in a PC matrix at this dol-P concentration was always less than 10% the rate of mannosyl transfer in a PE matrix. The rate of mannosyl transfer in PE/PC (1:1) mixtures was more variable and depended upon the preparation of enzyme and phospholipid utilized. In the present work, the PE/PC ratio was held constant by using a common phospholipid mixture for each experiment, and the rate of mannosyl transfer in PE/PC (1:1) dispersions reproducibly ranged between 30% and 40% of the control activity. Slightly modified procedures were used to introduce gramicidin and dodecane into the lipid matrix. Prior to the addition of these compounds to reaction mixtures containing sonically dispersed phospholipids, gramicidin was dissolved in trifluoroethanol and dodecane was diluted with tetrahydrofuran. Appropriate controls were included in each experiment.

Preparation of Lipid Vesicles. Lipid vesicles were prepared following the procedure of Monti et al. (1987). Lipid mixtures containing phospholipid (10 mg) and the indicated amounts of dol-P were added to 16 × 150 mm test tubes, and organic solvents were removed under a stream of dry nitrogen gas. The samples were held under continuous vacuum for 16 h to remove trace quantities of solvents and then hydrated for 20 min at room temperature in 1 mL of 0.1 M Tris/acetate buffer, pH 7.5, containing 1.3 mM EDTA and 1.6 mM reduced glutathione. The lipid mixtures were thoroughly suspended by using a vortex mixer and then transferred to 15-mL conical plastic tubes. Small vesicles were prepared by probe sonication using a Heat Systems Model W-225 sonifier equipped with a Model C-2 converter and a standard tapered microtip with the output control set between 2.5 and 3.0. The tube was suspended in an ice bath and the phospholipid dispersions were sonified for 10 min by using a 50% pulse cycle. No breakdown of the phospholipids, as judged by thin-layer chromatography, was observed by using this protocol. The vesicle preparations were centrifuged at 34000*g* for 30 min, and the supernatants were removed, stored at 3 °C, and used within 72 h.

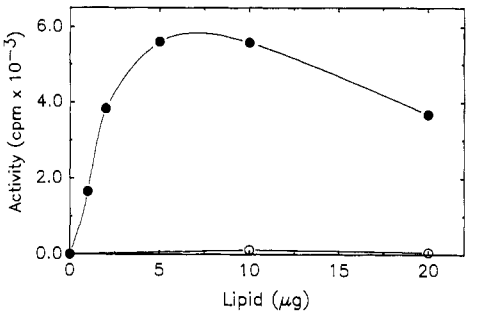

FIGURE 1: Activation of dolichyl-P-mannose synthase by glycoglycerolipids. Mannosyl transfer was assayed in reaction mixtures containing the indicated quantity of either sonically dispersed MGDG (●) or DGDG (○).

Density Gradient Centrifugation. Centrifugation on 10–40% sucrose gradients prepared in 0.1 M Tris/acetate buffer, pH 7.5, containing 1.3 mM EDTA, 1.6 mM reduced glutathione, and 5 mM $MnCl_2$, was used to separate lipid vesicles, protein lipid aggregates, and protein. Gradients were prepared for the TLS-55 rotor following a described protocol (Beckman application note DS-640A). Before layering samples (0.15 mL) onto the surface of the gradients, equal volumes of enzyme solution and lipid vesicles were incubated for 10 min at 37 °C. The composition of the samples applied to the gradients is detailed in the text and figure legends. All samples were centrifuged in the TLS-55 rotor at 55 000 rpm for 4 h at 4 °C by using the Beckman TL-100 tabletop ultracentrifuge with acceleration program 5 and deceleration program 9. Fractions (0.15 mL) were collected from each tube, after bottom puncture using a Beckman fraction recovery system. Appropriate aliquots (0.01–0.04 mL) of each fraction were assayed for enzyme activity in the presence of additional PE and dol-P.

