Activation of Mannosyltranferase II by Nonbilayer Phospholipids†

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ABSTRACT: Mannosyltransferase II catalyzes transfer from GDP-mannose to an oligosaccharide–lipid intermediate of glycoprotein biosynthesis with the formation of an α-1,3-mannosyl–mannose linkage [Jensen, J. W., & Schutzbach, J. S. (1981) *J. Biol. Chem. 256*, 12899–12904]. Highly purified mannosyltransferase II can be optimally reconstituted with phosphatidylethanolamine but not with other naturally occurring phospholipids [Jensen, J. W., & Schutzbach, J. S. (1982) *J. Biol. Chem. 257*, 9025–9029]. Phosphatidylethanolamine, unlike phosphatidylcholine, does not readily form bilayers in an aqueous environment but instead forms a nonbilayer or hexagonal phase, and our results suggested that mannosyltransferase II was optimally active in the presence of this nonbilayer phase. We now present evidence to demonstrate that mannosyltransferase activity can also be reconstituted with phospholipid mixtures that have been shown to form destabilized bilayers but not with mixtures that form stable bilayers [Cullis, P. R., & De Kruijff, B. (1978) *Biochim. Biophys. Acta 507*, 207–218]. The incorporation of increasing amounts of phosphatidylcholine into phosphatidylethanolamine dispersions promotes bilayer formation with a concomitant decrease in transferase activity. The addition of cholesterol to these phospholipid mixtures, however, destabilizes the bilayer structure, resulting in a stimulation of mannosyl transfer. Dolichol and dolichol-P were also found to restore mannosyltransferase activity in phosphatidylethanolamine–phosphatidylcholine mixtures, suggesting that dolichols might also destabilize phospholipid bilayer structures. ^{31}P NMR spectroscopy of phospholipid mixtures in the presence and absence of dolichol demonstrated that dolichol does have a significant effect on bilayer structure. Thus dolichols may play an important structural role in membranes, as well as being glycosyl carriers for the synthesis of oligosaccharide–lipid intermediates.

The asparagine-linked oligosaccharide side chains of mammalian glycoproteins originate from the en bloc transfer of precursor oligosaccharides, which are initially assembled on a dolichol–pyrophosphate carrier, to the nascent proteins (Struck & Lennarz, 1980; Hubbard & Ivatt, 1981). The enzymes that catalyze the incorporation of sugars into the oligosaccharide, as well as their lipid-linked acceptors, are intimately associated with membranes of the rough endoplasmic reticulum, although the functional organization of this complex has not been established.

We have previously reported the solubilization and substantial purification of one of the enzymes involved in synthesis of the oligosaccharide–lipid intermediate (Jensen & Schutzbach, 1981). Mannosyltransferase II[1] catalyzes transfer from GDP-mannose to a lipid-linked oligosaccharide, resulting in the formation of an α-1,3-mannosyl–mannose linkage. When enzymatic activity was reconstituted in the presence of phospholipids, it was found that species of PE containing unsaturated acyl chains provided optimal enzyme activity (Jensen & Schutzbach, 1982). Since phospholipids that preferentially form bilayer membranes were essentially inactive in promoting activity, we suggested that mannosyltransferase II activity was optimally stimulated by nonbilayer phospholipid phases. The presence of nonbilayer phases in hydrated phospholipid dispersions has been demonstrated by both ^{31}P NMR spectroscopy in conjunction with freeze–fracture electron microscopy (Cullis & De Kruijff, 1979; Hui et al., 1981) and by ^{2}H NMR spectroscopy (Tilcock et al., 1982). Discrete NMR signals have been correlated with bilayer and hexagonal phases as well as with an intermediate phase that has been characterized by the presence of lipidic particles (Cullis & De Kruijff, 1979; Hui et al., 1981; Tilcock et al., 1982). The bilayer to hexagonal phase transitions have a low energy barrier and the transition has been shown to be sensitive to temperature as well as to the presence of additional lipids and other agents.

We now present additional evidence showing that mannosyltransferase II is optimally active in the presence of hexagonal-phase phospholipids and that the enzyme is essentially inactive when reconstituted with phospholipids or phospholipid mixtures that form stable bilayers. Conditions that promote the formation of nonbilayer phases in PE–PC mixtures, however, induce the formation of a matrix that promotes optimal activity for mannosyltransferase II. On the basis of our results we suggest that dolichol and dolichol derivatives promote phase changes in membranes that may be similar to the hexagonal phase or to lipidic particles that have been observed in phospholipid mixtures.

