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The effect of acidic lipids on the activity of bovine milk galactosyltransferase in vesicles of different phosphatidylethanolamines

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Bovine milk galactosyltransferase was incorporated into vesicles prepared from different phosphatidylethanolamines which varied widely in both their gel-liquid crystalline and their lamellar-hexagonal phase transition temperatures. Although all phosphatidylethanolamines stimulated the activity of the enzyme the extent of stimulation varied. Acidic lipids phosphatidylserine and phosphatidic acid inhibited the activity of the enzyme incorporated into all of the phosphatidylethanolamines except when the enzyme was in soya PE in which the acidic lipids had no effect.

The galactosyltransferase of milk is considered to be a 'soluble' enzyme because detergents are not required for its solubilization. However, it exists in milk in a lipid-rich medium. Thus a study of the effects of different lipids on the activity of the enzyme was appropriate [1]. Neutral lipids such as phosphatidylcholine (PC) stimulated the activity of the enzyme while negatively charged lipids such as phosphatidic acid (PA) and phosphatidylserine (PS) inhibited the enzyme. The nature and charge of the lipid head group played a dominant role in the modulation of activity although the lipid fatty acyl composition influenced the activity also.

Some glycosyltransferases, specifically mannosyltransferase II [2–4] showed maximal activity in the presence of phosphatidylethanolamine (PE) containing unsaturated acyl chains. Since PE is known to adopt hexagonal (H_{II}) phase, a role for non bilayer phases was postulated [5]. Bovine milk galactosyltransferase is also stimulated by a variety of PEs (Moscarello, M.A., Mitranic, M.M. and Deber, C.M., unpublished data). However, the effect of the lipid on the enzyme activity could not be correlated with hexagonal phase. ^{31}P-NMR demonstrated the presence of an 'intermediate' phase, between lamellar and hexagonal which has not yet been characterized. In the present communication we studied the effect of increasing concentrations of PS or phosphatidic acid in mixtures with various PEs. Both these lipids had been shown to inhibit the activity of milk galactosyltransferase incorporated into PC vesicles [1].

Preparation of liposomes. The lyophilized lipids, were dissolved in chloroform so that the concentration of the stock solutions was 10 mg/ml. To prepare the mixed liposomes, aliquots of the lipids were added to large thin-walled test tubes containing 200 μl of chloroform. The concentration of one lipid was kept constant at 1.2 μmol while that of the second lipid was varied from 0.12 to 1.2 μmol so the second lipid represented 9, 17, 33 and 50% of the total lipid mixture. The lipids were mixed carefully in the chloroform and then dried in a shell around the bottom of the test tube under a stream of N_2. The dried lipids were resuspended in 1 ml of 1% sodium tetraborate (pH 9.2)

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Abbreviation: Mes, 4-morpholineethanesulfonic acid.

and sonicated in a Bransonic 220 bath sonifier until the solution was opalescent. The liposomes were dialysed overnight at 4°C against two changes of 0.05 M Mes buffer (pH 7.4), containing 10 mM sodium chloride and sonicated briefly after dialysis. Nitrogen was present at all steps in the preparation. Lipid concentrations were determined by measuring phosphorus [6]. In each assay, 4.7 pmol of bovine milk galactosyltransferase were used. After mixing on a Vortex, 25 μl of the vesicles were assayed for either lactose or lactosamine synthetase activity.

Galactosyltransferase assays. Lactose and lactosamine synthetase activities of bovine milk galactosyltransferase were measured as described previously [1]. The lactose synthetase assay contained 25 μl of liposomes, 100 mM Mes buffer (pH 7.4), 10 mM $MnCl_2$, 20 mM glucose, 1 mg/ml α-lactalbumin and 1 mM UDP galactose containing 10^5 dpm UDP [^{14}C]galactose in a total volume of 50 μl. The lactosamine synthetase activity was measured as described above except that 20 mM glucose and α-lactalbumin were replaced by 20 mM *N*-acetylglucosamine and water, respectively. After incubation at 37°C for 30 min, the reaction was stopped with 1 ml of 1 mM EDTA. The reaction mixture was then applied to the top of a column of Dowex 1-X8 (100–200 mesh) (H^+ form) in Pasteur pipettes. The product, either [^{14}C]lactose or *N*-acetyl[^{14}C]lactosamine washed through the column with a total of 4 ml of water and was collected directly into scintillation vials. After the addition of 10 ml of ACS counting fluid, the samples were counted in the Tracor liquid scintillation counter. The results are reported as nanomoles of galactose transferred at 37°C in 30 min.

