Biochimica et Biophysica Acta 859 (1986) 143–150
Elsevier

BBA 73149

The modulating effects of lipids on purified rat liver Golgi galactosyltransferase

P.E. Clark and M.A. Moscarello

Research Institute, The Hospital for Sick Children, Toronto, Ontario M5G 1X8 (Canada)

(Received January 23rd, 1986)

Key words: Galactosyltransferase; Lipid-protein interaction; Enzyme activity modulation; (Rat liver)

Galactosyltransferase was purified from rat liver Golgi membranes. The Triton X-100, used to solubilize the enzyme was removed immediately prior to the lipid interaction studies. In lipid vesicles, prepared from a variety of phosphatidylcholines (PCs), including egg PC, DOPC, DMPC, DPPC and DSPC, the ability of the lipids to stimulate the enzyme decreased in the order egg PC > DOPC > DMPC > DPPC > DSPC, i.e. the lower the transition temperature (T_c) the greater the stimulation of the enzyme. A second, neutral lipid, phosphatidylethanolamine was used to permit a comparison of the effect of a different head group of the same net charge at neutral pH. The PEs included, egg PE, soy PE, Pl-PE, PE(PC) and DPPE in order of increasing T_c. The effect of the PEs was opposite to that of the PCs, i.e. the higher the T_c, the greater the stimulation of the enzyme. In fact egg PE and soy PE which have the lowest T_c values were inhibitory. Thus the modulation of the Golgi membrane galactosyltransferase by these lipids was different from that reported earlier for the bovine milk galactosyltransferase. The effects of two acidic lipids, egg phosphatidic acid (PA) and egg phosphatidylglycerol (PG) were studied also. Both totally inhibited the enzyme even at low concentrations of lipid, however, the PA was more effective than PG. In mixtures of neutral lipid (PC) and acidic lipid (PA or PG), the effect of the acidic lipid dominated. Even in the presence of excess PC, total inhibition of the enzyme was observed. It was concluded that the enzyme bound the acidic lipid preferentially to itself. The choice of the lipids allowed us to make several direct comparisons concerning the effect of the nature of the lipid head group on the activity of the enzyme. For example PE(PC), egg PA and egg PG would have fatty acid chains identical to egg PC since these three lipids are all prepared by modification of egg PC. As well, DPPE differs from DPPC only by nature of the head group. These comparisons indicated that not only the net charge but also chemical nature of the head group were important in the lipid modulation of Golgi galactosyltransferase.

Introduction

The enzyme UDP galactose: *N*-acetylglucosamine β-1,4 galactosyltransferase catalyses the transfer of galactose from UDP-Gal to a suitable acceptor. In vivo this is a terminal GlcNAc residue on an N-linked oligosaccharide of a glyco-

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; PC, phosphatidylglycerol; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; soy PE, soybean PE; PE (PC), PE prepared by transphosphatidylation of egg PC; Pl-PE, plasmalogen PE; DPPE, dipalmitoylphosphatidylethanolamine; GlcNAc, *N*-acetyl-D-glucosamine.

protein [1]. In vitro it may transfer a galactose to free GlcNAc forming *N*-acetyllactosamine or to the terminal GlcNAc of a glycoprotein [1].

The control of the activity of this and other glycosyltransferases has been studied extensively at the substrate level and has been reviewed recently [2]. In most cases these studies have been carried out with solubilized (some purified) preparations of the enzymes. With such preparations most of the detailed enzymology of these reactions has been elucidated. Since the glycosyltransferases of the Golgi complex are membrane-bound enzymes, they function in a lipid-rich environment. It can be expected that lipids will exert a regulatory effect on these enzymes quite apart from those observed at the substrate level.

The lipid modulation of a number of enzymes, including bovine milk galactosyltransferase, a soluble galactosyltransferase of the mammary gland has been described [3,4]. Neutral lipids, such as egg PC stimulated the activity, while acidic lipids such as egg PA inhibited the activity. In mixtures of lipids the effect of the acidic lipid dominated over that of the neutral lipid. These studies emphasized the dominant role of the head group in modulating the activity of bovine milk galactosyltransferase although the nature of the fatty acyl chains had an effect also, since the activity decreased with increasing chain length and saturation.