Experimental Procedures

Materials. Mannosyltransferase II was solubilized and purified through the second hydroxylapatite column stage (hydroxylapatite II) as previously described (Jensen & Schutzbach, 1981), and the enzyme was stored at −20 °C in the presence of 10% (v/v) glycerol and 0.1% (v/v) Nonidet P-40 until used. The enzyme preparations used for these studies contained 20–40 μg of protein/mL and had specific activities of 2.5–3.7 nmol of mannose transferred min^{-1} (mg

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[1] Mannosyltransferase II is a trivial name for the enzyme that catalyzes mannosyl transfer from GDP-mannose to Manβ1–4GlcNAcβ1–4GlcNAc-P-P-dolichol, resulting in the formation of Manα1–3Manβ1–4GlcNAcβ1–4GlcNAc-P-P-dolichol. The enzyme can also utilize an isolated oligosaccharide–lipid with the structure Manα1–6Manβ–GlcNAcβ–GlcNAc-P-P-lipid as a substrate (Jensen et al., 1980). Other abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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of protein)$^{-1}$. The tetrasaccharide–pyrophosphoryllipid was isolated from porcine liver and analyzed as described (Jensen et al., 1980). Nonidet P-40 was obtained from Bethesda Research Laboratories, GDP-[^{14}C]mannose (192 mCi/mmol) was from New England Nuclear, dolichol and cholesterol (chromatographic grade) were from Sigma Chemical Co., chemically phosphorylated dolichol-P was from Calbiochem-Behring Corp., and phospholipids were from Avanti Polar Lipids, Inc. All other chemical and standard compounds were purchased from commercial sources or were prepared as previously described (Jensen et al., 1980; Schutzbach et al., 1980).

Analytical Methods. Protein and phosphate were determined as previously described, and the purity of the phospholipids was assessed by using thin-layer chromatography as before (Jensen & Schutzbach, 1982).

Enzyme Assays. Procedures for assay of mannosyltransferase II activity in the presence of nonionic detergents (Jensen & Schutzach, 1981) or phospholipid (Jensen & Schutzbach, 1982) followed previously described methods, and the latter assay is briefly described for convenience. Plant PE and plant PC were used throughout these studies unless otherwise indicated, and all assays were carried out in duplicate.

Tetrasaccharide–pyrophosphoryllipid (0.15 nmol) dissolved in chloroform–methanol–water (1:1:0.3), phospholipid (60 μg) dissolved in chloroform–methanol (2:1), and, when indicated, either dolichol or dolichol-P in chloroform–methanol (2:1) or cholesterol in the chloroform were added to 1.5-mL conical centrifuge tubes. The contents of the tubes were mixed and the organic solvents were removed under a stream of nitrogen. Water (0.11 mL), 0.1 M $MgCl_2$ (0.02 mL), and 0.1 M Tris–acetate,[2] pH 7.0 (0.05 mL), were added and the lipid components were dispersed by a 30-s immersion in an ultrasonic cleaning bath (Branson Model 7). Enzyme (0.01 mL) was then added and the mixture was incubated at 37 °C for 5 min. The transferase reaction was initiated by the addition of 0.01 mL of GDP-[^{14}C]mannose (0.1 μCi). After 15 to 30 min at 37 °C, the reactions were quenched by the addition of 0.3 mL of methanol–water (5:1) and the product was extracted into chloroform–methanol–water (1:1:0.3) and quantified as previously described (Schutzbach et al., 1980). The presence of excess phospholipids did not interfere with the precipitation or extraction of the reaction product. Control reactions incubated in the absence of added phospholipid had less than 5% of the activity noted in the standard assay. Assays carried out in the presence of detergent were linear for 20 min and in PE dispersions were linear for at least 40 min (Jensen & Schutzbach, 1982).