Materials. The lipids were purchased from Avanti Biochemicals Inc., Birmingham, AL. They included phosphatidylethanolamine, isolated from bovine brain, soybean, egg and pE formed by transphosphatidylation from egg phosphatidylcholine, egg phosphatidic acid and bovine brain phosphatidylserine. The Mes buffer, UDPgalactose and the bovine milk galactosyltransferase were purchased from Sigma. The milk galactosyltransferase consisted of two bands (45 K and 55 K) on polyacrylamide gels in the Laemmli [7] system with a continuous gradient of 7.5–15% acrylamide. No α-lactalbumin was present in the enzyme. After elution and reactivation from the gel, both bands were enzymatically active. The ACS scintillation fluid was purchased from the Amersham Corporation. The UDP[^{14}C]galactose (302 mCi/mmol) was purchased from the New England Nuclear Corporation.

Lactosamine synthetase activity. Bovine milk galactosyltransferase, catalyses the following reaction:

$$\text{GlcNAc} + \text{UDPgalactose} \xrightarrow{Mn^{2+}} \text{Lac NAc} + \text{UDP}$$

in which *N*-acetyllactosamine (LacNAc) is formed from *N*-acetylglucosamine (GlcNAc) and galactose. The effect of a number of PEs, chosen to encompass a wide range of gel → liquid-crystalline transition temperatures (T_c) and lamellar → hexagonal phase transitions (T_H) are shown in the upper panel of Fig. 1. All PEs stimulated the activity. The largest stimulation of activity was observed with PE obtained by transphosphatidylation from PC. The increase was more than 1000-fold while that for the other pEs was less than this amount. Concentrations of bovine brain PE above 0.4 mM were not possible because of aggregation of the vesicles.

The effect of phosphatidylserine (PS) on the activity of the enzyme in vesicles of the various PEs is shown in the center panel of Fig. 1. PS had no effect on the activity of the enzyme when soya PE was present. Therefore, soya PE was able to exclude PS from the environment of the enzyme. In the presence of egg PE, PS inhibited the enzyme in a linear fashion until at a 50 : 50 mixture of egg PE and PS, total inhibition of the enzyme was observed. PE obtained by transphosphatidylation of egg PC was not affected by PS until the concentration of PS in the mixture exceeded 20%. In the presence of bovine brain PE a rapid loss of activity was observed, which was almost complete at 35% PS.

When phosphatidic acid was the acidic lipid, soya PE was not affected at any concentration of phosphatidic acid, egg PE was not affected by phosphatidic acid until the concentration exceeded 20% phosphatidic acid but was completely inhibited when the vesicles were 35% phosphatidic acid. PE obtained from PC was very sensitive to

