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THE MODULATION OF BOVINE MILK D-GALACTOSYLTRANSFERASE BY VARIOUS PHOSPHATIDYLETHANOLAMINES

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ABSTRACT

To investigate the possible role of nonbilayer phases in the modulation of glycosyltransferase activity, bovine milk D-galactosyltransferase has been studied in phosphatidylethanolamine (PE)† membranes, including soybean PE, egg PE, PE prepared by transphosphatidylation of egg PC, bovine brain PE, plasmalogen PE, and DPPE. The gel-to-liquid crystalline transition (T_C) and the lamellar-to-hexagonal transitions (T_H) are known for most of the PE compounds. The lower the T_C (or T_H) value, the greater the stimulation of galactosyltransferase activity in both the lactose- and *N*-acetyllactosamine-synthetase reactions. No correlation was found between either T_C or T_H value and the break in the Arrhenius plots for the *N*-acetyllactosamine synthetase. In membranes consisting of mixtures of PE with PC, the dominant effect was that of PC. The stimulation of activity in the mixed-lipid systems was never greater than that produced by PC alone, therefore the enzyme showed a definite preference for PC in the mixtures.

INTRODUCTION

While the effects of lipid head group and fatty acyl chain composition on modulating the activities of several, membrane-bound and non-membrane-bound enzymes have been documented[1–3], the role of lipid-phase state is only beginning to be studied. In this context, the "soluble" glycosyltransferases of body fluids, such as milk, serum, and ascites fluid (so-called because detergents are not required for either their solubilization or stabilization) are of interest. The lipid requirements of bovine milk galactosyltransferase (milk Gal^T) have been reported recently[4]; neutral lipids such as PC stimulated the activity of the enzyme, while it was inhibited by the negatively charged lipids, PA and PS. In mixtures of PC and PA, the effects of PA dominated. Although the nature and charge on the lipid head group

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†Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; PA, phosphatidic acid; PS, phosphatidylserine.

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clearly played a role in the modulation of enzyme activity, the possible influence of the phase state of the lipid bilayer remained to be examined.

Mannosyltransferase II, purified from the rabbit liver microsomal fraction[5], catalyzes the synthesis of an α-D-Man*p*-(1→3)-Man linkage. More specifically, the enzyme catalyzes the transfer of a D-mannosyl group from GDP-D-mannose to an oligosaccharide-lipid intermediate at an early stage in the biosynthesis of asparagine-linked glycoproteins. A study of the lipid requirements of this enzyme showed that maximal activity was obtained in the presence of PE containing unsaturated acyl chains[6]. Since PE is known to form hexagonal (H_{II}) phases[7,8], a role for nonbilayer phases in the activation of mannosyltransferase II and dolichol-*P*-Man synthetase was postulated for these two enzymes[9]. Evidence from ^{31}P-n.m.r. spectroscopy demonstrated that the plant PE could indeed adopt the hexagonal phase under appropriate conditions[10]. However, since the solution conditions for the enzyme assays were different from those for the ^{31}P-n.m.r. studies, it remained to be demonstrated directly that hexagonal phase PE was present during the enzyme assays[11]. These circumstances are particularly important in the mannosyltransferase studies[9], where the PE used was obtained from bovine brain for which neither a gel–liquid crystalline transition nor a lamellar–hexagonal phase-transition temperature is known. In the absence of transition temperature data, it is critical to establish the nature of the phase present when mixtures of lipids are used, especially since phases "intermediate" between lamellar and hexagonal can occur[10,11].

In the present report, we have extended our studies on the effects of lipids on the activity of bovine milk D-galactosyltransferase to a systematic study of a number of PE compounds, of both plasmalogen and nonplasmalogen types. For many of these lipids, both the gel-liquid, crystalline transition temperature (T_C) and the temperature (T_H) at which the lamellar is converted into the hexagonal (H_{II}) phase is known, so that one can infer the phase state of the lipid at the temperature of assay. Since this enzyme transfers to both D-glucose and *N*-acetylglucosamine units depending on whether or not α-lactalbumin is present in the assay, we examined the effects of the various PE compounds on both lactose- and lactosamine-synthetase activities. Our data suggest that nonbilayer PE phases may play an important role in modulating the activity of milk galactosyltransferase, although it was not possible ultimately to correlate this modulation specifically with the presence of hexagonal phase.