Results

Enzyme Activity in Plant Glycoglycerolipid Dispersions. Previous studies had suggested that dolichyl-P-mannose synthase from mammalian liver had a requirement for highly unsaturated species of PE for activity (Jensen & Schutzbach, 1985). The results did not clearly distinguish, however, between a requirement for a specific phospholipid or for the unique macromolecular phase properties of unsaturated PE. In order to elucidate the lipid requirements for mannosyltransferase activity, the lipid specificity was further investigated. We found that dispersions of the plant glycoglycerolipid MGDG would support mannosyl transfer to dol-P while dispersions of DGDG would not (Figure 1). Maximal stimulation of mannosyl transferase in the presence of MGDG occurred at 5–10 μg of lipid/assay and was 50% of the activity observed in control reaction mixtures containing 20 μg of PE. MGDG was not a substrate for mannosyl transfer in the ab-

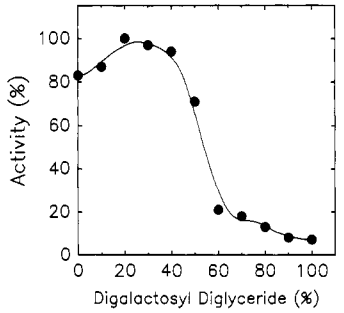

FIGURE 2: Mannosyltransferase activity in glycoglycerolipid dispersions of mixed composition. Mannosyl transfer was assayed in reaction mixtures containing the indicated proportions of MGDG and DGDG. The total amount of glycoglycerolipid was held constant at 10 μg. Activity is normalized to the MGDG/DGDG dispersion that stimulated the highest level of mannosyl transfer.

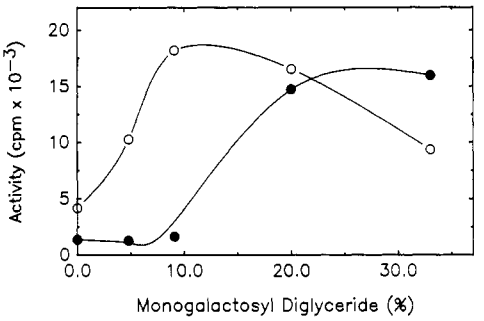

FIGURE 3: Effect of increasing concentrations of MGDG on mannosyltransferase activity in phospholipid dispersions. Dol-P-mannose synthase activity was assayed in reaction mixtures that contained either PE/PC (1:1) (○) or PC (●), and the quantity of MGDG was varied as indicated.

sence of dol-P. The concentration of dol-P required for half-maximal stimulation of mannosyl transferase in MGDG dispersions was 0.36 μM (apparent K_m) and was equivalent to that previously reported for dol-P in PE dispersions (0.3 μM) (Jensen & Schutzbach, 1985). The effect of increasing the percentage of DGDG in mixed dispersions of MGDG and DGDG is shown in Figure 2. Maximal activity was observed in MGDG/DGDG mixtures containing 20–40% DGDG. The reaction was progressively inhibited in mixtures containing more than 40% DGDG, and at concentations greater than 60% DGDG the rate of mannosyl transfer was reduced more than 5-fold. The ability of the glycoglycerolipid MGDG to replace phospholipid in providing a matrix for mannosyltranserase activity was also tested in lipid dispersions which normally supported less than optimal mannosyltransferase activity (Figure 3). Incorporation of MGDG into dispersions of either PE/PC (1:1 w/w) or PC stimulated the rate of mannosyl transfer. In both cases, the extent of enzyme activation observed was a function of the quantity of MGDG incorporated into the membranes. In the PE/PC (1:1) dispersions, maximal transferase activity occurred in the presence of 9% MGDG and was equivalent to that observed in control reactions containing PE alone. The incorporation of higher concentrations of MGDG into the matrix decreased the rate of mannosyl transfer. In the PC dispersions, incorporation of up to 9% MGDG had little effect on enzyme activity, but higher concentrations of MGDG stimulated enzyme activity. In PC dispersions containing 33% MGDG, the mannosyl-transfer rate was 89% of that observed in control reactions containing PE. Since MGDG could replace the requirement for PE in the reaction, the results clearly demonstrate that rat liver dolichyl-P-mannose synthase does not have a specific phospholipid requirement.