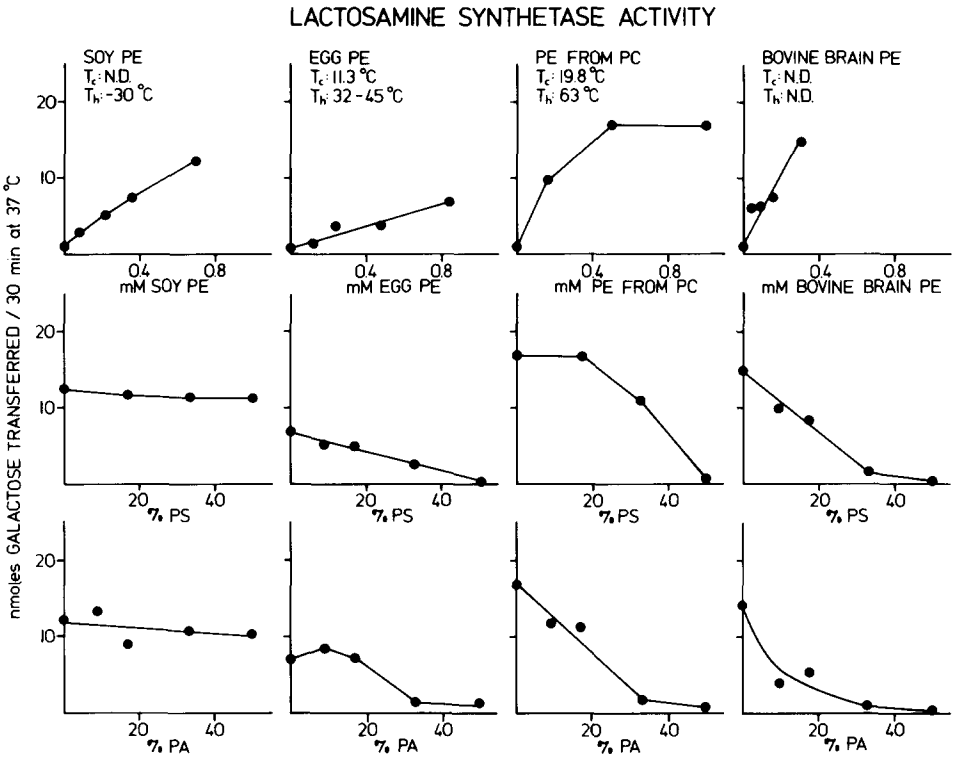

Fig. 1. Lactosamine synthetase activity. Upper panel. The effect of different phosphatidylethanolamines on lactosamine synthetase. The T_c and T_H values are shown for those PEs for which these numbers are known. Middle panel. The effect of increasing concentrations of phosphatidylserine (PS) on the activity of bovine milk galactosyltransferase incorporated into 0.8 mM of the different PEs. Lower panel. The effect of phosphatidic acid (PA). All conditions were the same as described for PS above. Each enzyme assay was carried out in duplicate. The data points represent means of the duplicates, which varied by 2–5%. All experiments were done three times with three independent batches of lipid vesicles. The enzyme content in each assay was 4.7 pmol.

the presence of phosphatidic acid. Inhibition of activity was observed at all concentrations of phosphatidic acid and complete inhibition at 35% phosphatidic acid. In bovine brain PE, the transferase activity was rapidly inhibited at low concentrations of phosphatidic acid.

Lactose synthetase activity. In the presence of α-lactalbumin, milk galactosyltransferase, transfers galactose to glucose (Glc) in the following reaction:

$$\text{Glc} + \text{UDPgalactose} \xrightarrow[\alpha\text{-lactalbumin}]{Mn^{2+}} \text{lactose} + \text{UDP}$$

This reaction is modulated by lipids in a manner similar to the lactosamine synthetase reaction above. In the top panel of Fig. 2, the effects of the different PEs on the synthesis of lactose are shown. The activation by the different PEs was similar to that observed in Fig. 1 for lactosamine synthesis, except that the percentage increase in activity was always considerably less.

In the center panel of Fig. 2 the effect of addition of increasing amounts of PS to the vesicles on enzyme activity is shown. The enzymic activity in soya PE vesicles was not affected by the addition of PS. Inhibition of activity was observed in egg PE and PE formed by transphosphatidylation of egg PC only at concentrations of PS greater than 30%. Complete inhibition was observed at 50% PS. The activity in bovine brain PE was sensitive to PS even at low concentrations of PS and was completely inhibited at 35% PS.