EXPERIMENTAL

Materials. — Uridine diphosphate-D-[^{14}C]galactose (sp. act., 11.2 GBq/nmol) was purchased from New England Nuclear, Boston MA 02118. Bovine milk galactosyltransferase, α-lactalbumin, and MES buffer [2(*N*-morpholino)ethanesulfonic acid] were purchased from Sigma Chemical Co., St. Louis, MO 63178. Dowex 1-X8 anion-exchange resin was obtained from BioRad Laboratories, Richmond, CA 94804. Lipids were obtained from Avanti Polar Lipids, Inc.,

Birmingham, AL 32516, and included PE isolated from bovine brain, soybean PE, egg PE, PE prepared by transphosphatidylation of egg lecithin [PE(PC)], plasmalogen PE from bovine brain, egg and bovine heart phosphatidylcholines, and synthetic DPPE.

Preparation of liposomes and lipid mixtures. — Lipids were received in the lyophilized form and were reconstituted in chloroform to give a stock solution of 10 mg/mL. For preparation of liposomes, the lipid (1.2 μmol) was dried under N_2 in a shell onto the bottom of a test tube, and $Na_2B_4O_7$ (1 mL of 1% solution at pH 9.2) was added. Sonication was performed in a Bransonic bath-type sonicator until the solution was clear or slightly opalescent, by the procedure of Stollery and Vail[12]. The liposomes were then dialyzed overnight against two changes of 0.05M MES buffer, pH 7.4, (200 mL) containing 10mM NaCl.

After dialysis, the liposomes were returned to a wide-bottom tube and sonicated briefly before removing aliquots for the lactose- and lactosamine-synthetase assays, and for the determination of P by the method of Bartlett[13]. In some experiments, the lipid was prepared by direct dispersion at pH 7.4 without dialysis in the buffer used for enzyme assay *(vide infra)*.

To prepare the lipid mixtures, one of the lipids was maintained at a proportion of 1.2 μmol while the proportion of the second was varied from 0.12 to 1.2 μmol, so that the concentration of the second lipid was 9, 17, 33, and 50% of the total lipid. The combined lipids were mixed in chloroform and dried in a shell around the bottom of a glass test tube under a stream of N_2. The vesicles were then prepared as described earlier.

Assays of enzymes. — Milk galactosyltransferase was added to the liposomes at ratios that varied between 800:1 to 12 000:1 of lipid to enzyme. The assays contained 2–5 pmol of milk Gal^T. Lactosaminesynthetase activity was measured under the following conditions. The assay mixture contained 10mM MES buffer, pH 7.4, 10mM $MnCl_2$, mM UDP-galactose containing UDP-D-[^{14}C]galactose (10^5 d.p.m.), and 20mM *N*-acetylglucosamine. To this mixture were added liposomes (25 μL) and water (5 μL) to a final volume of 50 μL. After incubation at 37° for 30 min, the reaction was stopped by the addition of mM EDTA, pH 7.2 (1 mL).

Lactosesynthetase activity was assayed by adapting the methods of Fitzgerald *et al.*[14] and Khatra *et al.*[15]. The reaction mixture contained bovine milk galactosyltransferase (2–5 pmol), 20mM D-glucose, mM UDP-galactose containing UDP-D-[^{14}C]galactose (10^5 d.p.m.), 10mM $MnCl_2$, 100mM MES buffer (pH 7.4), and bovine α-lactalbumin (50 μg) in a total volume of 50 μL. The standard assay was incubated for 30 min at 37°. The reaction was stopped by the addition of mM EDTA, pH 7.2 (1 mL).

The products of the two assays, *N*-acetyl-[^{14}C]lactosamine and [^{14}C]lactose were separated from UDP-[^{14}C]galactose by passing the reaction mixture through a column of Dowex 1-X8 (Cl^-) anion-exchange resin prepared in a Pasteur pipette. The column was washed with distilled water (3 mL) and a total of 4 mL containing the product was collected directly into plastic scintillation vials. After adding ACS

counting fluid (10 mL), the samples were counted in a Tracor Analytic, Betatrac liquid-scintillation counter 6895. The results are reported as nmol of galactose transferred/30 min at 37°.