Although this enzyme carries out its function in the lipid-rich environment of milk, it does not require detergents for solubilization and is readily incorporated into lipid vesicles. On the other hand the galactosyltransferase from rat liver Golgi membranes requires Triton X-100 for solubilization. In addition detergent is required for stabilization since the purified enzyme loses activity rapidly in its absence. Therefore, before studies of lipid modulation could be undertaken, it was necessary to remove the detergent while maintaining enzyme activity enabling the incorporation of active enzyme into lipid vesicles. This has been achieved with the galactosyltransferase of Golgi membranes purified in the presence of Triton X-100.

In this report we describe the effects on enzyme activity when galactosyltransferase free of Triton X-100 was incorporated into lipid vesicles. The results demonstrated that the Golgi enzyme was influenced in a different manner by various lipids than the bovine milk galactosyltransferase [4].

Materials and Methods

Isolation of galactosyltransferase from rat liver Golgi membranes. The Golgi membranes prepared from 15 male Wistar rats were suspended in 70 ml equilibrating buffer (15 mM sodium cacodylate (pH 7.5), containing 0.2% Triton X-100, 5 mM *N*-acetylglucosamine and 3 mM 2-mercaptoethanol). The suspended membranes were sonicated for 1 min using the Brinkman probe sonicator at the lowest setting and left to solubilize at 4°C overnight. The supernatant obtained after centrifuging at $155\,000 \times g$ for 60 min at 4°C in the Beckman Model L centrifuge was loaded on to an affinity column of α-lactalbumin coupled to agarose at a rate of 4 ml/h overnight at 4°C. The column was washed further with 50 ml of the equilibrating buffer followed by 50 ml of buffer (15 mM sodium cacodylate (pH 7.5), containing 0.1% Triton X-100, 5 mM *N*-acetylglucosamine, 0.1 M sodium chloride and 3 mM 2-mercaptoethanol). The enzyme activity was eluted from the column with eluting buffer (15 mM sodium cacodylate (pH 7.5), containing 0.1% Triton X-100, 0.1 M NaCl and 3 mM 2-mercaptoethanol). The active fractions were pooled and concentrated by Amicon ultrafiltration using an Amicon YM10 filter of 25 mM diameter. The concentrated enzyme was stored at 4°C. The average purification factor was 2000. The yield of galactosyltransferase was 20.8% of that of the Golgi membranes. The average final specific activity of the enzyme was 0.291 units per mg of protein, where one unit is equal to 1 μmol galactose transferred per min at 37°C.

Preparation of liposomes. Lipids, dissolved in chloroform were added to a large thin-walled test tube containing 200 μl of chloroform. After mixing the lipids were shell-dried under a stream of nitrogen. For phosphatidylethanolamine (PE) vesicles, 500 μl of 1% sodium tetraborate buffer (pH 9.2), was routinely added to the tube. Under these conditions PE assumes hexagonal (H_{II}) phase [5]. All other lipid preparations were mixed with 500 μl of 0.05 M Mes buffer (pH 7.4). After

addition of the appropriate buffer the suspensions were sonicated in a Bransonic 220 bath sonifier until opalescent. The bath temperature was maintained above the transition temperature (T_c) of the lipid. The liposomes made up in the Mes buffer were used immediately after sonication but the PE preparations were dialysed overnight against two changes of 200 ml of 0.05 M Mes buffer (pH 7.4). After dialysis these lipid preparations were vortexed and sonicated briefly before use. The liposomes were flushed with nitrogen during all stages of preparation. Detergent-free Golgi galactosyltransferase (60 μl, containing approx. 0.40 μg of protein) was added directly to 60 μl of vesicles. The lipid concentration was computed from the lipid phosphorus measured by the method of Bartlett [6].

