Stimulation of Collagen Galactosyltransferase and Glucosyltransferase Activities by Lysophosphatidylcholine

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Lysophosphatidylcholine stimulated the activities of collagen galactosyl- and glucosyltransferases in chick-embryo extract and its particulate fractions *in vitro*, whereas essentially no stimulation was noted in the high-speed supernatant, where the enzymes are soluble and membrane-free. The stimulatory effect of lysophosphatidylcholine was masked by 0.1 % Triton X-100. In kinetic experiments lysophosphatidylcholine raised the maximum velocities with respect to the substrates and co-substrates, whereas no changes were observed in the apparent K_m values. Phospholipase A preincubation of the chick-embryo extract resulted in stimulation of both transferase activities, probably by generating lysophosphatides from endogenous phospholipids. No stimulation by lysophosphatidylcholine was found when tested with 5000-fold-purified glucosyltransferase. The results suggest that collagen glycosyltransferases must be associated with the membrane structures of the cell in order to be stimulated by lysophosphatidylcholine. Lysophosphatidylcholine could have some regulatory significance *in vivo*, since its concentration in the cell is comparable with that which produced marked stimulation *in vitro*.

Collagen galactosyl- and glucosyl-transferases catalyse as post-translational modifications the transfer of galactose and glucose from their nucleotide-sugar donors, UDP-galactose and UDP-glucose, to collagen, forming hydroxylysine-linked mono- and di-saccharides, galactosylhydroxylysine and glucosylgalactosylhydroxylysine (for reviews, see Grant & Prockop, 1972*a,b,c*; Bornstein, 1974; Prockop *et al.*, 1976; Kivirikko & Risteli, 1976). Both reactions are specific, and require the presence of Mn^{2+} or certain other bivalent cations (R. G. Spiro & M. J. Spiro, 1971; M. J. Spiro & R. G. Spiro, 1971; Myllylä *et al.*, 1975*a*, 1976). These transferases have not yet been obtained in a completely pure state, although over 2000-fold purifications of collagen glucosyltransferase have been obtained by both conventional enzyme-purification methods and an affinity-chromatography procedure (Myllylä *et al.*, 1976; Anttinen & Kivirikko, 1976). Very little is known about the characteristics of these two enzymes, and almost nothing about their regulation in the cell.

Lysophosphatidylcholine is known to stimulate the activities of many enzymes (e.g. Fisher & Kaufman, 1973), but few reports have been published on the stimulation of glycosyltransferases (Kirschbaum & Bosmann, 1973; Mookerjee & Yung, 1974; Geren & Ebner, 1974; Shier & Trotter, 1976). Fisher & Kaufman (1973) suggested that lysophosphatidylcholine causes a conformational change in the structure of rat liver phenylalanine hydroxylase, but it is also possible that in other instances the

stimulation of some enzyme activities results from solubilization of enzymes from membrane structures. In the present work lysophosphatidylcholine stimulation of both collagen galactosyl- and glucosyltransferase in cellular particulate fractions is reported.

Experimental

Materials

Lysophosphatidylcholine (from egg yolk) was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. and phospholipase A (from *Crotalus terrificus terrificus*) from Boehringer Mannheim Corp., Heidelberg, West Germany. The sources of the other materials were the same as specified elsewhere (Myllylä *et al.*, 1975*a,b*, 1976).

Preparation of the crude glycosyltransferases

All procedures were carried out at 0–4°C. Chick embryos (10–12 days old) were homogenized in a Teflon/glass homogenizer with five strokes in a solution consisting of 225 mM-mannitol, 75 mM-sucrose, 50 μ M-dithiothreitol and 50 mM-Tris/HCl buffer adjusted to pH 7.4 at 4°C (2 or 3 ml of solution/g of embryo). The homogenate was filtered through cheesecloth and centrifuged at 500g for 20 min. The 500g supernatant was filtered to remove the lipid layer that had formed. To obtain the subcellular fractions, the 500g supernatant was centrifuged at

either 15000g or 110000g for 45 min. The pellets were washed once with the buffer used for homogenization, and suspended in the same buffer before assay.