For the determination of Arrhenius plots, lactosaminesynthetase activity was measured at various temperatures from 9 to 50°. Bath temperatures were controlled by a Braun circulating pump. Incubation times were 5 min. Activation energies were determined by equation *1*, where *Ea* represents the Arrhenius activation energy, k_1 and k_2 are rate constants at absolute temperatures T_1 and T_2 (ref. 16).

$$Ea = \frac{4.56 \cdot T_1 \cdot T_2 \cdot (\log k_2 - \log k_1)}{T_2 - T_1} \qquad (1)$$

RESULTS

The effect of lipid concentration on galactosyltransferase activity in various phosphatidylethanolamines. — The activation of the enzyme by various PE compounds, including soybean PE, plasmalogen PE, bovine brain PE, egg PE, PE formed from PC by transphosphatidylation [PE(PC)], and dipalmitoylphosphatidyl PE (DPPE), was studied and the (lamellar) gel-to-liquid, crystalline transition temperatures (T_C) and the lamellar-to-hexagonal transition temperatures (T_H) of these lipids are reported in Table I (see ref. 17).

PE(PC) is in liquid-crystalline lamellar phase at 37°, with its T_C and T_H at 19.8 and 63°, respectively (Table I). The effect of increasing concentrations of this PE on both the lactose- and *N*-acetyllactosamine-synthetase reactions is shown in

TABLE I

THE EFFECT OF VARIOUS LIPIDS ON LACTOSE AND *N*-ACETYLLACTOSAMINESYNTHETASE ACTIVITIES OF BOVINE MILK GALACTOSYLTRANSFERASE[a]

Lipid	T_C *(degrees)*	T_H *(degrees)*	*Lactosesynthetase*		N-*Acetyllactosamine-synthetase*	
			Activity[b]	*Control (%)*	*Activity*	*Control (%)*
Soybean PE	[c]	−30	18.18	153	14.12	665
Plasmologen PE	3	18	14.66	123	7.27	343
Bovine brain PE			9.66	81	6.55	309
Egg PE	11.3	32–45	9.86	83	5.97	281
PE(PC)	19.8	63	9.18	77	4.47	211
DPPE	63.7		2.91	24	0.03	2
Plasmalogen PC (bovine heart)			23.86	201	24.74	1166
Egg PC			14.16	119	13.51	637
Control (no lipid)			11.89	100	2.12	100

[a]Each assay contained 12.5 nmol of lipid per 2.4 pmol of enzyme (lipid-to-enzyme ratio 5200:1). See Experimental section for further details. [b]Activity = nmol of D-galactose transferred · 30 min^{-1} at 37°. [c]Not determined.

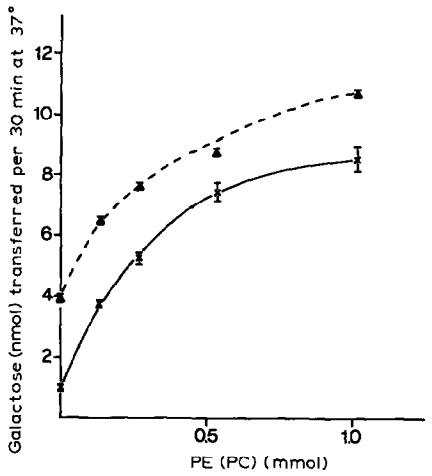

Fig. 1. Activity of milk galactosyltransferase in the presence of liposomes of PE prepared from egg PC [PE(PC)]. Liposomes were prepared by dialysis as described in the Experimental section. The molar ratio of PE(PC) to milk galactosyltransferase was varied from 1428:1 to 11 400:1. The amount of enzyme in each assay was 4.8 pmol. Both lactosesynthetase (--▲---▲--) and *N*-acetyllactosamine-synthetase (—x—x—), activities were measured. Error bars indicate duplicate assays.

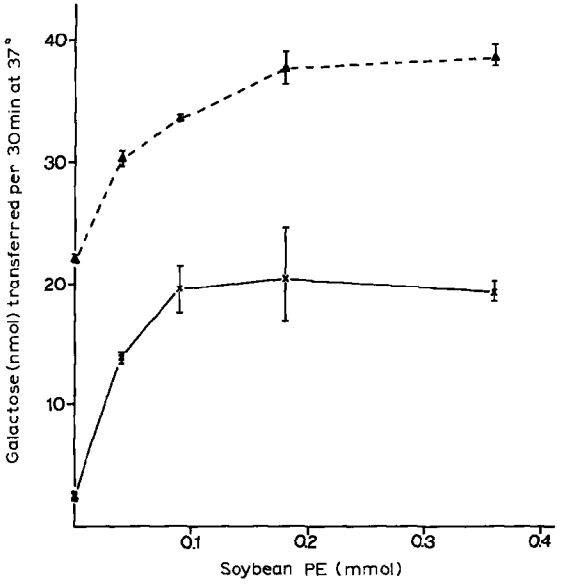

Fig. 2. Activity of milk galactosyltransferase in the presence of soybean PE liposomes. The molar ratio of soybean PE to milk galactosyltransferase was varied from 470:1 to 3757:1. The amount of enzyme in each assay was 4.8 pmol. Both lactosesynthetase (--▲---▲--) and *N*-acetyllactosaminesynthetase (—x—x—) activities were measured. Error bars indicate duplicate assays.