Removal of Triton X-100 by Exacti-Gel D. Golgi membrane galactosyltransferase, purified as described above [7] was applied to an Extracti-Gel D column (1 × 0.8 cm). A typical elution profile is shown in Fig. 1. The enzyme was applied in 15 mM sodium cacodylate buffer (pH 7.5) containing 3 mM 2-mercaptoethanol, 0.1 M NaCl and 0.1% Triton X-100. The enzyme was washed through with 0.05 M Mes buffer (pH 7.4). The enzyme activity was eluted in the void volume with the majority of the applied protein. Triton X-100 was eluted with the ethanol and butanol washes (2.6–2.8 ml). Although most of the protein was recovered in the non adsorbed fraction, only 12% of the enzyme activity was recovered. This loss of activity was attributed to irreversible denaturation on the column. The recovered enzyme was stimulated in the usual manner by re-addition of Triton X-100 demonstrating that it behaved in the same manner as the original enzyme. The enzyme activity after Extracti-Gel D, treatment was unstable in the absence of Triton X-100 making it necessary to carry out this procedure just prior to the addition to the liposomes.

The galactosyltransferase assay. The galactosyltransferase assay was performed as described previously [4]. The assay mixture contained 100 mM Mes buffer (pH 7.4), 10 mM $MnCl_2$, 20 mM *N*-acetylglucosamine and 1 mM UDP galactose containing [^{14}C]galactose (10^5 dpm), 30 μl liposomes in a total volume of 50 μl and 100 ng of enzyme. After incubation at 37°C for 30 min, the

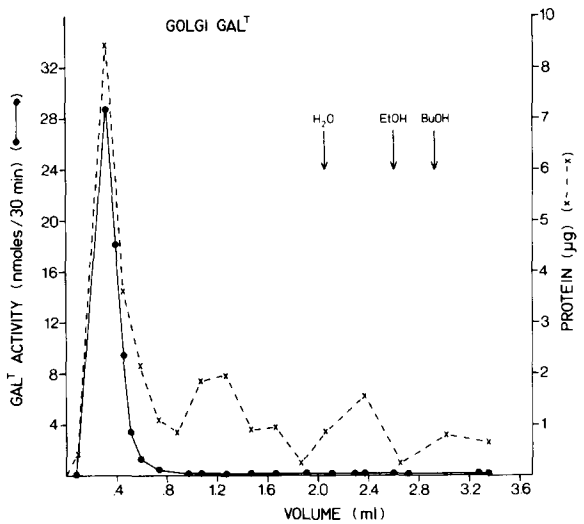

Fig. 1. The removal of Triton X-100 from purified rat liver Golgi membrane galactosyltransferase on Extracti-Gel D. Galactosyltransferase activity, O——O; protein, ×- - - - - ×. Triton X-100 was eluted with the ethanol and butanol washes.

reaction was stopped with the addition of 0.25 M EDTA in 2% sodium borate. The reaction mixture was chromatographed on column of AG-1X8 (100–200 mesh) in a Pasteur pipette (4 cm of gel column). The *N*-acetyl[^{14}C]lactosamine was eluted with 3.0 ml of distilled water directly into scintillation vials, 10 ml of ACS scintillation fluid was added and the vials were counted in Tracor Analytic Betatrac 6895 scintillation counter.

Phosphorus was measured by the method of Bartlett [6]. Protein concentrations were measured by the method of Peterson [8] using bovine serum albumin as a standard.

Materials. The lipids were purchased from Avanti Biochemicals, Birmingham, AL. They included egg PC, L-α-DMPC, L-α-DPPC, L-α-DSPC, L-α-DOPC, soy PE, egg PE, plasmalogen PE, PE produced by transphosphatidylation of egg PC, DPPE, L-α-PA, egg PG. The Mes buffer and UDP galactose were purchased from Boehringer Mannheim. ACS scintillation fluid was purchased from Amersham International. Uridine diphosphate [^{14}C]galactose (spec. act. 302 mCi/mmol) was purchased from New England Nuclear. Extracti Gel-D was obtained from Pierce, Rockwood, IL.