Preparation of the 5000-fold-purified collagen glucosyltransferase

Collagen glucosyltransferase was purified 5000-fold from the 15000g supernatant of the chick-embryo homogenate by using affinity chromatography on a UDP-glucose derivative coupled to agarose, as described by Anttinen & Kivirikko (1976).

Assays

In most of the experiments collagen galactosyltransferase activity was assayed in a final volume of 100 μ l containing 30 μ l of the enzyme fractions, 40 mg of calf skin gelatin substrate/ml, 30 μ M-UDP-[¹⁴C]-galactose [New England Nuclear Corp., Boston, MA, U.S.A., diluted with the unlabelled compound (Sigma Chemical Co., Kingston-upon-Thames, U.K.) to final specific radioactivity of 10 Ci/mol], 10 mM-MnCl₂, 100 μ M-dithiothreitol and 50 mM-Tris/HCl buffer adjusted to pH 7.4 at 20°C (Myllylä *et al.*, 1975a,b). The assay mixture for collagen glucosyltransferase was similar, except that 60 μ M-UDP-[¹⁴C]glucose [New England Nuclear, diluted with the unlabelled compound (Sigma) to final specific radioactivity of 3.3 Ci/mol] was used (Myllylä *et al.*, 1975a, 1976). The gelatin substrate was heated to 100°C for 10 min and rapidly cooled to 0°C immediately before addition to the incubation mixture (Myllylä *et al.*, 1975b; Risteli *et al.*, 1976). The samples were incubated at 37°C for 45 min, and the reactions terminated by adding 2 ml of 1% (w/v) phosphotungstic acid in 0.5 M-HCl. The products were purified and assayed as reported previously, including the ion-exchange-chromatography step (Myllylä *et al.*, 1975a), but the last step in the purification of the reaction products, paper electrophoresis, was omitted. The assays were almost specific without this, in that over 80% of the radioactivity found in the galactosylation reaction and over 95% of that found in the glucosylation reaction were recovered in the corresponding hydroxylysine-linked mono- and di-saccharides (Risteli & Kivirikko, 1976).

Phospholipase A preincubation

Phospholipase A preincubations were carried out for 15 min at 37°C in a final volume of 600 μ l, containing 500 μ l of the 500g supernatant of the chick-embryo homogenate (167 mg of chick embryo) and 1–100 μ l of phospholipase A (from *Crotalus*; 1 mg/ml). The reactions were stopped by cooling the

samples in an ice/water mixture to 0°C. The glycosyltransferase activity assays were performed with 30 μ l of the preincubation mixtures.

Results

Effect of mannitol/sucrose buffer on collagen glycosyltransferase activities

To test if the sugars used in the homogenization buffer were capable of affecting collagen glycosyltransferase activities, the activities of both transferases were measured with and without 30 μ l of the homogenization buffer added to the assay mixtures, the concentrations of mannitol and sucrose then being 68 and 23 mM respectively. A stimulation of 18% for galactosyltransferase and an inhibition of 21% for glucosyltransferase were observed. No effort was made to determine which of the sugars was mainly responsible for these effects. R. G. Spiro & M. J. Spiro (1971) and M. J. Spiro & R. G. Spiro (1971) reported an inhibition of 50% for galactosyltransferase and of 80% for glucosyltransferase in the presence of 300 mM-sucrose, a concentration much higher than that used here. In view of these effects the concentration of the homogenization buffer was kept the same in all assays within the same experiment, and never exceeded the concentration used in the above test. Thus the magnitude of all the changes and differences reported in the present study would have been the same if mannitol and sucrose had been omitted from the assays.

Effect of lysophosphatidylcholine on collagen glycosyltransferase activities in the 500g supernatant of chick-embryo homogenate

The effect of lysophosphatidylcholine on collagen glycosyltransferase activities was studied by adding various amounts of lysophosphatidylcholine directly to the assay mixtures. Lysophosphatidylcholine stimulated the activities of both galactosyl- and glucosyl-transferase in a dose-dependent manner (Fig. 1). The degree of stimulation found in different experiments was somewhat variable, possibly owing to minor differences in the extent of homogenization of the chick embryos. Maximum stimulation was obtained with lysophosphatidylcholine concentrations of 0.9 mM for galactosyltransferase and of 0.6 mM for glucosyltransferase (Fig. 1). These values are in both cases lower than the 7.5 mM-lysophosphatidylcholine required for a galactosyltransferase (Mookerjee & Yung, 1974) and the 2 mM-lysophosphatidylcholine required for a sialyltransferase (Shier & Trotter, 1976) of rat liver microsomal fraction. Stimulation remained the same until the lysophosphatidylcholine concentration reached

