Phospholipase-Induced Modulation of Dolichyl-Phosphomannose Synthase Activity†

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ABSTRACT: Rat liver dolichyl-phosphomannose synthase is optimally active when the enzyme is reconstituted with lipids that prefer a nonlamellar macroscopic organization in isolation, such as phosphatidylethanolamine (PE), but the enzyme is only negligibly active in the presence of lipids that normally form stable bilayers, such as phosphatidylcholine (PC) [Jensen, J. W., & Schutzbach, J. S. (1985) *Eur. J. Biochem. 153*, 41–48]. We now report that the activity of the synthase can be modulated by incorporating diacylglycerol and lysophosphatidylcholine into the lipid matrix. Enzyme activity in PC bilayers was stimulated by the presence of diacylglycerol, a lipid that has a conical dynamic molecular shape and disrupts bilayer stability. In PC/diacylglycerol mixtures the apparent K_m for dolichyl-P was 30-fold lower than the apparent K_m for the polyprenol acceptor in PC membranes. Enzyme activity was also stimulated when diacylglycerol was generated in situ by incubation of PC vesicles with phospholipase C. In contrast, the activity of enzyme reconstituted in PE dispersions, or in PE/PC bilayers, was markedly inhibited by the presence of lysophospholipids. Enzyme activity was also reduced by the in situ generation of lysophospholipids in PE/PC vesicles by incubation with phospholipase A_2. Since lysophospholipids and diacylglycerols arise in vivo as products of phospholipid metabolism, modulation of enzyme activity by these compounds may represent a potential regulatory mechanism for the synthesis of oligosaccharide lipids.

Dolichyl-phosphomannose synthase (EC 2.4.1.83) is a membrane enzyme that catalyzes the synthesis of an important intermediate in the pathway for the formation of asparagine-linked oligosaccharides (Hubbard & Ivatt, 1981; Snider, 1984). The metabolic regulation of dolichyl-P-mannose synthase is not well understood but could possibly involve covalent modification of the synthase (Banerjee et al., 1987) or changes in the concentrations of the glycosyl donor, GDP-mannose, the glycosyl acceptor, dol-P,[1] or the synthase itself (Struck & Lennarz, 1980). Our results suggest that rat liver dolichyl-P-mannose synthase activity might also be regulated by changes in the membrane lipid microenvironment, since reconstituted preparations of this enzyme were shown to be very sensitive to the composition of the lipid matrix (Jensen & Schutzbach, 1985; Schutzbach et al., 1987]. In this paper, we describe the effects of incorporating either DAG or lyso-PC into the lipid matrix on dolichyl-P-mannose synthase activity. Low concentrations of both of these lipids are known to be present in biological membranes as the result of phospholipid metabolism (Preiss et al., 1986), and the presence of either DAG or lyso-PC would be expected to disturb lipid packing and alter local membrane architecture. DAG is also known to activate protein kinase C and has been implicated as a second messenger in the transduction of membrane receptor mediated signals (Nishizuka, 1984). In this investigation, the activity of dolichyl-P-mannose synthase was stimulated when DAG was incorporated into PC, PE/PC, or PC/PE/PI membranes, whereas the rate of mannosyl transfer was decreased in lipid matrices containing lysophospholipids. Generation of the same lipids in situ by incubation with either phospholipase C or phospholipase A_2 resulted in a corresponding activation or repression of synthase activity. These results are consistent with the lipid matrix requirements for activity and suggest that phospholipase-induced alterations in the lipid microenvironment may play a role in the in vivo regulation of dolichyl-P-mannose synthase activity.

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. Phospholipase C (*Bacillus cereus*), phospholipase A_2 (*Naja naja*), and diacylglycerols were obtained from Sigma Chemical Co. Dolichyl-P-mannose synthase was purified from rat liver microsomes through the DEAE-cellulose step as previously described (Jensen & Schutzbach, 1985). After removal of detergent, the enzyme was stored frozen in the presence of 10% glycerol. The specific activity of the preparations ranged from 8 to 21 nmol of mannose transferred min^{-1} (mg of protein)$^{-1}$. All other chemicals and standard compounds were purchased from commercial sources.