Results

The effect of various phosphatidylcholines on the activity of rat liver Golgi galactosyltransferase

Rat liver galactosyltransferase is a membrane-bound enzyme of the Golgi complex. A number of phospholipids, especially phosphatidylcholines are present in its natural environment [9–11]. Thus the effects of phosphatidylcholine on the enzyme in vitro are of importance for an understanding of the mechanisms of lipid modulation. In these studies several phosphatidylcholines of increasing fatty acyl chain lengths from 14–18 carbons were used. These included DMPC, DPPC, and DSPC. The effects of a single unsaturated, synthetic PC (DOPC) and the natural egg PC of mixed fatty acyl composition were also studied. Except for egg PC, they all have defined transition temperatures.

Typical lipid activation curves are shown in Fig. 2. The galactosyltransferase activity is plotted as a percentage of the change in the presence of lipid compared to the activity of the enzyme in the absence of lipid. In all cases, the PCs stimulated the activity of the enzyme at low lipid concentration. Only the longer chain length PCs, DPPC and DSPC inhibited the activity at high lipid concentrations. For ease of comparison the data are collected in Table I. These data demonstrate that maximum stimulation was observed at higher lipid concentrations as the T_c of the lipid decreased, implying greater interaction of the lipids of low T_c with the enzyme.

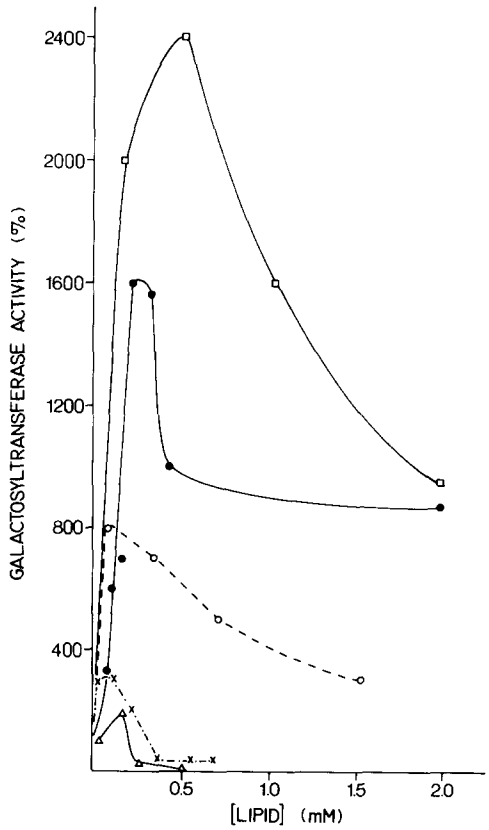

Fig. 2. The effect of increasing concentrations of various phosphatidylcholines on the activity of rat liver Golgi membrane galactosyltransferase. Each assay contained 100 ng of enzyme. DSPC, △———△; DPPC, ×–·–×; DMPC, - - - - - -; DOPC, ————; egg PC, □———□. The activity of the galactosyltransferase is expressed as a percent of the activity in the absence of lipid.

The effect of various phosphatidylethanolamines on rat liver Golgi galactosyltransferase

Phosphatidylethanolamine (PE) represents a second major phospholipid of the Golgi membranes [9–11]. It differs in several aspects from PC. It has a smaller head group since the amino group is not methylated. Because of this smaller head group, it can, in principle, adopt a non-bilayer phase (hexagonal or H_{II}) in aqueous solutions. A number of PEs were studied including soy, plasmalogen, egg, PE obtained by transphosphatidylation of PC and diplamitoyl PE. The temperatures at which the gel to liquid crystalline (T_c) and lamellar to hexagonal (T_H) occur are known for most of these lipids. The results obtained with the various PEs were markedly different from those with PC since the correlation with T_c was opposite that observed with the PCs. These data are shown in Fig. 3A. Soy PE inhibited the activity of the enzyme at all concentrations. In the presence of 0.5 mM lipid the activity was about 20% of the control. Plasmalogen PE had little effect on the enzyme below 0.2 mM but inhibited the enzyme at higher concentrations. At 0.5 mM the activity of the enzyme was about 50% of the enzyme in the absence of lipid. Egg PE inhibited at all concentrations but only to about 60% of the control. These three lipids were in liquid-crystalline phase at the temperature of assay and all were above the lamellar to hexagonal phase transitions. These temperatures are shown in Fig. 3B.