Nuclear Magnetic Resonance Spectroscopy. Phospholipids (50 mg total) were dried from chloroform solution under a stream of nitrogen, and the remaining solvent was removed under high vacuum for 4 h. The phospholipids were hydrated in 0.9 mL of 10 mM Tris-HCl, pH 7.0, containing 100 mM NaCl and 2 mM EDTA by blending on a vortex mixer. Samples containing PE or dolichol required prolonged blending, and hydration was facilitated by the presence of a small glass shard in the tube. Deuterated water (0.1 mL) was added to the samples prior to NMR spectroscopy. ^{31}P NMR spectra were obtained by using a JEOL FX-100 nuclear magnetic resonance spectrometer operating at 40.3 MHz. Spectra were obtained by collecting from 1000 to 5000 free induction decays by using a 90° pulse of 25-μs width and a recycle time of 2 s. The spectral width was 10 kHz and an exponential window corresponding to a 1.2-Hz line broadening was applied to the free induction decay before Fourier transformation. The temperature of the 10-mm probe was maintained at 39 ± 2 °C by a JEOL VT-3B variable-temperature unit and was measured directly in the sampling area subsequent to each data acquisition.

[2] The 0.1 M Tris–acetate, pH 7.0, contained 1.3 mM EDTA and 1.6 mM reduced glutathione. The presence of EDTA was found to be essential for mannosyltransferase II activity in either nonionic detergent or in phospholipid dispersions.

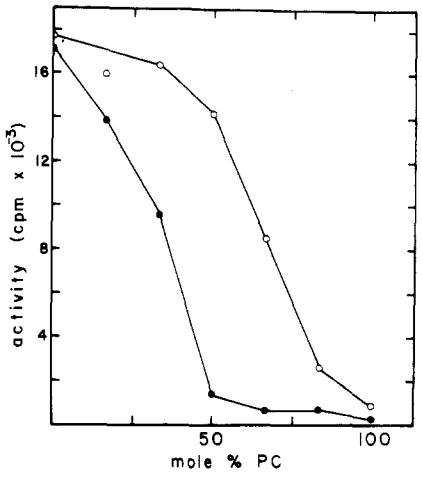

FIGURE 1: Effect of cholesterol on enzyme activity in phospholipid vesicles of mixed composition. Mannosyltransferase II was assayed as described under Experimental Procedures except that solutions of the phospholipids were mixed in the indicated proportions, dried under a stream of nitrogen, and sonically dispersed. The total amount of phospholipid was held constant at 60 μg. Activity is expressed as cpm of [^{14}C]mannose transferred in 30 min in PE–PC mixtures (●) and in PE–PC mixtures containing 31 μg of cholesterol (○).

Results

Effect of PE Acyl Chains on Activity. We have previously shown that sonified disperions of PE derived from egg yolk, mammalian tissues, plants, and synthetic dioleoyl-PE ($18:1_c/18:1_c$) were able to promote full mannosyltransferase activity at 37 °C (Jensen & Schutzbach, 1982). All of these phospholipids contain unsaturated acyl chains and all form a hexagonal phase at the assay temperature. We now report that dielaidoyl-PE ($18:1_t/18:1_t$) dispersions provide only 32% activity (6500 cpm of [^{14}C]mannose transferred/30 min) when compared with dioleoyl-PE (20 200 cpm transferred/30 min) and when assayed under the same experimental conditions. Since dioleoyl-PE undergoes a bilayer to hexagonal phase transition between 9 and 12 °C, whereas dielaidoyl-PE does not undergo this transition until 60 °C (Tilcock & Cullis, 1982), this experiment provides additional evidence that mannosyltransferase II activity is optimal when the enzyme is reconstituted with phospholipids that form nonbilayer phases. The results also demonstrate that phospholipid phase properties are more important than head-group specificity for enzyme activity.

Enzyme Activity in Membranes of Mixed Composition. The addition of increasing quantities of unsaturated PC to dispersions of unsaturated PE has been shown to induce a transition from hexagonal phase to stable bilayers (Tilcock et al., 1982; Cullis & De Kruijff, 1978a). The further addition of an equimolar concentration of cholesterol to the binary mixtures of PE and PC promoted a phase change that was described as bilayer destabilization. We have previously shown that increasing the molar percentage of PC in PE–PC mixtures suppressed mannosyltransferase II activity (Jensen & Schutzbach, 1982). When an equimolar amount of cholesterol is added to the phospholipid mixtures, however, the activity