Fig. 1. The presence of mM PE(PC), giving a lipid-to-enzyme ratio of 11 300:1, increased the lactosesynthetase activity by 275% *vs.* the enzyme activity in the absence of lipid. The stimulation of the *N*-acetyllactosaminesynthetase activity was 930%, a four-fold greater effect.

The lamellar-to-hexagonal phase transition for soybean PE occurred at −30° (Table I), and, therefore, at the assay temperature of 37°, this lipid is in principle entirely in the H_{II} phase. As shown in Fig. 2, lactosesynthetase activity increased from 24 nmol to 38 nmol of galactose transferred/30 min at 37°, representing a stimulation (*vs.* free enzyme) of ~175% at 0.35mM soybean PE. Under the same conditions, *N*-acetyllactosamine synthetase activity increased from 2.5 to 20 nmol of galactose transferred/30 min at 37°, a stimulation of ~700%. As with PE(PC) (Fig. 1), soybean PE had a greater effect on *N*-acetyllactosamine than on lactose synthesis.

Several PE types given in Table I were studied in the same manner as described earlier for soybean PE, *i.e.*, the lipid-concentration dependency was determined for each lipid (data not shown). In each case, lactose- and *N*-acetyllactosamine-synthetase activities were measured in the same experiment, at a lipid-to-enzyme ratio of 5200:1 (12.5 nmol of lipid/2.5 pmol of enzyme); maximal activity was attained at this lipid-to-enzyme ratio for all lipids. Egg PC was included for comparison since its properties are well-established[4].

Results from Table I suggest that the lower the T_H value, the greater the

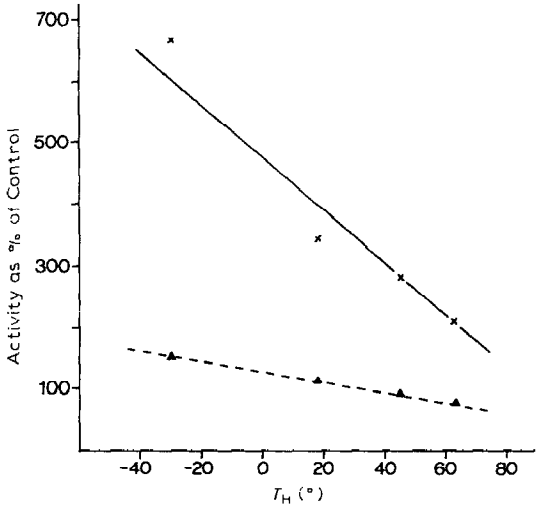

Fig. 3. Relationship of the activity of milk galactosyltransferase in liposomes of various PE compounds as a function of the hexagonal-phase transition temperatures of the lipids. Enzyme activity stimulated by PE compounds is expressed as percentage *vs.* enzyme activity in the absence of lipid. Both lactose-(--▲---▲--) and *N*-acetyllactosamine- (—×—×—) -synthetase activities are shown. The PE compounds used and their lamellar-to-hexagonal phase transition temperatures (T_H) are soybean PE (T_H −30°), plasmalogen PE (T_H 18°), egg PE (T_H 32–45°), and PE(PC) (T_H 63°). The molar ratio of PE to galactosyltransferase was 5200:1. The concentration of galactosyltransferase per assay was 2.4 pmol.

stimulation of the enzyme. The correlation observed with T_H was also observed with T_C, *i.e.*, the lower the T_C value, the greater the stimulation of enzyme activity. These findings are presented graphically in Fig. 3, where the percent of stimulation of both lactose- and *N*-acetyllactosamine-synthetase by PE compounds from soybean, plasmalogen, and egg, and PE(PC), is shown as a function of T_H values. Although the effect on the lactosesynthetase reaction is less pronounced than the effect on *N*-acetyllactosaminesynthetase, parallel trends were observed. Bovine brain PE is a mixture of plasmalogen and nonplasmalogen PE compounds for which