TABLE I

THE EFFECT OF VARIOUS PHOSPHATIDYLCHOLINES ON RAT LIVER GOLGI GALACTOSYLTRANSFERASE

In all cases the lipid vesicles were prepared and the detergent-free enzyme was added to aliquots of the liposomes. After 5 min, the galactosyltransferase activity was measured as described in Materials and Methods, after incubation at 37°C for 30 min. The activity was expressed as nmoles of galactose transferred to *N*-acetyl-D-glucosamine in 30 min. The percent increase of activity was calculated relative to the activity in the absence of lipid.

Lipid	T_c (°C)	$\frac{\mu M \text{ Lipid}}{\mu M \text{ E}^*}$ at peak	% Stimulation at peak
DMPC	23	$3 \cdot 10^3$	780
DPPC	41	$2 \cdot 10^3$	320
DSPC	58	$1 \cdot 10^3$	200
DOPC	−22	$7 \cdot 10^3$	1600
Egg PC	unknown	$20 \cdot 10^3$	2400

* The molecular weight of the enzyme was estimated to be 50000.

The effect of PE formed by transphosphatidylation from PC was different. The lipid stimulated the activity of the enzyme to 280% of the lipid free enzyme at 0.25 mM lipid. The activity was less at higher concentrations of lipid and at 1.1 mM the activity was the same as that of the lipid free enzyme (data not shown).

The effect of DPPE was noteworthy. Stimula-

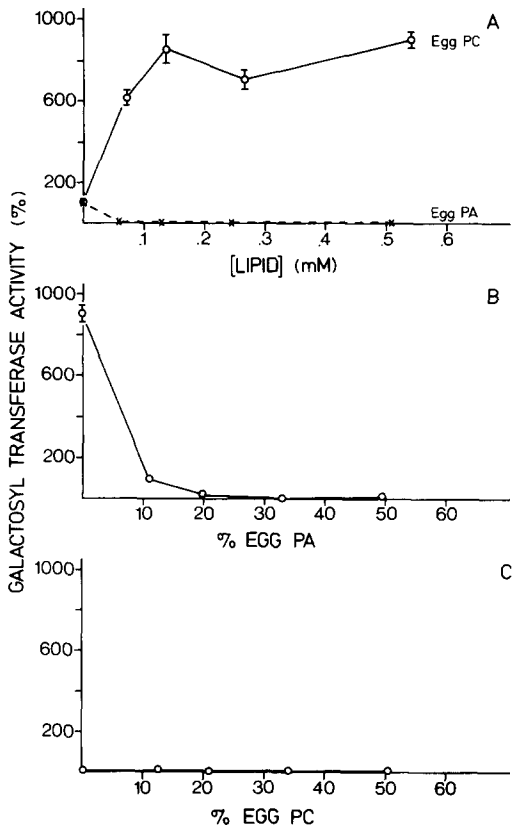

Fig. 4. The effect of an acidic lipid (PA) alone and in mixtures with PC on the activity of rat liver Golgi membrane galactosyltransferase. Each assay contained 100 ng of enzyme. (A) The effect of egg PC alone and egg PA alone on the activity of the enzyme. (B) In the presence of maximal egg PC (0.54 mM), increasing amounts of egg PA were added up to 50% (w/w). (C) In the presence of maximal egg PA (0.54 mM) increasing amounts of egg PC were added to a maximum of 50% (w/w).