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

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[1] Abbreviations: DAG, diacylglycerol; DEPE, dielaidoylphosphatidylethanolamine; diolein, 1,2-dioleoylglycerol; DLPC, dilaurylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; dol-P, dolichyl monophosphate; DOPC, dioleoylphosphatidylcholine; DOPE, dioleoylphosphadtidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; EDTA, ethylenediaminetetraacetic acid; lyso-PC, 1-palmitoyllysophosphatidylcholine; PC, plant phosphatidylcholine; PE, plant phosphatidylethanolamine; PI, plant phosphatidylinositol; Tris, tris(hydroxymethyl)aminomethane.

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Chromatography. Thin-layer chromatography was performed on 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 with a radiometric assay that depended upon the differential partitioning of products and substrates in a biphasic scintillation cocktail (Jensen & Schutzbach, 1985). All assays were performed in duplicate. The compositions of lipid mixtures used to reconstitute the synthase are expressed as weight/weight ratios. The hydrated lipid components in the assay were prepared either by immersion in a water-bath sonicator (Branson Model 7) or by probe sonication. For the first method, organic solvents were removed under a stream of nitrogen, and the lipids were sonically dispersed for 30 s in 25 mM Tris/acetate buffer, pH 7.5, containing 0.3 mM EDTA, 0.4 mM reduced glutathione, and 2.5 mM $MnCl_2$. The rate of mannosyl transfer in the presence of PE (20 μg) and dol-P (0.5 μg) was used as the standard reference to compare the effects of other lipid environments on enzyme activity. The rate of mannosyl transfer in PC dispersions at this dol-P concentration was always 10% or less than that observed in control PE/dol-P mixtures. Transferase activity in PE/PC (1:1) mixtures usually ranged between 30 and 50% of the control activity, depending on the enzyme preparation and the phospholipid mixture used in the experiment. All assays within an experiment used a common phospholipid mixture to maintain a constant PE/PC ratio. The ability of lysophospholipids and diacylglycerols to modulate mannosyltransferase activity was assessed by adding increasing quantities of these compounds to lipid mixtures that contained fixed quantities of phospholipid (20 μg) and dol-P (0.5 μg) before removing the organic solvent.

For some assays, small lipid vesicles were used for reconstituting active mannosyltransferase. Phospholipid vesicles containing dol-P were prepared by probe sonication in 0.1 M Tris/acetate buffer, pH 7.5, containing 1.3 mM EDTA and 1.6 mM reduced glutathione (buffer A), using the method of Monti et al. (1987). The vesicles contained 10 mg/mL phospholipid, and the dol-P concentration ranged from 0.25 to 2.5 mg/mL. Further details regarding vesicle composition are given in the appropriate figure and table legends.

Phospholipase C activity was assayed by measuring the concentration of water-soluble organic phosphate liberated by the hydrolysis of PC. The activity of phospholipase C generates equal molar quantities of phosphorylcholine and DAG. Reaction mixtures containing water (0.14 mL), buffer A (0.04 mL), 0.1 M $MnCl_2$ (0.005 mL), and lipid vesicles (0.01 mL, 125 nmol of phosphatidylcholine, 1.8 nmol of dol-P) were vortexed. The reactions were initiated by the addition of phospholipase C (0.005 mL, 0.3–5 ng). After 15 min at 37 °C, the incubations were terminated by the addition of 0.5 mL of chloroform/methanol (2:1) and 0.1 mL of water. The tubes were centrifuged for 2 min at 10000*g*, and the aqueous phases were saved. The lower phase of each tube was extracted with 0.25 mL of Folch upper phase (Folch et al., 1957). The aqueous phases were combined and extracted twice with 0.25 mL of chloroform/methanol, dried under vacuum, and analyzed for phosphate. Under these conditions the phospholipase (1 ng) catalyzed the release of 0.28 nmol of phosphorylcholine/min.