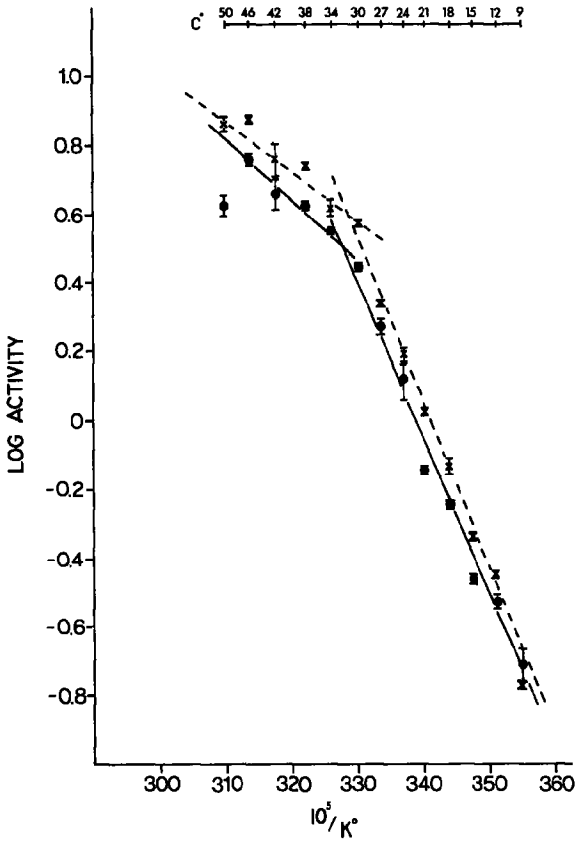

Fig. 4. Arrhenius plots of milk galactosyltransferase activity between 9–50° in the presence of plasmalogen PE. Liposomes of plasmalogen PE were prepared by two procedures: (—●—●—) the dried lipid was sonicated until clear in 1% $Na_2B_4O_7$ buffer, and then dialyzed overnight against two changes of 0.05M MES (pH 7.4) buffer containing 10mM NaCl. The molar ratio of plasmalogen PE to enzyme was 3700:1. (---×---×---) The dried lipid was sonicated until clear in 0.05M MES (pH 7.4) buffer containing 10mM NaCl. The molar ratio of plasmalogen PE to enzyme was 4500:1. The concentration of the galactosyltransferase was 5 pmol per assay. The *N*-acetyllactosaminesynthetase activity of the enzyme was measured. The energies of activation for the lower and higher temperatures derived from curve (—●—●—) were 5000 and 1940 J, respectively; the break temperature was 32.3°. Corresponding values derived from curve (---×---×---) were 5500 and 1960 J, respectively; the break temperature was 31.6°. Error bars indicate duplicate assays.

no transition temperature data are available; DPPE, which is in gel phase at 37°, produced nearly complete inhibition of the enzyme. The phosphatidylcholines [plasmalogen PC (bovine heart), and egg PC] stimulated the activity by more than 600 and 1100%, respectively (Table I).

Arrhenius plots. — If the transition from lamellar-to-hexagonal phase plays a functional role in the activation of the enzyme, a sharply defined change in enzyme activity should be seen at the temperature at which this transition occurs. For such studies, Arrhenius plots, *i.e.*, log [activity] *vs.* $1/T$ (K), have proven valuable[16]. Two of the PE compounds studied herein, PE(PC) (T_H 63°) and plasmalogen PE (T_H 18°) represent species which are totally in lamellar phase, and hexagonal phase, respectively, under the assay conditions at 37°. To test if the lamellar-to-hexagonal phase transition was an important factor in the regulation of the galactosyltransferase, Arrhenius plots were used to measure the modulation of enzyme