Fig. 1. Effect of different concentrations of lysophosphatidylcholine on collagen (a) galactosyltransferase and (b) glucosyltransferase activities in the 500g supernatant of the chick-embryo homogenate

Lysophosphatidylcholine was added directly to the assay mixtures. Synthesis of (a) [^{14}C]galactosylhydroxylysine and of (b) [^{14}C]glucosylgalactosylhydroxylysine was measured in the absence (○) and presence (●) of 0.1% Triton X-100 in the assay mixture.

Table 1. Effect of temperature on the stimulation of collagen glycosyltransferase activities by lysophosphatidylcholine

Collagen galactosyl- and glucosyl-transferase activities in the 500g supernatant of chick-embryo homogenate were assayed in the presence of lysophosphatidylcholine (1.3 mM) at 37°C and 25°C as described in the Experimental section. Lysophosphatidylcholine was added directly to the assay mixtures. The stimulation of the transferase activity is expressed as a percentage of the activity observed in the absence of lysophosphatidylcholine.

Temperature (°C)	Stimulation of activity (%)	
	Galactosyl- transferase	Glucosyl- transferase
37	+250	+180
25	+180	+160

phosphatidylcholine and Triton X-100 are mediated similarly.

Effect of lysophosphatidylcholine on K_m values for glycosyltransferases in the 500g supernatant of the chick-embryo homogenate

The stimulation obtained with 1.3 mM-lysophosphatidylcholine was little lower at 25°C than at 37°C (Table 1). There were no changes in the apparent K_m values for gelatin and UDP-sugar donors as substrates when tested in the presence and absence of 1.3 mM-lysophosphatidylcholine (Figs. 2 and 3). The apparent K_m values obtained in this work without lysophosphatidylcholine all agreed, within a factor of two, with reported values (Myllylä *et al.*, 1975a,b; Risteli *et al.*, 1976). The small differences may be caused by differences in the purity of the transferase fractions. The apparent K_m values for gelatin vary considerably depending on the extent of the gelatinization process, and probably also depending on the amounts of free hydroxylysine and galactosylhydroxylysine in different gelatin preparations. The apparent K_m values obtained here for gelatin were 24 g/litre for galactosyltransferase and 44 g/litre for glucosyltransferase, and those for UDP-galactose and UDP-glucose were 40 μM for galactosyltransferase and 60 μM for glucosyltransferase.

With certain enzymes, lysophosphatidylcholine not only increases the maximum velocities of the reactions but also alters the kinetics with respect to substrates by lowering the K_m values and converting the shape of the initial-velocity curves (Fisher & Kaufman, 1973). No such changes in the kinetics were observed here, and lysophosphatidylcholine always raised the maximum velocities of the collagen glycosyltransferase reactions.

2.5 mM, but declined slightly with higher concentrations.

The presence of Triton X-100 in the reaction mixture masked the stimulatory effect of lysophosphatidylcholine (Fig. 1), a finding also noted for the galactosyltransferase (Mookerjee & Yung, 1974) and the sialyltransferase (Shier & Trotter, 1976) of rat liver microsomal fractions. Since lysophosphatidylcholine is known to possess detergent properties, it is possible that the effects of lyso-

Fig. 2. Double-reciprocal plots of initial velocity and gelatin concentration in the synthesis of (a) [¹⁴C]galactosylhydroxylysine and of (b) [¹⁴C]glucosylgalactosylhydroxylysine with the corresponding glycosyltransferase in the 500g supernatant of the chick-embryo homogenate

The assays were performed in the absence (○) and presence (●) of 1.3 mM-lysophosphatidylcholine.

Fig. 3. Double-reciprocal plots of initial velocity and (a) UDP-galactose or (b) UDP-glucose concentration in the synthesis of (a) [¹⁴C]galactosylhydroxylysine or (b) [¹⁴C]glucosylgalactosylhydroxylysine with the corresponding glycosyltransferase in the 500g supernatant of the chick-embryo homogenate

The assays were performed in the absence (○) and presence (●) of 1.3 mM-lysophosphatidylcholine.