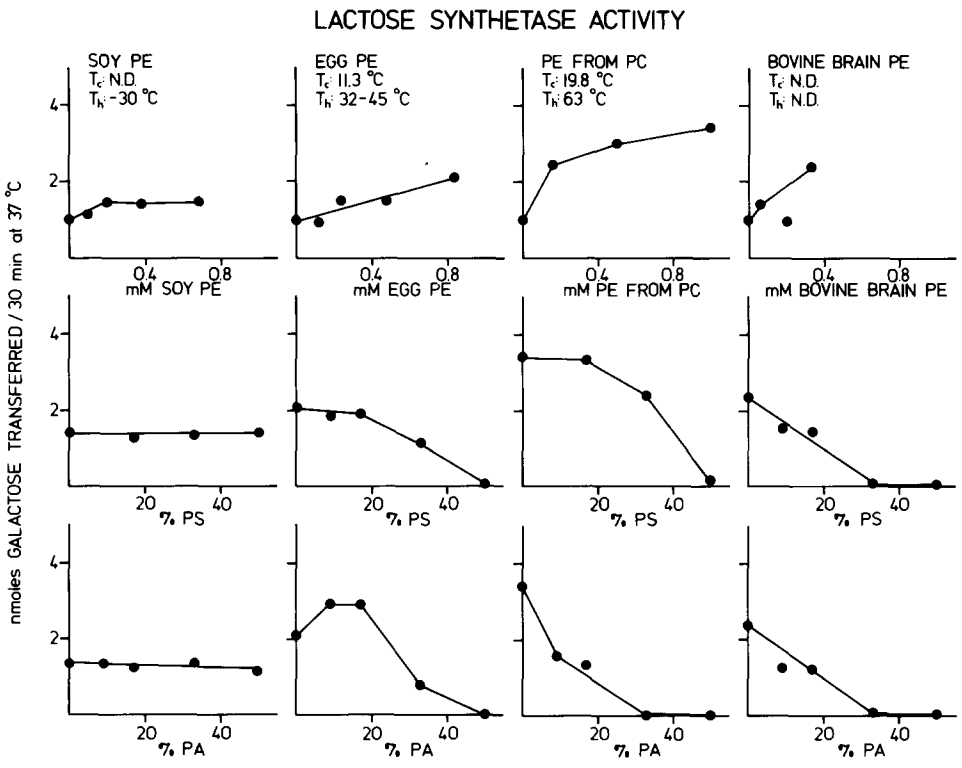

Fig. 2. Lactose synthetase activity. Upper panel. Effect of different phosphatidylethanolamines on lactose synthetase. Middle panel. Effect of increasing concentrations of PS on activity of bovine milk galactosyltransferase incorporated into 0.8 mM of the different PEs. Lower panel. Effect of phosphatidic acid (PA). All conditions were the same as for PS above. As in Fig. 1 each assay was carried out in duplicate with a similar variation among them. Again these experiments were repeated three times.

The effect of phosphatidic acid on transferase activity is shown in the lower panel of Fig. 2. In soya PE, no effect on activity was found. In egg PE a small activation was observed at low phosphatidic acid concentrations. Inhibition occurred at phosphatidic acid concentrations greater than 20% and complete inhibition at 50%. In both PE from egg PC and bovine brain PE, rapid inhibition was observed, and complete inhibition at 35% phosphatidic acid.

PE stimulated the activity of bovine milk galactosyltransferase in both the lactosamine and lactose synthetase reactions. The greatest stimulation of activity was observed with PE obtained by transphosphatidylation of egg PC, in which the stimulation observed was similar to that reported for egg PC (Moscarello, M.A., Mitranic, M.M. and Deber, C.M., unpublished data). Since the assay temperature of 37°C was well above the T_c but well below T_H the large stimulation may be related to a requirement for lamellar phase lipid.

Both the acidic lipids inhibited the activity of the transferase in all PEs except soya PE in which they had no effect. The dominant role of the acidic head group reported earlier when acidic lipids were mixed with PC [1] was not observed with soya PE. No explanation can be suggested for this observation at this time. The dominant role of the lipid head group on enzyme activity is demonstrated for all other PEs since the enzyme could be totally inhibited by the acidic lipid, whether PS or phosphatidic acid. Inhibition of enzyme activity in various PCs by PS and phosphatidic acid has been reported [1]. In the present study soya PE stands out since it was the only lipid in which the enzyme activity was not affected by PS or phosphatidic acid. Further studies are required to determine if the physical structure of this lipid differs from the others preventing the acidic lipids from interacting with the enzyme.

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