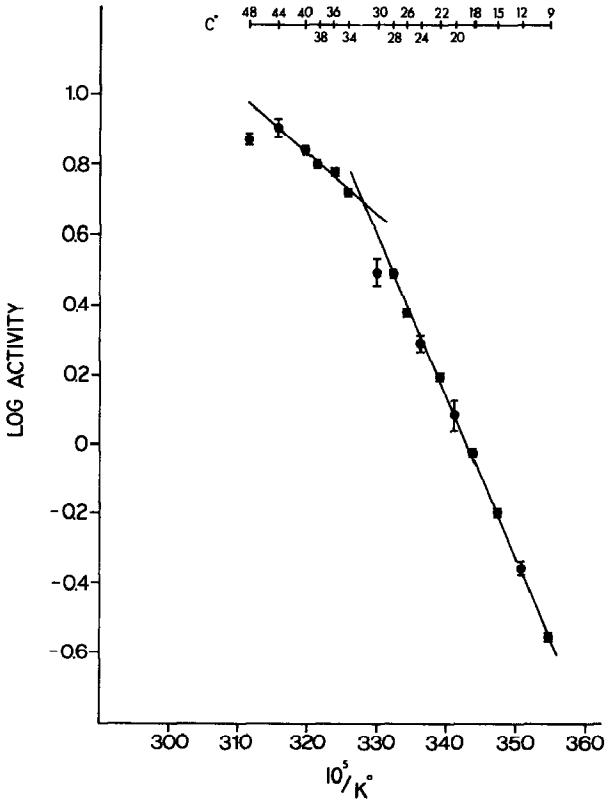

Fig. 5. Arrhenius plot of milk galactosyltransferase (*N*-acetyllactosaminesynthetase) activity in the presence of PE(PC) liposomes prepared by drying the lipid into a tube under a stream of N_2, sonicating in 1% $Na_2B_4O_7$ buffer above 25° until clear, and dialyzing overnight against 2 changes of 0.05M MES (pH 7.4) containing 10mM NaCl. The molar ratio of PE(PC) to enzyme was 5800:1. The concentration of galactosyltransferase per assay was 4.8 pmol. The energies of activation derived from the curve at the lower and higher temperatures were 5000 and 1900 J, respectively; the break temperature was 31.7°. Error bars indicate duplicate assays.

activity by plasmalogen PE between 9 and 50°, thereby spanning a temperature range on either side of the T_H value. Since no break occurred at 19° in the *N*-acetyllactosamine synthetase reaction, it was concluded that the transition to H_{II} phase at 19° did not affect the activity of the enzyme. The resulting Arrhenius plots for stimulation by plasmalogen PE of *N*-acetyllactosamine synthetase activity are shown in Fig. 4. To rule out any effect of method of preparation the PE compound was prepared by both the dialysis procedure (see Experimental section) and by direct dispersion. The general shapes of the curves, the break temperatures (32.3 and 31.6°), and the activation energies (reported in the legend to the figure) were very similar in both experiments. The Arrhenius plot for PE(PC) showed a break at 31.7° (Fig. 5). Again, the break in the Arrhenius plot did not correspond to the phase transitions.

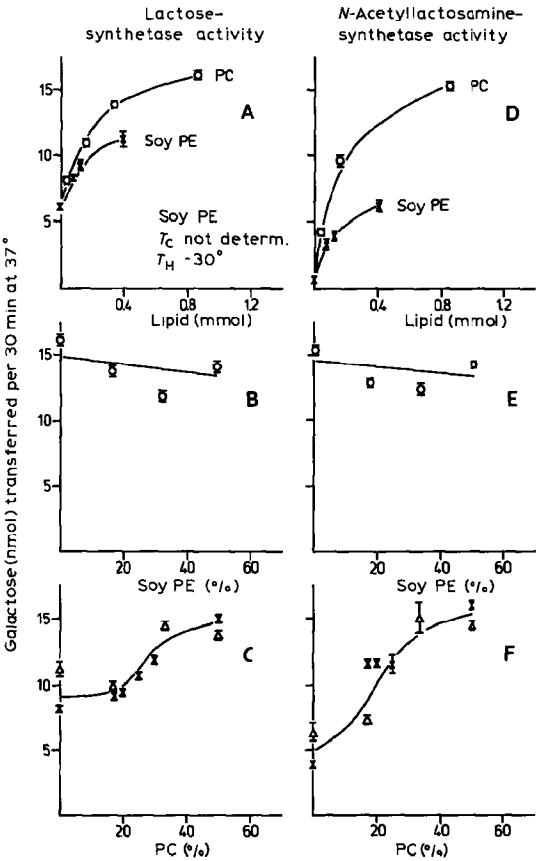

Fig. 6. Galactosyltransferase activity in vesicles of mixed-lipid compositions. The preparation of the mixed-lipid vesicles is described in the Experimental section. A and D: Effects of the pure lipids PE and PC alone on lactose- and *N*-acetyllactosamine-synthetases. B and E: Effect of adding increasing amounts of soy PE to 1.2 μmol of PC, up to a maximum of 50%. C and F: Effect of adding increasing amounts of PC to 1.2 μmol of PE.