Subcellular localization of glycosyltransferase activities for lysophosphatidylcholine stimulation

The 500g supernatant of the chick-embryo homogenate was fractionated by centrifugation at 110000g into a soluble and a particulate fraction, which were assayed in the presence and absence of 1.5 mM-lysophosphatidylcholine (Table 2). In the absence of lysophosphatidylcholine the subcellular distribution of collagen glycosyltransferase activities was essentially the same as those previously described (Myllylä *et al.*, 1976; Risteli *et al.*, 1976). In the presence of lysophosphatidylcholine in the assay mixture there was a marked stimulation in the particulate fraction but little stimulation in the 110000g supernatant

fraction. When the soluble and particulate fractions were combined in different ratios, the degree of stimulation obtained with lysophosphatidylcholine in the assay mixture was similar to that calculated for the combinations (results not shown). When the 500g supernatant was preincubated with 1.5 mM-lysophosphatidylcholine for 30 min at 37°C before the centrifugation at 110000g, there was an increase in collagen glycosyltransferase activities in the soluble fraction (Table 3), presumably owing to solubilization of the enzymes from membranes. However, an increase was also observed in the activities in the particulate fraction. This may be due

Table 2. *Distribution and stimulation by lysophosphatidylcholine of collagen glycosyltransferase activities in the fractions of the 500g supernatant of the chick-embryo homogenate*

The 500g supernatant of the chick-embryo homogenate was fractionated by centrifugation at 110000g, and the pellet and supernatant fractions were assayed in the presence and absence of 1.5 mM-lysophosphatidylcholine as described in the Experimental section. Synthesis of [^{14} C]galactosylhydroxylysine or [^{14} C]glucosylgalactosylhydroxylysine is expressed as d.p.m. per corresponding fraction obtained from 1 mg of chick embryo. The distribution values are expressed as percentages of the activity in the 500g supernatant of the chick-embryo homogenate. Stimulation of activity is expressed as a percentage of the activity in a corresponding fraction measured in the absence of lysophosphatidylcholine.

Enzyme	Fraction	Without lysophospha- tidylcholine (d.p.m./mg)	Distribution (%)	Plus lysophospha- tidylcholine (d.p.m./mg)	Stimulation (%)
Collagen galactosyltransferase	500g supernatant	141.7	100	289.1	+104
	110000g supernatant	102.7	72	119.0	+16
	110000g pellet	33.2	23	152.3	+359
Collagen glucosyltransferase	500g supernatant	57.3	100	130.4	+128
	110000g supernatant	36.4	64	39.4	+8
	110000g pellet	21.0	37	72.3	+244

Table 3. *Effect of preincubation with lysophosphatidylcholine on the sedimentation of collagen glycosyltransferases at 110000g*

The 500g supernatant of the chick-embryo homogenate was preincubated for 30 min at 37°C in the presence and absence of 1.5 mM-lysophosphatidylcholine, and centrifuged at 110000g. The pellet and supernatant fractions were assayed as described in the Experimental section. Synthesis of [^{14} C]galactosylhydroxylysine or [^{14} C]glucosylgalactosylhydroxylysine is expressed as d.p.m. per corresponding fraction obtained from 1 mg of chick-embryo homogenate.

Enzyme	Fraction	Without lysophospha- tidylcholine (d.p.m./mg)	Plus lysophospha- tidylcholine (d.p.m./mg)	Increase (%)
Collagen galactosyltransferase	110000g supernatant	106.6	185.9	+74
	110000g pellet	26.4	73.8	+180
Collagen glucosyltransferase	110000g supernatant	40.4	104.4	+158
	110000g pellet	19.7	31.3	+59

to partial solubilization of the membranes, so that the enzymes which were still membrane-bound could interact with their macromolecular substrate.

Preincubation of collagen glycosyltransferases in the 500g supernatant of the chick-embryo homogenate with phospholipase A

Samples of the 500g supernatant of the chick-embryo homogenate were preincubated with various amounts of phospholipase A for 15 min at 37°C before the assays. Both transferases were stimulated in a manner dependent on the amount of phospholipase A used in the preincubations (Fig. 4, open symbols). The stimulation was assumed to reflect the hydrolytic action of phospholipase on phospholipids, generating lysophosphatides. The presence of 1.3 mM-lysophosphatidylcholine in the assay always gave the same increase in stimulation (Fig. 4, closed symbols), which might also indicate partly different

mechanisms of stimulation by phospholipase A preincubation and by lysophosphatidylcholine.