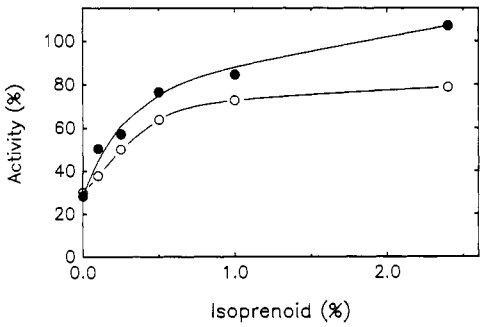

FIGURE 4: Effect of increasing concentrations of isoprenoid compounds on mannosyltransferase activity in PE/PC (1:1) dispersions. Dol-P-mannose synthase activity was assayed in reaction mixtures containing PE/PC (1:1) and the indicated amounts of either ubiquinone 50 (●) or C-95 dolichol (○).

Effect of Bilayer-Destabilizing Agents on Enzyme Activity. Since the physical properties of the lipid matrix appeared to be a primary determinant of enzyme activity, the rate of mannosyl transfer to dol-P was monitored in the presence of several other lipophilic compounds known to physically alter bilayer structure in model membrane systems, including gramicidin (Van Echteld et al., 1981), dodecane (Kirk & Gruner, 1985), cholesterol (Cullis & De Kruijff, 1978), dolichol (Jensen & Schutzbach, 1984; Gruner, 1985; Valtersson et al., 1985; Monti et al., 1987), and dol-P (Van Duijn et al., 1986). The effects of these agents on synthase activity are presented below.

Mannosyl transfer was stimulated by the inclusion of isoprenoid compounds in PE/PC (1:1) mixtures (Figure 4). Under these conditions, the rate of mannosyl transfer in the presence of 2.4% ubiquinone 50 was equivalent to that observed in PE controls, and the incorporation of 2.4% C-95 dolichol into PE/PC membranes supported a level of mannosyl transfer which was 79% of that observed in PE controls. Mannosyl transfer was inhibited by the incorporation of higher concentrations of dolichol, but not ubiquinone, into PE/PC (1:1) dispersions. Concentrations of dol-P that were found to support optimal rates of mannosyl transfer in PE dispersions supported less than 10% of the control activity when incorporated into a PC matrix. We found, however, that the mannosyl-transfer rate in PC membranes could be stimulated by greatly increasing the concentration of dol-P present in the lipid matrix. Maximal mannosyl transfer in PC dispersions occurred in the presence of 33 μM dol-P and was equivalent to 64% of the enzyme activity in control reactions. The quantity of dol-P required for half-maximal activation in PC membranes was 9 μM, which was 30-fold higher than the apparent K_m for dol-P in PE alone (Jensen & Schutzbach, 1985). The apparent K_m for dol-P in PC membranes (9 μM) was 5 times higher than the concentration of dol-P (1.75 μM) used in standard assays, accounting for previous results.

The hydrophobic cyclic peptide gramicidin has been shown to significantly alter the physical structure of some phospholipids organized in the bilayer phase. Incorporation of this compound into DOPC membranes was shown to induce the formation of a hexagonal (H_{II}) phase, but no such effect was noted when the peptide was incorporated into DLPC membranes (Van Echteld et al., 1981). Dolichyl-P-mannose synthase was minimally active when assayed in the presence of 10 μg of DOPC and 0.5 μg of dol-P, but the incorporation of increasing quantities of gramicidin into this lipid matrix significantly stimulated the rate of mannosyl transfer (Figure 5). Maximal activity occurred at a gramicidin/DOPC ratio of 0.2 and was equivalent to 46% of the activity observed in control