Table I: Activity of Mannosyltransferase II When Enzyme and Oligosaccharide-Lipid Acceptor Are Present in Separate Phospholipid Vesicles[a]

vesicle 1	vesicle 2	activity cpm	activity %
PE-OL[b]	PE-MT II	14 300[c]	100
PE-OL	PC-MT II	2 300	16
PC-OL	PE-MT II	800	6
PC-OL	PC-MT II	60	0
PE-OL-MT II	PE	14 300	100
PE-OL-MT II	PC	11 400	80
PC-OL-MT II	PE	1 900	13
PC-OL-MT II	PC	100	1
PE-PC-OL-MT II	none	900	6

[a] Sonified dispersions of PE that are predominantly in the hexagonal phase are difficult to visualize as vesicles, and under standard assay conditions, the PE probably does not form structures that resemble typical closed unilamellar or multilamellar liposomes (Szoka & Papahadjopoulos, 1980; Stollery & Vail, 1977). Sonified PC or equimolar mixtures of PE and PC would, however, be expected to form typical multilamellar liposomes under the conditions employed in these studies. Phospholipid vesicles of the indicated composition were prepared as follows. The indicated phospholipid (75 μg of each) and tetrasaccharide-pyrophosphoryllipid (0.75 nmol where indicated) were dried under a stream of nitrogen. Enzyme (2 μg of protein where indicated), 0.05 M Tris-acetate,[2] pH 7.0, and Nonidet P-40 at a final concentration of 0.05% (v/v) were added to a total volume of 0.1 mL. The mixture was solubilized by immersion in an ultrasonic water bath for 15 s and then diluted 10-fold by the addition of Tris-acetate, pH 7.0, containing 10 μmol of $MgCl_2$. The vesicle mixtures were prepared by combining 0.195 mL of the indicated populations, or 0.195 mL of buffer, and after 5 min at 37 °C, the reactions were initiated by the addition of 0.01 mL of GDP-[^{14}C]mannose (0.1 μCi). Reactions were quenched by the addition of 0.4 mL of methanol, and the [^{14}C]pentasaccharide-lipid product was quantified as previously described (Schutzbach et al., 1980). [b] Abbreviations: OL, tetrasaccharide-pyrophosphoryllipid; MT II, mannosyltransferase II. [c] Activity is expressed as cpm of [^{14}C]-mannose transferred in a 30-min incubation.

of the enzyme is increased substantially over that observed in the absence of cholesterol (Figure 1). Activity stimulated by PE alone was relatively unaffected by the addition of cholesterol. PC, either by itself or in combination with cholesterol, did not support enzyme activity. The progressive loss of transferase activity observed in phospholipid mixtures as the mole percentage of PC was increased can be correlated with results showing the formation of a bilayer phase under these conditions (Tilcock et al., 1982; Cullis & De Kruijff, 1978a). Likewise, the sparing effect of cholesterol at inhibitory concentrations of PC can be related to the ability of this molecule to destabilize bilayers composed of the mixed phospholipids.

Although we interpreted these results as an effect of PC on the phase properties of the phospholipid matrix, it was also possible that the PC head group was a direct inhibitor of the mannosyltransferase. This possibility was tested by incorporating enzyme and oligosaccharide–lipid acceptor into different phospholipid dispersions and then testing for transferase activity (Table I). Full enzyme acitvity was obtained only when the enzyme and the acceptor were prepared in PE dispersions. Similar results were obtained whether the enzyme and acceptor were reconstituted into the same PE dispersion or were added together in separate dispersions. When either enzyme or acceptor was incorporated into PC vesicles, however, enzyme activity was never higher than 16% of the maximum. More importantly, when the enzyme and acceptor were incorporated together in the same PE dispersion, the addition of separate vesicles of PC did not inhibit the enzyme more than 20%. Thus the addition of PC did not significantly inhibit PE-stimulated

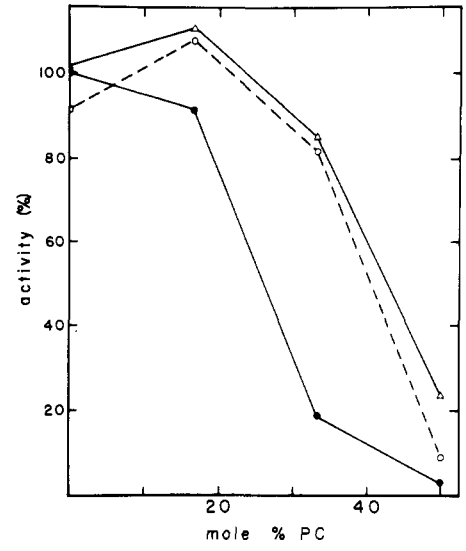

FIGURE 2: Effect of dolichol and dolichol-P on enzyme activity in phospholipid vesicles of mixed composition. Mannosyl transfer was assayed as described under Experimental Procedures except that the phospholipids were mixed in the indicated proportions. Activity was measured as cpm of [^{14}C]mannose transferred in 30 min in PE–PC mixtures (●) or in PE–PC mixtures containing either 1 μg of dolichol (△) or 10 μg of dolichol-P (○).