Effects of lysophosphatidylcholine on 5000-fold-purified collagen glycosyltransferase

To test whether purified collagen glycosyltransferase could still be stimulated by lysophosphatidylcholine, samples of 5000-fold-purified glucosyltransferase were incubated with 1 mM- and 5 mM-lysophosphatidylcholine in the standard assay. There was no stimulation of the activity of purified glucosyltransferase by lysophosphatidylcholine, indicating that in the purification process glucosyltransferase loses receptivity to lysophosphatidylcholine stimulation. The result agrees with the finding that collagen glycosyltransferases could not be stimulated by lysophosphatidylcholine in the high-speed-supernatant fraction, where the transferases are presumably soluble and not associated with membranes.

Fig. 4. Effect of phospholipase A preincubation on collagen (a) galactosyltransferase and (b) glucosyltransferase activities in the 500g supernatant of chick-embryo homogenate

The preincubations were carried out with various amounts of phospholipase A for 15 min at 37°C in the final volume of 600 μl. Synthesis of (a) [¹⁴C]galactosylhydroxylysine and (b) [¹⁴C]glucosylgalactosylhydroxylysine was measured in the absence (○) and presence (●) of 1.3 mM-lysophosphatidylcholine in the assay mixture.

Discussion

Recent studies have suggested that collagen glycosyltransferases in the cell are associated with membrane structures (Harwood *et al.*, 1975; Myllylä *et al.*, 1976; Risteli *et al.*, 1976). Studies on the fractionation of embryonic chick-tendon cells have indicated that most of the collagen glycosyltransferase activity was associated with the rough-endoplasmic-reticulum fraction, but significant amounts were also present in the smooth-endoplasmic-reticulum fraction

(Harwood *et al.*, 1975). Studies on the effect of substrate helical conformation on collagen glycosylation *in vitro* (Myllylä *et al.*, 1975b; Risteli *et al.*, 1976) and the intracellular site of collagen glycosylation (Brownell & Veis, 1975; Harwood *et al.*, 1975; Oikarinen *et al.*, 1976) likewise give evidence for the location of these transferases within the cisternae of the endoplasmic reticulum, where collagen proline hydroxylase and lysine hydroxylase are also located (Diegelmann *et al.*, 1973; Olsen *et al.*, 1973; Cutroneo *et al.*, 1974; Harwood *et al.*, 1974a,b; Prockop *et al.*, 1976).

The data obtained in the present work indicate that collagen galactosyl- and glucosyl-transferase in particulate fractions of chick-embryonic extracts are very sensitive to stimulation by exogenously added lysophosphatidylcholine. The stimulation obtained by phospholipase A preincubation is assumed to be caused by the hydrolytic release of lysophosphatides from endogenous phospholipids. It has been demonstrated that lysophosphatidylcholine and other phospholipids alter the kinetics of some enzymes with respect to substrates, acting like a positive allosteric effector (Cunningham & Hager, 1971; Fisher & Kaufman, 1973). In the present work no changes caused by lysophosphatidylcholine were observed in the apparent K_m values for either gelatin or UDP-sugar donors as substrates for the transferases, indicating no facilitation of the binding of either of these substrates to the enzymes.

The stimulation obtained by using lysophosphatidylcholine is probably mainly due to solubilization of the enzymes from membrane structures. However, the possibility cannot be excluded that part of the stimulation is mediated by true activation of the membrane-associated enzymes. Lysophosphatidylcholine is a normal membrane component (Ray *et al.*, 1969) and a naturally occurring detergent (Helenius & Simons, 1975). Its concentration in membranes is determined by a phosphoglyceride deacylation-reacylation cycle catalysed by membrane-associated phospholipase A₂ and acyl-CoA lysophosphoglyceride acyltransferases (van den Bosch, 1974). Ray *et al.* (1969) reported that about 2% of liver phospholipid is lysophosphatidylcholine and that assuming uniform distribution, its concentration in the liver cell would then be 3 mM. However, since lysophosphatidylcholine is mainly located in membranes at correspondingly higher concentration, changes in lysophosphatidylcholine concentration in the cell could regulate the activity of glycosyltransferases *in vivo*.