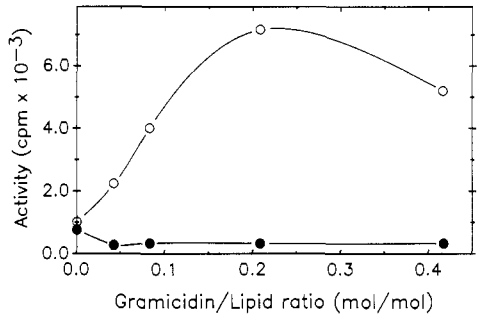

FIGURE 5: Effect of increasing concentrations of gramicidin on mannosyltransferase activity in phosphatidylcholine dispersions. Dol-P-mannose synthase activity was assayed in reaction mixtures that contained 10 μg of either DOPC (○) or DLPC (●) and the indicated quantity of gramicidin.

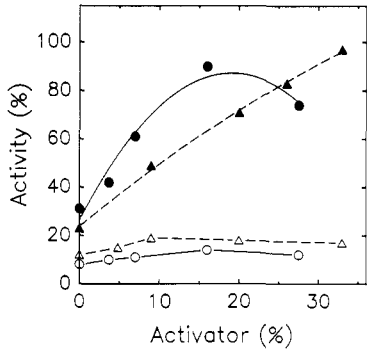

FIGURE 6: Effect of increasing concentrations of dodecane and cholesterol on mannosyltransferase activity in phospholipid dispersions. Dol-P-mannose synthase activity was assayed in reaction mixtures containing either PE/PC (1:1) (closed symbols) or PC (open symbols) and the indicated quantities of either dodecane (circles) or cholesterol (triangles).

reactions containing PE. Addition of gramicidin to dispersions of DLPC did not stimulate enzyme activity at gramicidin/DLPC ratios ranging from 0.05 to 0.3. In the absence of phospholipids, gramicidin was unable to provide a matrix capable of supporting mannosyl transfer to dol-P.

Alkanes are known to partition readily into hydrated lipid dispersions and have been shown to induce alterations in the phase properties of lipid systems containing unsaturated PE (Kirk & Gruner, 1985). The addition of 16% dodecane to PE/PC (1:1) mixtures was found to increase the rate of mannosyl transfer to a level that was 90% of that observed in control reactions containing PE, but dodecane had essentially no effect on the rate of mannosyl transfer when the enzyme was assayed in PC dispersions (Figure 6). Dodecane did not support mannosyl transfer to dol-P in the absence of phospholipids. Cholesterol has also been shown to destabilize phospholipid bilayers composed of equal amounts of PE and PC (Cullis & De Kruijff, 1978). PE/PC (1:1) dispersions containing equimolar cholesterol supported a level of synthase activity which was equivalent to that observed in PE dispersions, but the mannosyl-transfer rate was not stimulated by similar concentrations of cholesterol in PC dispersions (Figure 6).

Association of Dolichyl-P-Mannose Synthase with Phospholipids. Previous work had suggested that dolichyl-P-mannose synthase formed a productive association with phospholipid in the presence of the acceptor dol-P (Jensen & Schutzbach, 1985). The method used in those experiments to demonstrate enzyme/phospholipid interactions, i.e., cosedimentation of the enzyme and lipid by ultracentrifugation, could not unambiguously distinguish between the formation