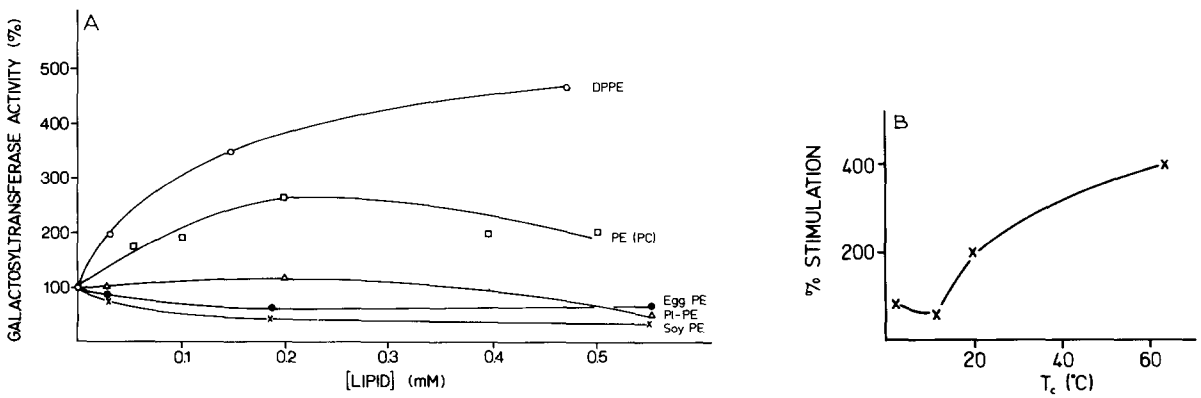

Fig. 3. The effect of increasing concentrations of various phosphatidylethanolamines on the activity of purified rat liver Golgi membrane galactosyltransferase (A). Each assay contained 100 ng of enzyme. DPPE, ○———○; PE (PC), □———□; egg PE ●———●; PI-PE, △———△; soy PE, × ———— ×. (B) The relationship between T_c of the lipid and the activity of the enzyme.

tion of enzyme activity was observed at all concentrations to a maximum of 400% at 0.45 mM. The percent stimulation of enzyme activity increased with increasing lipid transition temperature, in contrast to the results obtained with the various PCs (Table I).

The effect of mixtures of acidic lipids with PC on the activity of rat liver Golgi galactosyltransferase

The effects of two acidic lipids egg PA and egg PC alone and in combination with egg PC were studied to determine if the acidic lipids which strongly inhibit the enzyme dominate in a mixture of lipids. When the enzyme was added to vesicles of egg PC, the expected stimulation of activity was observed. In vesicles of egg PA total inhibition of enzyme activity was observed (Fig. 4A) even at low lipid concentrations. When increasing amounts of egg PA was added to egg PC total inhibition of enzyme activity was found at 20% PA (0.11 mM egg PA in 0.54 mM egg PC). When PC was added to vesicles of egg PA only the inhibitory effect of PA was seen, i.e. PC was not able to stimulate the enzyme probably because it was unable to displace PA from the enzyme (Fig. 4C). When egg PG was used instead of PA, similar results were obtained (Fig. 5), i.e. egg PG alone totally inhibited the enzyme. Addition of egg PG to PC vesicles showed that the effect of PG dominated, totally inhibiting the enzyme at 30% PG (a significantly higher concentration than required for egg PA). Addition of PC to egg PG failed to reverse the inhibition, as demonstrated for egg PA (Fig. 4).