Density Gradient Centrifugation. Lipid vesicles, protein/lipid aggregates, and protein were separated by centrifugation on sucrose gradients (10–40%) prepared in buffer A. 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 legend to Figure 5. Samples were centrifuged in the TLS-55 rotor at 55 000 rpm for 4 h at 4 °C 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, and aliquots (0.01 mL) of each fraction were assayed for enzyme activity and lipid phosphorus.

Results

Activation of Dolichyl-P-mannose Synthase by Diacylglycerol. It was previously demonstrated that optimal dolichyl-P-mannose synthase activity required reconstitution in a lipid matrix containing either PE or the plant glycoglycerolipid monogalactosyl diglyceride (Schutzbach et al., 1987). Although these lipids have dissimilar headgroups, both have a dynamic conical molecular shape and preferentially organize in the hexagonal (H_{II}) phase (De Kruijff et al., 1985). The results suggested that the physical properties of the lipid matrix were more important than specific lipids in the activation of synthase activity. DAG is another lipid that has a similar dynamic conical molecular shape, but this lipid displays no long-range organization when hydrated (Das & Rand, 1986). We found that sonicated dispersions of diolein did not support mannosyl transfer to dol-P when tested at concentrations ranging from 1 to 20 μg in the presence of 0.5 μg of dol-P. Thus, the ability of a lipid to organize in a nonlamellar phase appears to be an important factor for the reconstitution of an active ternary complex of synthase, dol-P, and lipid matrix.

Although nonbilayer lipids provide a suitable matrix for synthase activity, the highest levels of enzyme activity were found to occur when bilayer-forming lipids were combined with nonbilayer-forming lipids at ratios expected to promote the formation of destabilized bilayers (Schutzbach et al., 1987). Since the incorporation of low concentrations of DAG into phospholipids organized in the bilayer phase has been shown to promote the formation of destabilized bilayers (Das & Rand, 1986), the synthase was reconstituted in either PE/PC (1:1) or PC membranes containing increasing concentrations of diolein (Figure 1). The results demonstrate that the incorporation of diolein into either lipid matrix stimulated the rate of mannosyl transfer to dol-P. In PE/PC (1:1) membranes, 5% diolein stimulated mannosyl transfer to a level equivalent to that observed in control reactions containing PE alone. The incorporation of diolein into PC bilayers also promoted enhanced synthase activity, although neither of these lipids provides a good matrix for activity when tested by itself. In PC/diolein (2:1) mixtures the rate of mannosyl transfer was 86% of the control activity. It was previously shown that acidic phospholipids, by themselves, did not provide a good matrix for mannosyltransferase activity (Jensen & Schutzbach, 1985), and the addition of diolein to either PI or dioleoylphosphatidylglycerol did not enhance transferase activity (Table I). Although the enzyme has negligible activity when reconstituted in the presence of PC/PE/PI (2:1:0.3) mixtures, the incorporation of 10% diolein into this lipid mixture provided a matrix that supported full mannosyltransferase activity (Figure 1). The phospholipid ratio in this mixture is approximately the same as that reported for rat liver microsomal membranes (Whitmer et al., 1986).

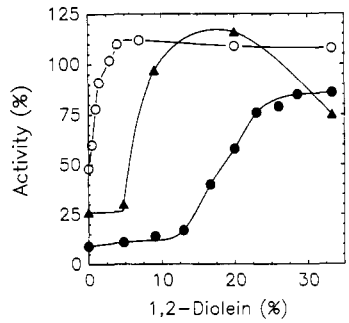

FIGURE 1: Effect of increasing concentrations of diolein on mannosyltransferase activity in phospholipid dispersions. Dolichyl-P-mannose synthase activity was assayed in reaction mixtures containing the indicated quantity of diolein. Activity is plotted as a percentage of mannosyltransferase activity in the presence of PE dispersions in the absence of diolein. (○) PE/PC (1:1); (●) PC; (▲) PC/PE/PI (2:1:0.3).