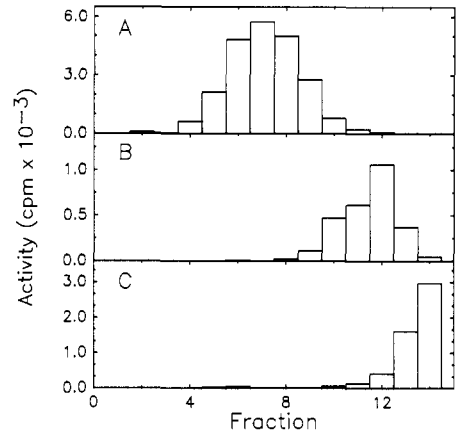

FIGURE 7: Effect of lipid on the buoyant density of dolichyl-P-mannose synthase. Enzyme preparations were layered on 10–40% sucrose gradients and centrifuged at 4 °C for 4 h at 55 000 rpm in the absence of added lipid (panel A) or in the presence of phospholipid vesicles comprised of either PE/PC (1:1) (panel B) or PE/PC/dol-P (1:1:0.05) (panel C). Activity of the synthase was determined in reaction mixtures containing PE (40 μg) and dol-P (1 μg). Recovery of total enzyme activity compared with that initially applied to the gradients was respectively 46%, 26%, and 100% for the experiments depicted in panels A, B, and C. In the experiments depicted in panels B and C, phospholipid was quantitatively recovered in fractions 13 and 14.

of a lipid/enzyme complex and the sedimentation of an aggregated protein induced by the presence of phospholipid. In addition, the low recovery of activity in control experiments made it difficult to clearly interpret the results. In the present work, we found that free enzyme could be clearly distinguished from enzyme associated with phospholipid vesicles on the basis of centrifugation in 10–40% sucrose gradients. When centrifugation was carried out in the absence of nonionic detergent or phospholipid, all of the recovered enzyme activity was found as a broad peak in a position midway through the gradient (Figure 7A). The recovery of enzyme activity ranged between 40% and 60% of that applied to the gradient. Incubation of the synthase (0.004 mg) with small sonified vesicles comprised of 0.375 mg of PE, 0.375 mg of PC, and 0.019 mg of dol-P prior to centrifugation resulted in nearly quantitative recovery of enzyme activity at the top of the gradient associated with phospholipid (Figure 7C). These results clearly demonstrated association of dolichyl-P-mannose synthase with phospholipid/dol-P vesicles. The amount of total phospholipid/dol-P vesicles required for complete association of 0.004 mg of enzyme protein was 0.4 mg, while 0.1 mg of the lipid vesicles was sufficient to complex 50% of the enzyme activity. It was also found that the enzyme activity could be quantitatively recovered associated with lipid after centrifugation in 10–40% sucrose gradients containing 0.2 M KCl, indicating that the association of enzyme with lipid vesicles containing dol-P was not simply an ionic interaction. The extent to which the synthase interacted with PE/PC vesicles in the absence of polyprenol-P acceptor was also determined. When enzyme was incubated with PE/PC vesicles in 0.1 M buffer prior to centrifugation, the recovered enzyme activity was found in a single peak that was intermediate between the position of free enzyme and lipid-associated enzyme (Figure 7B). Although the enzyme was not found to be physically associated with lipid vesicles at the top of the gradient under these conditions, the change in sedimentation velocity indicates that either the size or the buoyant density of the enzyme was altered by interactions with substrate-free phospholipid vesicles. In the presence of 0.2 M KCl, however, all of the recovered enzyme activity was found to be associated with lipid vesicles at the top of the gradient, indicating that the enzyme was able to

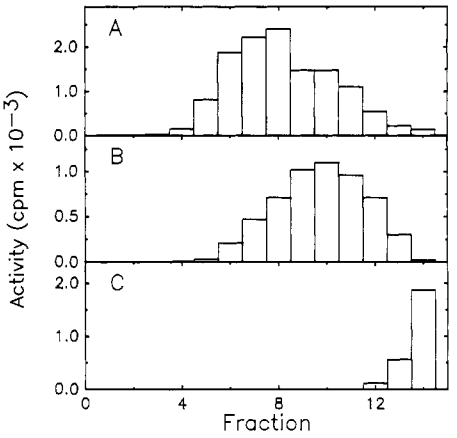

FIGURE 8: Effect of dol-P concentration on the buoyant density of enzyme/PC mixtures. Synthase preparations were incubated with lipid vesicles containing PC (panel A), PC/dol-P (1:0.025) (panel B), and PC/dol-P(1:0.25) (panel C) for 10 min at 37 °C and layered on the surface of 10–40% sucrose gradients. The samples were centrifuged at 4 °C for 4 h at 55 000 rpm. Enzyme activity was determined in reaction mixtures containing PE (80 μg) and dol-P (2 μg). The respective recovery of enzyme initially applied to gradients for the experiments depicted in panels A, B, and C was 47%, 100%, and 100%. Phospholipid was quantitatively recovered in fractions 13 and 14.