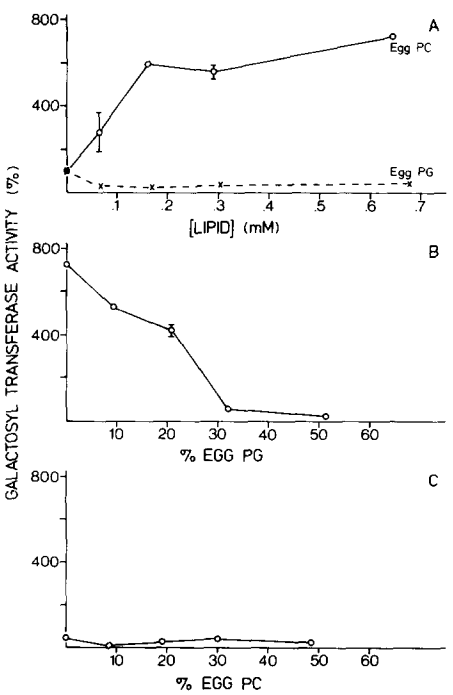

Fig. 5. The effect of PG alone and in mixtures with PC on the activity of the rat liver Golgi membrane galactosyltransferase. Each assay contained 100 ng of enzyme. (A) The effect of egg PC alone and egg PG alone on the activity of the enzyme. (B) In the presence of maximal amounts of egg PC (0.54 mM), increasing amounts of egg PG were added to a maximum of 50% (w/w). (C) In the presence of maximal amounts of egg PG (0.54 mM), increasing amounts of egg PC were added to a maximum of 50% (w/w).

Discussion

The biosynthesis of asparagine-linked oligosaccharides involves several distinct stages including the en bloc transfer of the lipid linked oligosaccharide in the rough endoplasmic reticulum, processing of the oligosaccharide by glycosidases and the synthesis of the final oligosaccharide in the Golgi complex by the various glycosyltransferases. The enzymological reactions required to produce the final oligosaccharide structure have been largely elucidated [1]. The isolation and purification of glycosyltransferases has been of considerable importance although crude enzyme preparations have contributed significantly to these studies. Substrate level control has been elucidated with these preparations. An extensive discussion has been published recently [2].

The glycosyltransferases are membrane-bound enzymes of the Golgi complex and must function in the lipid-rich environment of the bilayer. Therefore the nature of the lipid in the immediate environment of the enzyme most probably exerts some regulatory control over the activity of the enzyme. In earlier studies lysoPC and lysoPA were shown to stimulate and inhibit, respectively, the galactosyltransferase activity of rat liver microsomal membranes [12]. In a further study PI and PS were shown to inhibit lysoPC and Triton X-100 solubilized galactosyltransferase [13]. The interpretation of lipid effects on such detergent solubilized membranes is complex because of the pres-

ence of both detergent and lipid in the same mixture. Recently the effects of detergents and lysophosphatides on α-2,3 sialyltransferase activity have been reported [14]. Lysophosphatides with acidic head groups were inhibitory to both forms A and B of the enzyme from porcine submaxillary gland.

In several earlier studies the lipid-modulation of bovine milk galactosyltransferase was investigated [3,4,15]. This enzyme was chosen since it could be obtained readily in soluble form in the absence of detergents allowing unambiguous interpretation of the effects of added lipids. These results provided a basis for the present work in which lipid effects on purified Golgi membrane galactosyltransferase are reported. Significant differences in the effects of lipids on the Golgi enzyme compared to the milk enzyme were observed. The relative stimulation for a series of PCs was DOPC > egg PC > DMPC > DPPC > DSPC with a maximum increase of 640% for the milk enzyme in DOPC vesicles [4]. The order of stimulation for the Golgi enzyme was egg PC > DOPC > DMPC > DPPC > DSPC with a maximum stimulation of 2400% for egg PC. While only 4% of the enzyme activity was observed in DSPC vesicles with the milk enzyme which represented almost total inhibition, the Golgi enzyme was stimulated by about 200%.

The most dramatic difference in the response to lipids of the two enzymes (bovine milk and rat liver Golgi galactosyltransferase) was observed with the phosphatidylethanolamines. The order of stimulation for the Golgi enzyme was DPPE > PE(PC) > Pl-PE > Egg PE > Soy PE which is the reverse of the order observed with bovine milk galactosyltransferase [16]. PE, prepared by transphosphatidylation from egg PC, stimulated much less than egg PC. Since the fatty acyl chains of these two are identical, the head group must be responsible for the difference in activity observed. Both lipids were in liquid crystalline phase at the temperature of assay since the T_c for egg PC was below 9°C while that for PE (PC) was 19.8°C.