Table I: Effect of 1,2-Diolein on the Activity of Dolichyl-Phosphomannose Synthase in the Presence of Phospholipids

	synthase activity[a]		
phospholipid	minus 1,2-diolein	plus 1,2-diolein	concn[b] (%)
none	NA	0.1	100.0
plant PE	1.0	NA	NA
DOPE	0.8	1.0	0.5
DEPE[c]	0.4	1.1	5.0
plant PC	0.1	0.9	33.0
DOPC	0.2	1.1	20.0
DPPC	0.2	0.4	50.0
DMPC	0.1	0.3	50.0
DLPC	0.1	0.3	43.0
plant PI	0.25	0.3	20.0

[a] All assays contained 20 μg of phospholipid and 0.5 μg of dol-P. Activity is expressed relative to that observed in control reactions containing PE. Dioleoylphosphatidylglycerol did not support more than 10% of the control activity in the absence or presence of 1,2-diolein. [b] Concentration of 1,2-diolein (%, w/w) required for maximal stimulation of enzyme activity. [c] Since DEPE has a gel to liquid-crystalline transition temperature of 37 °C, reactions containing DEPE were incubated at 41 °C.

Since the naturally occurring plant phospholipids used for the reconstitution of enzyme activity in the experiments described above contain a heterogeneous mixture of highly unsaturated acyl chains (Monti et al., 1987), the ability of diolein to activate mannosyl transfer in the presence of phospholipids that contain acyl chains of defined composition was also examined (Table I). In the absence of 1,2-diolein, there was a 2-fold difference in the abilities of DOPE and DEPE to activate the reaction. Incorporating diolein into dispersions of either DOPE or DEPE stimulated the rate of mannosyl transfer to a level equivalent to that observed in PE controls; however, the quantity of diolein required for full activation in a DOPE matrix (0.5%) was much less than that required in DEPE dispersions (5%). We also examined the ability of diolein to activate mannosyl transfer in dispersions of DOPC, which contains a single double bond, and in dispersions of phosphatidylcholines, containing saturated acyl chains. In the absence of diolein, none of these lipids provided more than 20% activity (Table I). Inclusion of diolein (20%) in DOPC dispersions stimulated rates of mannosyl transfer comparable to those observed in PE controls. The mannosyl transfer rate was stimulated to a lesser extent by the addition of diolein to dispersions of phosphatidylcholines having saturated acyl chains. Lipid mixtures containing 40–50% diolein promoted enzyme activities that were 30–40% of that observed in PE controls. These results demonstrate that the rate of mannosyl transfer in phosphatidylcholine/diolein dispersions was dependent upon both the length and degree of unsaturation of the acyl chains, factors that are known to have an effect on the physical properties of a lipid matrix. In contrast, there was little indication that the types of acyl chains in the DAG moiety were important factors in activation of the enzyme, since 1,2-dipalmitoylglycerol, 1,3-dipalmitoylglycerol, 1,3-dioleoylglycerol, 1,3-dilinoleoylglycerol and 1-oleoyl-2-acetyl-glycerol were found to stimulate the rate of mannosyl transfer to the same extent as diolein when incorporated into membranes of PC or PE/PC (1:1) (data not shown).

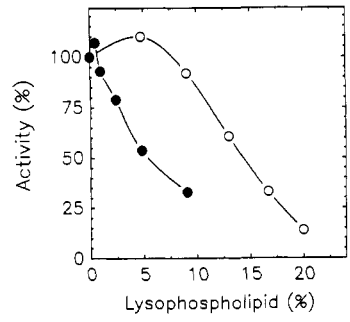

FIGURE 2: Effect of increasing concentrations of lyso-PC on dolichyl-P-mannose synthase activity in phospholipid dispersions. Mannosyltransferase activity was assayed in reaction mixtures containing the indicated quantity of lyso-PC. Activity is plotted as a percentage of mannosyltransferase activity in the presence of either (○) PE (●) or PE/PC (1:1) dispersions in the absence of lyso-PC.