transferase activity unless the PC was incorporated into the same vesicle along with either enzyme or substrate, a condition that would have affected the phase properties of the mixed phospholipid matrix.

Effect of Dolichols on Activity in Mixed Phospholipid Vesicles. The lipid component of the oligosaccharide–lipid acceptor used in these studies is probably a dolichol although this has yet to be established, but the possibility that dolichol or dolichol derivatives might influence the formation of an optimal phospholipid phase or matrix for transferase activity was considered. Thus the addition of the oligosaccharide–lipid acceptor to the assay mixtures might also have resulted in destabilization of membrane structures that would normally have been in the bilayer configuration.

Enzyme activity in phospholipid mixtures of varying proportions was measured at two concentrations of oligosaccharide–lipid that approximate 0.2 and 0.4 μg of dolichol assuming C-95 dolichol as the average in the acceptor preparation. Doubling the acceptor concentration in PE dispersions had no effect on enzyme activity, indicating that the acceptor was present at saturating concentrations in the PE dispersion. When the amount of acceptor present in PE–PC mixtures was doubled, however, enzyme activity was increased 28% at a PE:PC ratio of 2:1 and was increased over 2-fold at PE:PC ratio of 1:1 although the basal activity at this phospholipid ratio is very low (see Figure 1). A much larger effect was noted when either dolichol (1 μg) or dolichol-P (10 μg), which are not substrates for mannosyltransferase II, was added to the phospholipid mixtures (Figure 2). The dolichols had little effect on the PE stimulated activity, although 10 μg of dolichol-P gave a slight but variable (up to 25%) inhibition in the presence of PE alone. Both dolichol and dolichol-P, however, markedly stimulated mannosyl transfer in the presence of inhibitory concentrations of PC. The effects on activity noted for the dolichols when they were added to phospholipid mixtures were similar to that noted with the addition of cholesterol. Therefore, it was possible that dolichols promoted the formation of unique phospholipid phases that provided the optimal environment for mannosyltransferase activity.

Nuclear Magnetic Resonance Studies. ^{31}P NMR spectra obtained in our studies on pure preparations of PE and PC

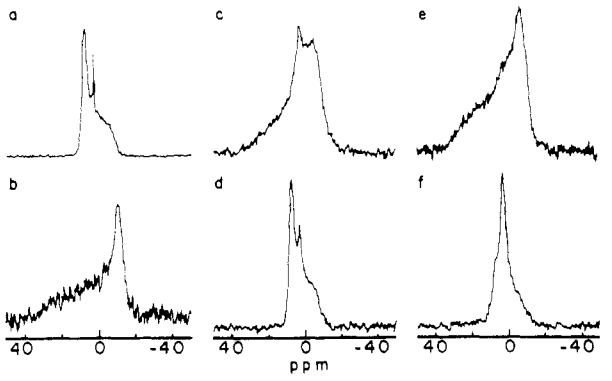

FIGURE 3: 40.3-MHz NMR spectra at 39 °C. The samples were (a) PE, (b) PC, (c) PE–PC (2:1), (d) PE–PC (2:1) plus 10 mg of dolichol, (e) PE–PC (1:1), and (f) PE–PC (1:1) plus 10 mg of dolichol. Samples were prepared as described under Experimental Procedures and each sample contained a total of 50 mg of phospholipid.

and on PE–PC mixtures (Figure 3) are similar to those spectra obtained by other investigators (Cullis & De Kruijff, 1979) and are consistent with the signals expected for PE in the hexagonal phase (Figure 3a) and for PC and PE–PC (1:1) mixtures in the bilayer phase (parts b and e of Figure 3). At a PE:PC ratio of 2:1, the phospholipid mixture produces an NMR signal with an isotropic component (Figure 3c) that has been suggested to represent an unstable bilayer or the presence of lipidic particles (Cullis & De Kruijff, 1979; Hui et al., 1981; Tilcock et al., 1982). Upon addition of dolichol to the phospholipids, the NMR signal of the PE–PC (2:1) mixture (Figure 3d) was shifted to a spectrum expected for hexagonal-phase phospholipid, and the 1:1 mixture (Figure 3f) was shifted from bilayer to a signal suggesting the formation of destabilized bilayers. Thus dolichol produces an effect on phospholipid phases similar to that described for cholesterol (Tilcock et al., 1982; Cullis & De Kruijff, 1978a).