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References

- Anttinen, H. & Kivirikko, K. I. (1976) *Biochim. Biophys. Acta* **429**, 750–758
- Bornstein, P. (1974) *Annu. Rev. Biochem.* **43**, 567–603
- Brownell, A. G. & Veis, A. (1975) *Biochem. Biophys. Res. Commun.* **63**, 371–377
- Cunningham, C. C. & Hager, L. P. (1971) *J. Biol. Chem.* **246**, 1583–1589
- Cutroneo, K. R., Guzman, N. A. & Sharawy, M. M. (1974) *J. Biol. Chem.* **249**, 5989–5994
- Diegelmann, R. F., Bernstein, L. & Peterkofsky, B. (1973) *J. Biol. Chem.* **248**, 6514–6521
- Fisher, D. B. & Kaufman, S. (1973) *J. Biol. Chem.* **248**, 4345–4353
- Geren, L. M. & Ebner, K. E. (1974) *Biochem. Biophys. Res. Commun.* **59**, 14–21
- Grant, M. E. & Prockop, D. J. (1972a) *N. Engl. J. Med.* **286**, 194–199
- Grant, M. E. & Prockop, D. J. (1972b) *N. Engl. J. Med.* **286**, 242–249
- Grant, M. E. & Prockop, D. J. (1972c) *N. Engl. J. Med.* **286**, 291–300
- Harwood, R., Grant, M. E. & Jackson, D. S. (1974a) *Biochem. Biophys. Res. Commun.* **59**, 947–954
- Harwood, R., Grant, M. E. & Jackson, D. S. (1974b) *Biochem. J.* **144**, 123–130
- Harwood, R., Grant, M. E. & Jackson, D. S. (1975) *Biochem. J.* **152**, 231–302
- Helenius, A. & Simons, K. (1975) *Biochim. Biophys. Acta* **415**, 29–79
- Kirschbaum, B. B. & Bosmann, H. B. (1973) *FEBS Lett.* **34**, 129–132
- Kivirikko, K. I. & Risteli, L. (1976) *Med. Biol.* **54**, 159–186
- Mookerjee, S. & Yung, J. W. M. (1974) *Biochem. Biophys. Res. Commun.* **57**, 815–822
- Myllylä, R., Risteli, L. & Kivirikko, K. I. (1975a) *Eur. J. Biochem.* **52**, 401–410
- Myllylä, R., Risteli, L. & Kivirikko, K. I. (1975b) *Eur. J. Biochem.* **58**, 517–521
- Myllylä, R., Risteli, L. & Kivirikko, K. I. (1976) *Eur. J. Biochem.* **61**, 59–67
- Oikarinen, A., Anttinen, H. & Kivirikko, K. I. (1976) *Biochem. J.* **156**, 545–551
- Olsen, B. R., Berg, R. A., Kishida, Y. & Prockop, D. J. (1973) *Science* **182**, 825–827
- Prockop, D. J., Berg, R. A., Kivirikko, K. I. & Uitto, J. (1976) in *Biochemistry of Collagen* (Ramachandran, G. N. & Reddi, A. H., eds.), Plenum Publishing Corp., New York, in the press
- Ray, T. K., Skipski, V. P., Barclay, M., Essner, E. & Archibald, F. M. (1969) *J. Biol. Chem.* **244**, 5528–5536
- Risteli, J. & Kivirikko, K. I. (1976) *Biochem. J.* **158**, 361–367
- Risteli, L., Myllylä, R. & Kivirikko, K. I. (1976) *Biochem. J.* **155**, 145–153
- Shier, W. T. & Trotter, J. T., III, (1976) *FEBS Lett.* **62**, 165–168
- Spiro, M. J. & Spiro, R. G. (1971) *J. Biol. Chem.* **246**, 4910–4918
- Spiro, R. G. & Spiro, M. J. (1971) *J. Biol. Chem.* **246**, 4899–4909
- van den Bosch, H. (1974) *Annu. Rev. Biochem.* **43**, 243–277