We recently reported that enzyme activity in PC dispersions could be stimulated by greatly increasing the concentration of the lipophilic acceptor, dol-P, in the PC matrix (Jensen & Schutzbach, 1988). This effect presumably derives from the ability of dolichyl compounds to promote the formation of destabilized bilayers (Jensen & Schutzbach, 1984; Gruner, 1985; Valtersson et al., 1985; Van Duijn et al., 1986; Chojnacki & Dallner, 1988). In PC membranes the apparent K_m for dol-P was 9 μM. The apparent K_m for dol-P in PC/diolein (2:1) mixtures was 0.3 μM, which was the same as that observed in PE dispersions. The results suggest that incorporation of diolein into PC membranes alters the physical properties of the lipid matrix in a manner that increases the apparent affinity of the enzyme for the polyprenol substrate. By comparison, at saturating concentrations of dol-P, the K_m for the water-soluble substrate, GDP-mannose, was not significantly different when the enzyme was reconstituted in PC/diolein (2:1) mixtures, in PC bilayers, or in PE dispersions. The respective K_m values were 0.29, 0.24, and 0.7 μM.

Enzyme Activity in Membranes Containing Lysophosphatidylcholine. Sonicated dispersions of lyso-PC failed to support mannosyl tranfer to dol-P when tested at concentrations ranging from 1 to 20 μg in the presence of 0.5 μg of dol-P. When the synthase was reconstituted either with PE or in PE/PC (1:1) mixtures, a progressive reduction in enzyme activity was observed when increasing concentrations of lyso-PC were incorporated into the lipid matrix (Figure 2). The rate of mannosyl transfer was halved when 14% lyso-PC was incorporated into PE dispersions and when 5% lyso-PC was incorporated into PE/PC (1:1) membranes. Although the incorporation of lyso-PC into PE dispersions decreased the rate of mannosyl transfer, lyso-PC did not affect the apparent K_m for dol-P. The concentration of dol-P that provided half-maximal activity in PE/lyso-PC (9:1) mixtures (0.7 μM) was not significantly different from the apparent K_m for dol-P in PE dispersions (0.3 μM).

Enzyme Activity in Phospholipid Vesicles Incubated with Phospholipases. The preceding results demonstrate that synthase activity could be modulated by incorporation of either

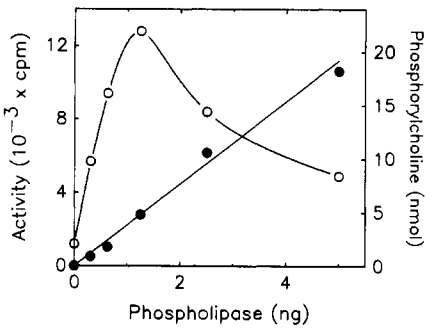

FIGURE 3: Effect of phospholipase C on mannosyl transfer to dol-P incorporated into phospholipid vesicles. Reaction mixtures containing water (0.12 mL), buffer A (0.05 mL), 0.1 M $MnCl_2$ (0.005 mL), liposomes (0.01 mL, 125 nmol of phosphatidylcholine, 1.8 nmol of dol-P), dolichyl-P-mannose synthase (0.005 mL, 0.25 μg of protein), and phospholipase C (0.005 mL, 0.3–5 ng) were incubated 5 min at 37 °C. The mannosyltransferase reactions were initiated by the addition of GDP-[^{3}H]mannose (0.005 mL), and the amount of dolichyl-P-mannose synthesized, expressed as cpm/10-min assay, was quantified with the two-phase assay (○). Phospholipase C activity (●) was assayed as described under Materials and Methods.

Table II: Effect of Phospholipase A_2 on Mannosyl Transfer to Dolichyl-P Incorporated into Phospholipid Vesicles

	synthase activity[b]	
phospholipase[a] (units)	PC/dol-P vesicles	PE/PC/dol-P vesicles
0.000	7000	7300
0.025	6900	7300
0.050	5500	6600
0.100	3000	800
0.500	600	80

[a] One unit of the *Naja naja* enzyme catalyzes the formation of 1 μmol/min of lysophosphatidylcholine and free fatty acid from phosphatidylcholine at pH 8.9, 25 °C. [b] Incubation mixtures contained water (0.12 mL), buffer A (0.05 mL), 0.1 M $MnCl_2$ (0.005 mL), dolichyl-P-mannose synthase (0.005 mL, 0.25 μg of protein), phospholipase A_2 (0.005 mL) at the indicated dilution, and phospholipid/dol-P vesicles (0.01 mL, 0.1 mg of phospholipid). PC/dol-P vesicles contained 20% dol-P, and PE/PC/dol-P vesicles contained 2.4% dol-P. The incubation mixtures were held for 5 min at 37 °C before mannosyltransferase reactions were initiated by the addition of GDP-[^{3}H]-mannose (0.005 mL). The amount of dolichyl-P-mannose synthesized, expressed as cpm/10-min assay, was quantified by using the two-phase assay.