Discussion

Hydrated dispersions of PE that contain unsaturated acyl chains adopt a nonbilayer or hexagonal configuration (Cullis & De Kruijff, 1978b, 1979). We have previously reported that unsaturated species of PE supported maximal mannosyltransferase II activity, whereas phospholipids that form stable bilayers did not support enzyme activity (Jensen & Schutzbach, 1982). The addition of nonionic detergent to PE in the hexagonal phase has been shown to induce the formation of a PE bilayer phase (Madden & Cullis, 1982; Hornby & Cullis, 1981), and detergent was found to inhibit PE-stimulated mannosyltransferase activity. In this paper, we show that dioleoyl-PE, which is in the hexagonal phase at the assay temperature, supports full enzyme activity but dielaidoyl-PE, which does not form a hexagonal phase at 37 °C, does not support full enzyme activity, again suggesting that mannosyltransferase II prefers a nonbilayer phase for activity.

The inclusion of PC into PE dispersions has also been shown to promote bilayer formation, and equimolar mixtures of these phospholipids form normal multilamellar bilayers (Hui et al., 1981; Tilcock et al., 1982; Cullis & De Kruijff, 1978a). Our results show that equimolar mixtures of PE and PC significantly depress mannosyltransferase II activity, but the inhibitory effect of PC was only noted when PE and PC were present in the same phospholipid vesicles. Mannosyltransferase activity was optimal when the enzyme and lipid-linked acceptor were incorporated into PE, but there was essentially no difference in activity if enzyme and substrate were in the same dispersion or in separate dispersions that were mixed. If either enzyme or acceptor was incorporated into PC vesicles, however, activity was reduced more than 80%. When the enzyme and acceptor were incorporated into PE, the addition of separate PC vesicles did not substantially reduce the rate of mannosyl transfer. This experiment demonstrated that both enzyme and substrate had to be incorporated into lipid dispersions that prefer the nonbilayer phase and that PC inhibited the reaction only under conditions where it could affect the phase properties of the PE, i.e., when it was incorporated into a mixed phospholipid membrane.

Thus mannosyltransferase II was essentially inactive under experimental conditions that favored the formation of normal bilayer structures, since neither sonified PC dispersions nor mixed membranes composed of equimolar concentrations of PE and PC supported full enzyme activity. In the latter case, the addition of agents such as cholesterol, which destabilize the bilayer (Tilcock et al., 1982; Cullis & De Kruijff, 1978a) and promote the formation of lipidic particles (De Kruijff et al., 1979), greatly enhanced mannosyl transfer. Dolichol, dolichol-P, and increasing concentrations of oligosaccharide–lipid acceptor also activated the enzyme in PE–PC mixtures. The effect of dolichol on membrane structure was examined by ^{31}P NMR spectroscopy, and the results of these experiments demonstrated that dolichol had a large effect on the phase properties of the phospholipids. The NMR spectra obtained in the presence of dolichol are consistent with the suggestion that dolichols destabilize bilayer membrane structures. A destabilized bilayer, characterized by the presence of hexagonal-phase lipids or lipidic particles, might more realistically incorporate the long dolichol chain in a membrane structure. Conceivably, unique lipidic phases may also be involved in the transmembrane movement of sugars from the cytoplasmic to the luminal surface of the rough endoplasmic reticulum. The sugars could be transported as either glycosyl-enzyme intermediates or as lipid-bound sugars. This mechanism would be consistent with either the results of Haselbeck & Tanner (1982) or the mechanism proposed by Hanover & Lennarz (1981, 1982).

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Registry No. Dielaidoyl-PE, 16777-83-6; dioleoyl-PE, 2462-63-7; cholesterol, 57-88-5; dolichol, 11029-02-0; dolichol phosphate, 12698-55-4; mannosyltransferase II, 81181-76-2.