Galactosyltransferase activity in vesicles of mixed-lipid compositions. — Jensen and Schutzbach[11] reported that in mixtures of PE and PC, the effect of PC on the activity of mannosyltransferase dominated, which resulted in an inhibition of the activity of the enzyme that correlated with the appearance of the hexagonal phase. We had reported earlier[4] that galactosyltransferase was stimulated by PC. In the present study, the effect of PE on enzyme activity in mixed vesicles of soybean PE and egg PC is shown in Fig. 6. Both lactose- and *N*-acetyllactosamine-synthetase activities were studied.

PE from soybean stimulated both activities, but to a lesser extent than egg PC (Fig. 6, A and D). When galactosyltransferase was incorporated into PC vesicles under conditions giving maximal stimulation, increasing concentrations of soybean PE up to 50% (w/w) had little or no effect on the enzyme activity, demonstrating the dominant role of PC over PE (Fig. 6, B and E). However, when increasing amounts of egg PC were added to PE, further stimulation of activity greater than that due to PE alone occurred, which reached a maximum when the composition of the vesicles was 50% of PC. The shape of the curves (Fig. 6, C and F) were sigmoidal, showing a sharp increase in activity at 20–30% of PC in PE vesicles. The maximum stimulation observed was the same as shown in Fig. 6, A and D, where PC was the only lipid present. These results demonstrate a dominant role for the PC lipid head-group.

DISCUSSION

Recent reports have suggested that phospholipids in nonbilayer structures may have important functional roles to play in the control of synthesis of asparagine-linked glycoproteins. For instance, both mannosyltransferase II, which forms the (1→3)-α-D-mannopyranosyl linkage in α-D-Man*p*-(1→3)-[α-D-Man*p*-(1→6)]-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-α-D-Glc*p*NAc-*P*-*P*-lipid; and dolichol-*P*-Man-synthetase, which transfers a D-mannopyranosyl residue from GDP-Man to dolichol-*P*, are stimulated by nonbilayer (*i.e.*, hexagonal phase) phospholipids[5,6,9,11]. Although the correlation with hexagonal-phase lipid is implied by the data, confirmation must await a consideration of other possible phase-related correlations, as well as a definition of the lipid phases under which the enzymic assays are actually performed.

In continuation of our studies of the role of lipid dynamics in bovine milk galactosyltransferase function and activity, we selected several PE compounds for most of which the T_C and T_H have been reported[17]. Results in Table I showed that soybean PE (T_H −30°, the lowest value among PE compounds studied) stimulated both lactose- and *N*-acetyllactosamine-synthetases to the greatest extents. Since both soybean PE and plasmalogen PE (T_H 18°) are in hexagonal phase in principle at the temperature of assay (37°), the presence of the hexagonal phase cannot be responsible solely for the enzyme activation and the degree of its stimulation. While a correlation was found between T_H and degree of stimulation (Fig. 3), *i.e.*, the

higher the T_H the lower the stimulation, a corresponding correlation was observed between T_C and enzyme activity, *i.e.*, the activity was lower with those lipids exhibiting a high T_C value. In the most striking case, DPPE (T_C 63.7°, thus producing a gel state for this lipid at 37°), showed 24 and 2% of the activity in lactose- and *N*-acetyllactosamine-synthetase reactions, respectively, *vs.* activity in the absence of lipid.

Arrhenius plots showed no breaks in the degree of lipid-stimulated activity through the plasmalogen PE lamellar-to-hexagonal phase transition (Fig. 4, 18°); however, whether this PE or PE(PC) (T_H 63°) was used, a break in the curves was consistently observed at 31–32°, a temperature that corresponded to neither the T_C nor the T_H. Furthermore, a break at this temperature was observed also for rat serum galactosyltransferase[16]. We concluded, tentatively, that (a) either some intermediate phase[10] was going through its transition at the break temperatures for both plasmalogen PE and PE(PC); or (b) assuming that the enzyme does not undergo a change in conformation or degree of self-association at 31–32°, the origin of these phenomena lies in a transition common to both PE compounds, possibly the dissociation of intermolecular hydrogen bonds in head-group regions and their reformation with water, or sites on the enzyme, or both. Alternatively, a change in K_m at this temperature may be responsible for the break[18].