DAG or lyso-PC into the lipid matrix. When dolichyl-P-mannose synthase was reconstituted with PC vesicles containing 2.4% dol-P, the rate of mannosyl transfer was significantly increased by the addition of phospholipase C (Figure 3). Under these conditions, the rate of mannosyl transfer observed was proportional to the quantity of DAG, generated by phospholipase C, in the lipid matrix. DAG concentrations of 0.75 mol % stimulated a 5-fold increase in enzyme activity, and maximal levels of enzyme activity were noted at 4 mol % DAG. The production of higher concentrations of DAG in the lipid matrix resulted in a decrease in the rate of mannosyl transfer.

In contrast, the enzyme was inhibited by phospholipase A_2 when the mannosyltransferase was reconstituted either in PC vesicles containing 20% dol-P or in PE/PC vesicles containing 2.4% dol-P. Mannosyltransferase activity was inversely proportional to the amount of phospholipase A_2 added to the reaction mixture (Table II). The modulation of dol-P-mannose synthase activity observed when phospholipases were added to the reaction mixtures is consistent with the results found when either DAG or lyso-PC was directly incorporated into the lipid matrix.

Physical Association of Dolichyl-P-mannose Synthase with Phospholipid Vesicles. Since previous results had suggested

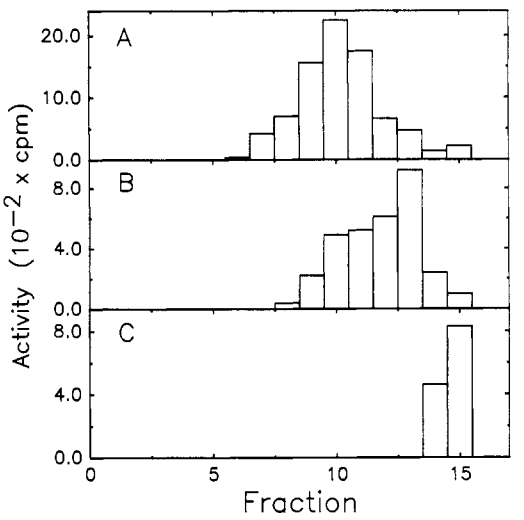

FIGURE 4: Effect of lipid on the bouyant of dolichyl-P-mannose synthase. Enzyme preparations incubated in the absence of added lipid (panel A), in the presence of PE vesicles containing 20% lyso-PC and 2.4% dol-P (panel B), and in the presence of PC vesicles containing 10% diolein and 2.4% dol-P (panel C) were layered on 10–40% sucrose gradients and centrifuged at 4 °C for 4 h at 55 000 rpm. Aliquots of each fraction (10 μL) were assayed for enzyme activity in reaction mixtures containing PE (80 μg) and dol-P (2 μg). The respective recovery of enzyme initially applied to the gradients for the experiments depicted in panels A–C was 75%, 53%, and 55%. In the experiments depicted in panels B and C, lipid phosphorus was quantitatively recovered in fractions 14 and 15.