The dominant role of the lipid head group is further emphasized when the effects of DPPE are compared to those of DPPC. In this case both lipids, DPPE and DPPC, are in gel phase at the temperature of assay. DPPC stimulated the enzyme only at 0.1 μM and inhibited at higher concentrations of lipid. DPPE continued to stimulate the enzyme at concentrations in excess of 0.1 mM and had not attained maximal values even at 0.5 mM. At 0.5 mM, DPPC almost totally inhibited the enzyme. These data emphasize the important role of the chemical nature of the head group since both DPPC and DPPE have net charges of zero at pH 7.4.

The dominant role of the lipid head group was demonstrated further by the studies with egg PG, egg PA and PE (PC) all of which were derived by chemical modification of the head group of the egg PC, so that in all lipids the fatty acyl chain compositions were identical. Both egg PC and PE (PC) stimulated the galactosyltransferase while egg PA and egg PG both inhibited the activity of the enzyme. Egg PA which has a greater negative charge than egg PG inhibited the enzyme totally while egg PG inhibited the enzyme to 34%. In mixtures of egg PC and egg PA (or egg PG), the effect of the acidic lipid dominated emphasizing the importance of the chemical nature of the lipid head group.

These studies of lipid modulation of the Golgi membrane galactosyltransferase demonstrated that the lipid effects on this enzyme are quite distinct from those observed previously for the bovine milk galactosyltransferase. Although the two enzymes carry out similar reactions (in vitro at least) the different responses to lipids implies differences in primary or secondary structures which affect the binding properties of the lipids. Elucidation of the primary structures of these enzymes represents an important future study.

Acknowledgements

This work was supported by an operating grant from the Medical Research Council of Canada (MA-8679). Financial support in the form of an Ontario Graduate Scholarship to one of us (P.E.C.) during the graduate program is greatly appreciated. The technical assistance of Mrs. L. Kashuba and the helpful comments of Mrs. M. Mitranic are acknowledged.

References

1 Kornfeld, R. and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664
2 Schachter, H. (1984) Biol. Cell 51, 133–146
3 Paquet, M.R. and Moscarello, M.A. (1986) in Membrane Fluidity and Biology: Cellular Aspects and Disease Processes (Aloia, R. and Boggs, J.M., eds.), Vol. 4, pp. 209–246, Academic Press, New York
4 Mitranic, M.M., Boggs, J.M. and Moscarello, M.A. (1983) J. Biol. Chem. 258, 8630–8636
5 Cullis, P.R., Hope, M.M., De Kruijff, B., Verkleij, A.J. and Tilcock, C.A.S. (1985) in Phospholipids and Cellular Regulation (Kuo, J.F., ed.), CRC Press, Boca Raton
6 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
7 Paquet, M.R. and Moscarello, M.A. (1984) Biochem. J. 218, 745–751
8 Peterson, G.L. (1977) Anal. Biochem. 83, 346–356
9 Keenan, T. and Morré, D. (1970) Biochemistry 9, 19–25
10 Glaumann, H. (1975) J. Cell Biol. 64, 356–377
11 Zambrono, F. (1975) Biochim. Biophys. Acta 380, 357–369
12 Mookerjea, S. (1979) Can. J. Biochem. 57, 66–71
13 Ratnam, S., Fraser, I.H. and Mookerjea, S. (1980) Can. J. Biochem. 58, 58–66
14 Westcott, K.R., Wolf, C.C. and Hill, R.L. (1985) J. Biol. Chem. 260, 13109–13115
15 Mitranic, M.M. and Moscarello, M.A. (1980) Can. J. Biochem. 58, 809–814
16 Mitranic, M.M. and Moscarello, M.A. (1986) Carbohydr. Res., in the press