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Thermal Behavior of Cores of Human Serum Triglyceride-Rich Lipoproteins: A Study of Induced Circular Dichroism of β-Carotene†

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ABSTRACT: Induced circular dichroism (CD) of β-carotene has been used to study the physical state in the cores of three classes of triglyceride-rich lipoproteins from human serum: intermediate-density lipoproteins (IDL) ($1.006 < d < 1.019$ g/mL) and subfractions of the $d < 1.006$ g/mL lipoproteins of β and pre-β electrophoretic mobility. Effects on the physical state in the cores attributable to the ratio of triglycerides to cholesteryl esters and particle diameters were assessed by comparing the temperature-dependent CD spectra of β-carotene with those of low-density lipoproteins (LDL). Lipoproteins were prepared from serum by sequential ultracentrifugation after the donors were given supplemental dietary β-carotene (60 mg/day) for 2 weeks. The β- and pre-β-migrating $d < 1.006$ g/mL lipoproteins were separated by starch block electrophoresis and were then individually separated into subfractions by agarose gel filtration chromatography. Between 7 and 30 °C, four subfractions of the β-migrating $d <$ 1.006 g/mL lipoproteins and IDL exhibited reversible, temperature-dependent induced CD of β-carotene, with contours similar to those of LDL but with smaller magnitudes and much broader transitions of the CD bands than those of LDL. In contrast, subfractions of the pre-β-migrating $d < 1.006$ g/mL lipoproteins showed no detectable induced CD of β-carotene. These results show that the cores of triglyceride-rich lipoproteins can exist in some ordered state between 7 and 30 °C if they have a relatively low ratio of triglycerides to cholesteryl esters (mass ratio <1.6) and relatively small particle diameter (<60 nm).

The cores of human serum very low density lipoproteins (VLDL)[1] are enriched in triglycerides, whereas those of high-density lipoproteins (HDL) and low-density lipoproteins (LDL) chiefly contain cholesteryl esters (Skipski, 1972). LDL undergo a reversible thermal transition around body temperature, reflecting a change in the physical state of their core cholesteryl esters from an ordered liquid-crystalline phase to a disordered liquid phase, as shown by studies of differential scanning calorimetry, X-ray scattering, polarizing microscopy, nuclear magnetic resonance spectroscopy, and a β-carotene circular dichroism (CD) probe technique (Deckelbaum et al., 1975, 1977a; Sears et al., 1976; Atkinson et al., 1977; Laggner et al., 1977; Kroon, 1981; Chen & Kane, 1974). Unlike LDL, normal human VLDL and HDL do not undergo similar thermal changes in the physical state of their core cholesteryl esters (Deckelbaum et al., 1977b; Tall et al., 1977b).

A change in the physical state of the cores in lipoproteins is greatly influenced by the composition of the core lipids and by the diameter of the lipoprotein particles (Deckelbaum et al., 1977a; Laggner et al., 1977; Tall et al., 1977b; Tall, 1980). The transition temperature depends on the ratio of triglycerides to cholesteryl esters and on the degree of saturation of cholesteryl ester fatty acids (Deckelbaum et al., 1977a; Pownall et al., 1980a,b; Tall et al., 1977a, 1978; Kirchhausen et al., 1979; Kroon & Seidenberg, 1982). Thus, the absence of a transition in normal human VLDL has been attributed to their high content of triglycerides, indicating that the core cholesteryl esters are dissolved in the liquid triglycerides (Deckelbaum et al., 1977b). Furthermore, studies of the physical state of the cores in cholesteryl ester rich lipoproteins from various species have shown that their core cholesteryl esters can undergo an ordered/disordered state transition if the particle diameter is greater than 14 nm (Deckelbaum et al., 1977a; Laggner et al., 1977; Tall, 1980). Thus, the absence of a transition in human HDL has been attributed to their diameter, which is too small to accommodate the formation of an ordered state of cholesteryl esters in the cores (Tall et al., 1977b; Tall, 1980), or the domain of cholesteryl esters in HDL is too small to exhibit cooperative melting. It is not yet known whether the core cholesteryl esters in some triglyceride-rich

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[1] Abbreviations: VLDL, very low density lipoproteins; HDL, high-density lipoproteins; LDL, low-density lipoproteins; IDL, intermediate-density lipoproteins; CD, circular dichroism; EDTA, ethylenediaminetetraacetic acid.

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