that dolichyl-P-mannose synthase physically interacted only with lipid matrices capable of stimulating synthase activity (Jensen & Schutzbach, 1985; Schutzbach et al., 1987), the ability of the enzyme to form a stable physical association with phospholipid vesicles containing either DAG or lyso-PC was examined. When aliquots of the synthase were centrifuged on 10–40% sucrose gradients in the presence of PC vesicles containing 10% diolein and 2.4% dol-P, all of the recovered enzyme activity was found at the top of the gradient associated with phospholipid, indicating that the synthase formed a stable physical association with this phospholipid matrix (Figure 4C). When the synthase was mixed with PE vesicles containing 20% lyso-PC and 2.4% dol-P and centrifuged on 10–40% sucrose gradients, the recovered enzyme activity was not associated with phospholipid at the top of the gradient (Figure 4B). Instead, a single peak of enzyme activity, which partially overlapped the sedimentation position of enzyme centrifuged in the absence of phospholipid (Figure 4A), was noted. The shift in sedimentation position over that seen for free enzyme indicates that interaction between the synthase and the lipid vesicles altered either the size or bouyant density of the enzyme. These data demonstrate, however, that the synthase did not form a stable physical association with PE/lyso-PC/dol-P vesicles.

Discussion

In this paper, we have shown that the activity of rat liver dolichyl-P-mannose synthase reconstituted in a lipid matrix can be modulated by the incorporation of either DAG or lyso-PC. The enhanced mannosyltransferase activity observed when DAG was incorporated into the lipid matrix is believed to be a consequence of the ability of this conically shaped lipid to physically alter the structure of bilayer membranes. The incorporation of DAG into lipid bilayers has been shown to destabilize the bilayer phase and promote the formation of nonlamellar phases in model lipid systems (Dawson et al., 1984; Epand, 1985; Das & Rand, 1986). Since DAG has a

small headgroup, incorporation of this lipid into bilayer structures results in an increase in the membrane hydrocarbon volume, which is not balanced by a commensurate increase in the cross-sectional area occupied by polar headgroups. The primary effect of this imbalance is to spread the polar headgroups and expose the acyl chains to the aqueous environment (Epand, 1985; Das & Rand, 1986). Activation of dolichyl-P-mannose synthase in dispersions of plant PC occurred at DAG concentrations reported by Das and Rand (1986) to induce bilayer destabilization and the formation of nonlamellar phases in egg PC membranes. The apparent increased affinity of the enzyme for dol-P in PC/DAG mixtures may be the result of DAG-induced phospholipid headgroup spreading, which would allow the synthase increased access to the polar phosphate group of the polyprenol substrate. When diolein was directly incorporated into PC dispersions, optimal enzyme activity occurred at a diolein concentration of 33%. Much lower amounts of DAG stimulated optimal mannosyl transfer in PC vesicles when the DAG was generated by digestion with phospholipase C. Optimal enzyme activity under these conditions was observed in the presence of 4 mol % DAG, and the rate of mannosyl transfer was stimulated 5-fold by as little as 0.75 mol % DAG. This latter concentration is within the physiological concentration range, since Preiss et al. (1986) have reported that the concentration of DAG in resting and vasopressin-stimulated hepatocytes was, respectively, 0.4 and 1.2 mol % of the total phospholipid. DAG has also been shown to stimulate the activity of Ca^{2+}-ATPase (Cheng & Hui, 1986) and several phospholipases (Dawson et al., 1984). The increased enzyme activity was proposed to be the result of DAG-induced bilayer destabilization.

The present results demonstrate that lyso-PC/dol-P mixtures do not provide a matrix capable of supporting synthase activity when the lysolipid is present at micelle-forming concentrations. However, the diminished mannosyltransferase activity noted in the presence of phospholipid dispersions containing low concentrations of lyso-PC is not the result of micelle formation. Madden and Cullis (1982) have demonstrated a bilayer phase organization for egg PE dispersions containing 10 mol % lysophospholipid, and up to 35 mol % lyso-PC can be incorporated in DOPC dispersions before the onset of micelle formation (Van Echteld et al., 1981). Thus, the inhibitory effects of lyso-PC on mannosyltransferase activity can be ascribed to the ability of lysolipids to stabilize the bilayer phase in phospholipid mixtures containing PE (Madden & Cullis, 1982).