Preliminary ^{31}P-n.m.r. studies supported these overall conclusions. The n.m.r. spectra were characteristic of relatively mobile bilayers undergoing isotropic motion; under the sample conditions used, which resembled closely the conditions of the enzyme assay, neither soybean PE or 7:3 mixture of soybean PE–egg PC showed hexagonal phase, nor, along with egg PC, showed spectra characteristic of pure lamellar phase[10]. In all cases, spectra that have been attributed to "intermediate" phases were observed[19]. These preliminary studies are being extended to include not only further n.m.r. but also X-ray diffraction experiments to establish the nature of the lipid phase present in the various vesicle preparations.

Jensen and Schutzbach[11] reported that mannosyltransferase II, which catalyzes the transfer of mannose from GDP-mannose to a lipid-linked oligosaccharide in the rough endoplasmic reticulum, was inhibited by increasing amounts of PC in PE membranes. After transfer of this lipid-linked oligosaccharide to the peptide backbone, processing occurs, followed by the addition of the terminal sugar residues, galactose and sialic acid, in the Golgi complex. At this stage, mannosyltransferase II activity is not required, so that the inhibition of this enzyme by PC accompanied by the stimulation of galactosyltransferase by PC are consistent with the biosynthetic requirements and represent a mechanism for the modulation of glycoprotein biosynthesis at the level of the glycosyltransferases. The dominant role played by the lipid-head group, reported earlier[4] and observed in this study, supports the mechanism of modulation referred to earlier and is not related to the presence of hexagonal phase.

REFERENCES

1 M. D. HOUSLAY AND K. K. STANLEY, *Dynamics of Biological Membranes*, Wiley–Interscience, New York, 1982.
2 R. B. FREEDMAN in J. B. FINEAN AND R. H. MICHELL (Eds.), *New Comprehensive Biochemistry*, Vol. 1, Elsevier, Amsterdam, 1981, pp. 161–214.
3 M. R. PAQUET AND M. A. MOSCARELLO, in R. C. ALOIA AND J. M. BOGGS (Eds.), *Membrane Fluidity in Biology*, Vol. 3, Academic Press, New York, 1985.
4 M. M. MITRANIC, J. M. BOGGS. AND M. A. MOSCARELLO, *J. Biol. Chem.*, 258 (1983) 8630–8636.
5 J. W. JENSEN AND J. S. SCHUTZBACH, *J. Biol. Chem.*, 256 (1981) 12899–12904.
6 J. W. JENSEN AND J. S. SCHUTZBACH, *J. Biol. Chem.*, 257 (1982) 9025–9029.
7 P. R. CULLIS AND B. DE KRUIJFF, *Biochim. Biophys. Acta*, 559 (1979) 399–420.
8 S. W. HUI, T. P. STEWART, P. L. YEAGLE, AND A. D. ALBERT, *Arch. Biochem. Biophys.*, 207 (1981) 227–240.
9 J. S. SCHUTZBACH AND J. W. JENSEN, *Proc. Int. Symp. Glycoconjugates, VIIth*, (1983) 689a.
10 P. R. CULLIS AND B. DE KRUIJFF, *Biochim. Biophys. Acta*, 507 (1978) 207–218.
11 J. W. JENSEN AND J. S. SCHUTZBACH, *Biochemistry*, 23 (1984) 1115–1119.
12 J. G. STOLLERY AND W. J. VAIL, *Biochim. Biophys. Acta*, 471 (1987) 372–390.
13 G. R. BARTLETT, *J. Biol. Chem.*, 234 (1959) 466–468.
14 D. K. FITZGERALD, B. COLVIN, R. MAWAL. AND K. EBNER, *Anal. Biochem.* 36 (1970) 43–61.
15 B. S. KHATRA, D. G. HERRIES. AND K. BREW, *Eur. J. Biochem.*, 44 (1974) 537–560.
16 M. MITRANIC, J. M. STURGESS. AND M. A. MOSCARELLO, *Biochim. Biophys. Acta*, 443 (1976) 190–197.
17 J. M. BOGGS, D. STAMP, D. W. HUGHES. AND C. M. DEBER, *Biochemistry*, 20 (1981) 5728–5735.
18 H. K. KIMELBERG, in G. POSTE AND G. L. NICOLSON (Eds.), *Dynamic Aspects of Cell Surface Organization*, Vol. 3, North–Holland; Amsterdam, 1977, Chap. 4, pp. 205–293.
19 B. DE KRUIJFF, A. J. VERKLEY, C. I. A. VAN ECHTELD, W. J. GERRITSEN, C. MOMBERS, P. C. NOORDAM. AND J. DE GIER, *Biochim. Biophys. Acta*, 555 (1979) 200–209.