physically interact with the vesicles under conditions expected to promote greater hydrophobic interaction.

The relationship between enzyme activity in a PC matrix and the ability of the enzyme to form a physical association with PC/dol-P vesicles was investigated by subjecting dol-P-mannose synthase to centrifugation after incubating enzyme with PC vesicles that contained varying quantities of dol-P (Figure 8). When the enzyme was centrifuged in the presence of PC vesicles that did not contain dol-P, the enzyme activity profile was very similar to that of enzyme centrifuged in the absence of phospholipid vesicles, indicating that the bulk of the enzyme did not interact with the PC vesicles (Figure 8A). As shown earlier, mannosyltransferase activity in the presence of PC vesicles required much higher concentrations of dol-P than when the enzyme was reconstituted with PE. When the enzyme was centrifuged in the presence of PC vesicles containing 2.4% dol-P, all of the recovered activity was found in a single peak overlapping the sedimentation position of free enzyme (Figure 8B). However, with PC vesicles containing 20% dol-P, all of the recovered activity was found associated with lipid at the top of the gradient (Figure 8C). These experiments demonstrate that the synthase did not substantially associate with PC vesicles until the concentration of dol-P in the vesicles was increased to a level capable of supporting enzyme activity.

Discussion

The activity of partially purified rat liver dolichyl-phosphomannose synthase was previously shown to be dependent upon reconstitution of the enzyme with specific phospholipids (Jensen & Schutzbach, 1985). In the present study several different lipid matrices were found to activate the synthase reaction to the same extent as PE, demonstrating that the synthase does not have a specific lipid requirement. The plant glycoglycerolipid MGDG, which has phase properties similar to PE (Shipley et al., 1973), supported synthase activity. High rates of mannosyl transfer were also observed in the presence of bilayer-forming lipids when the composition of the lipid matrix was altered by the addition of various lipotropic compounds including polyisoprenoids, cholesterols, dodecane, and gramicidin, which have been shown to destabilize bilayer membrane structure and to induce the formation of nonlamellar phases in model lipid systems. The insertion of bilayer-destabilizing agents into bilayer structures expands the cross-sectional area occupied by the hydrocarbon chains without commensurately affecting the cross-sectional area occupied by the polar head groups (De Kruijff et al., 1985). The primary effects of this imbalance are to increase the order of the acyl chains and to expose the polar head groups to the aqueous environment (Cullis et al., 1986).

Although PE and MGDG have dissimilar head groups, both lipids have a conical shape and prefer the hexagonal phase in isolation (De Kruijff et al., 1985). In contrast, DGDG and PC, lipids that did not support the synthase reaction, are cylindrically shaped and adopt the bilayer phase upon hydration (De Kruijff et al., 1985). Although dispersions containing only MGDG supported enzyme activity, higher rates of mannosyl transfer were observed in MGDG/phospholipid and in MGDG/DGDG mixtures. The results were very similar to those previously reported for the synthase in the presence of PE/PC dispersions (Jensen & Schutzbach, 1985). In both cases the enzyme was activated by the conically shaped lipid but was virtually inactive in the presence of the cylindrically shaped lipid. The phase properties of model lipid mixtures containing both the conical and cylindrical lipids have been shown to be dependent upon the relative proportion of conical lipid in the mixture (De Kruijff et al., 1985). In the present study the highest rate of mannosyl transfer occurred when conical lipids were combined with cylindrical lipids at ratios expected to induce alterations in the physical properties of the lipid matrix previously defined as bilayer destabilization (Cullis & De Kruijff, 1978). It has also been found that MGDG could replace the quantitative requirement for PE in the reconstitution of sarcoplasmic reticulum Ca^{2+}-ATPase (Navarro et al., 1984).