The present study has shown that the activity of dolichyl-P-mannose synthase was stimulated by incorporation of DAG into the lipid matrix but that enzyme activity was decreased by the presence of low concentrations of lyso-PC. These results are consistent with our previous conclusion that optimal dolichyl-P-mannose synthase activity requires a unique lipid microenvironment best provided by destabilized bilayers (Schutzbach et al., 1987). Since both DAG and lyso-PC arise in membranes as the result of phospholipid metabolism, the results suggest that these lipids may potentially regulate the in vivo formation of dolichyl-P-mannose.

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References

Banerjee, D. K., Kousvelari, E. E., & Baum, B. J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6389–6393.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Cheng, K., & Hui, S. N. (1986) *Arch. Biochem. Biophys. 244*, 382–386.

Chojnacki, T., & Dallner, G. (1988) *Biochem. J. 252*, 1–9.

Cullis, P. R., & De Kruijff, B. (1978) *Biochim. Biophys. Acta 507*, 207–218.

Das, S., & Rand, R. P. (1986) *Biochemistry 25*, 2882–2889.

Dawson, R. M. C., Irvine, R. F., Bray, J., & Quinn, P. J. (1984) *Biochem. Biophys. Res. Commun. 125*, 836–842.

De Kruijff, B., Cullis, P. R., Verkleij, A. J., Hope, M. J., Van Echteld, C. J. A., & Taraschi, T. F. (1985) in *The Enzymes of Biological Membranes* (Martonosi, A. N., Ed.) pp 131–204, Plenum, New York.

Dittmer, J. C., & Lester, R. C. (1964) *J. Lipid Res. 5*, 126–127.

Duck-Chong, C. G. (1979) *Lipids 14*, 492–497.

Epand, R. M. (1985) *Biochemistry 24*, 7092–7095.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957) *J. Biol. Chem. 226*, 497–509.

Gruner, S. M. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 3665–3669.

Hubbard, S. C., & Ivatt, R. J. (1981) *Annu. Rev. Biochem. 50*, 555–583.

Jensen, J. W., & Schutzbach, J. S. (1984) *Biochemistry 23*, 1115–1119.

Jensen, J. W., & Schutzbach, J. S. (1985) *Eur. J. Biochem. 153*, 41–48.

Jensen, J. W., & Schutzbach, J. S. (1988) *Biochemistry 27*, 6315–6320.

Kirk, G. L., & Gruner, S. M. (1985) *J. Phys. (Les Ulis, Fr.) 46*, 761–769.

Madden, T. D., & Cullis, P. R. (1982) *Biochim. Biophys. Acta 684*, 149–153.

Nishizuka, Y. (1984) *Nature (London) 308*, 693–698.

Preiss, J., Loomis, C. R., Bishop, W. R., Stein, R., Niedel, J. E., & Bell, R. M. (1986) *J. Biol. Chem. 261*, 8597–8600.

Schutzbach, J. S., Jensen, J. W., Lai, C. S., & Monti, J. A. (1987) *Chem. Scr. 27*, 109–118.

Snider, M. D. (1984) in *Biology of Carbohydrates* (Ginsburg, V., & Robbins P. W., Eds.) Vol. 2, pp 163–198, Wiley, New York.

Struck, D. K., & Lennarz, W. J. (1980) in *The Biochemistry of Glycoproteins and Proteoglycans* (Lennarz, W. J., Ed.) pp 35–83, Plenum, New York.

Valtersson, C., van Duyn, G., Verkleij, A. J., Chojnacki, T., De Kruijff, B., & Dallner, G. (1985) *J. Biol. Chem. 260*, 2742–2751.

Van Duijn, G., Valtersson, C., Chojnacki, T., Verkleij, A. J., Dallner, G., & De Kruijff, B. (1986) *Biochim. Biophys. Acta 861*, 211–223.

Van Echteld, C. J. A., De Kruijff, B., Mandersoot, J. G., & De Gier, J. (1981) *Biochim. Biophys. Acta 649*, 211–220.

Whitmer, D. I., Russell, P. E., Ziurys, J. C., & Gollan, J. L. (1986) *J. Biol. Chem. 261*, 7170–7177.

Zweig, G., & Whittaker, J. R. (1967) in *Paper Chromatography and Electrophoresis*, Vol. 1, pp 232–266, Academic, New York.