Dol-P-mannose synthase was previously shown to be optimally active in PE dispersions containing 2.5% dol-P. In contrast, PC dispersions containing this concentration of dol-P supported very low levels of enzyme activity (Jensen & Schutzbach, 1985). The present results indicate that PC dispersions provided a matrix capable of supporting increased rates of mannosyl transfer when the concentration of dol-P in the dispersion was increased more than 10-fold. The enzyme did not physically associate with PC vesicles containing 2.4% dol-P but did form a physical association with PC vesicles containing 20% dol-P, suggesting that dol-P, incorporated into PC vesicles, was not accessible to the enzyme as a substrate until the structure of the bilayer was physically altered. The effects of dol-P on the physical properties of PC membranes have not been well-defined, but several laboratories have reported that dolichols destabilize bilayer membranes containing both PE and PC (Jensen & Schutzbach, 1984; Kirk & Gruner, 1985; Valtersson et al., 1985; Van Duijn et al., 1986; Monti et al., 1987; Schutzbach et al., 1987). The presence of dol-P in DOPE/DOPC (2:1 mol/mol) mixtures was shown to alter the structural organization of the phospholipids (Van Duijn et al., 1986).

Cholesterol has been shown to destabilize PE/PC (1:1 mol/mol) bilayers presumably as a consequence of its wedgelike shape (Cullis & De Kruijff, 1978; Tilcock et al., 1982). The incorporation of equimolar concentrations of cholesterol into PE/PC (1:1) mixtures stimuated an increased rate of mannosyl transfer, but cholesterol incorporation into PC membranes had no effect on the mannosyl-transfer rate. These results are consistent with those previously reported for another mannosyltransferase with a similar phospholipid

specificity (Jensen & Schutzbach, 1984). The effects on enzyme activity induced by the addition of dodecane to PE/PC (1:1) and PC dispersions can be correlated with the effects of alkanes on the phase properties of phospholipid mixtures. Kirk and Gruner (1985) reported that incorporation of 5% (w/w) dodecane into DOPE/DOPC (3:1) mixtures reduced the bilayer to hexagonal phase transition temperature for the lipid mixture by more than 50 °C. The addition of nonpolar compounds such as dodecane or dolichol to PE/PC mixtures is believed to relax the hydrocarbon packing energy, allowing a nonbilayer phase to form. However, phase transformation would not be expected to occur under the same conditions in PC membranes, because PC has a high intrinsic radius of curvature (Gruner, 1985). In addition, Wieslander et al. (1986) have shown that the ratio of conical to cylindrical lipids in *A. laidlawii* membranes decreased when the organism was grown in the presence of nonpolar organic solvents.

The present results demonstrate that activation of dol-P-mannose synthase does not require specific lipids but that the reaction requires a lipid matrix having a specific physical organization. Since the structural state of the lipids in enzyme/lipid complexes could not be determined by using physical methods, due to limiting quantities of enzyme, we have correlated our results with the conclusions obtained by others using model lipid systems. Our results indicate that while the enzyme was active in the presence of conical lipids such as PE or MGDG, optimal activity was associated with lipid matrices which would be expected to form destabilized bilayers. Further work will be required to establish whether the in vivo mannosyl-transfer reaction requires specific lipid structures.

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Registry No. EC 2.4.1.83, 62213-44-9; dolichyl-P, 12698-55-4; dodecane, 112-40-3; cholesterol, 57-88-5; gramicidin, 1405-97-6; ubiquinone 50, 303-98-0; C-95 dolichol, 42436-66-8.

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