Biochimica et Biophysica Acta 856 (1986) 392–394
Elsevier

BBA 70295

BBA Report

The effect of retinoids on the activity of the membrane form of galactosyltransferase, studied in an enzyme / liposome model system

Bernhard M.K. Gmeiner

Institute of Tumorbiology - Cancer Research of the University of Vienna, Borschkegasse 8a, A-1090 Vienna (Austria)

(Received October 21st, 1985)

Key words: Retinoid; Galactosyltransferase; Phosphatidylserine; Liposome

In the present study the effect of retinoids on the membrane form of galactosyltransferase was tested. A model system consisting of pure bovine milk galactosyltransferase and phosphatidylserine vesicles was used for this investigation. Retinol, retinal and retinylphosphate were able to overcome the modulating effect of phosphatidylserine, that is, activated the enzyme. Retinoic acid and retinylpalmitate were ineffective in this system.

It has been established that retinoids are involved in glycosylation reactions, acting as lipid intermediates, transferring mannosyl and galactosyl residues onto glycoconjugates [1,2]. Plotkin and Wolf [3] have reported that retinoids can stimulate the enzyme galactosyltransferase in microsomal preparations from rat tracheal epithelium. Similar retinoid effects were observed in preparations from human urinary bladder tumor cells (Gmeiner, B. and Wolf, G., unpublished data). It is not known by which mechanism retinoids can stimulate galactosyltransferase activity in microsomal preparations. Taking into consideration, that (i) in microsomes the galactosyltransferase is membrane-bound [4], that (ii) the lipid structure of the membrane is an important determinant for the activity of several membrane-bound enzymes [5] and (iii) that retinoids can effect biological membranes [6,7], one can suppose that the lipophilic retinoids may interfere with the lipid microenvironment of the enzyme, ultimately leading to an altered enzyme activity. Studying the modulating effect of lipids on the activity of membrane-bound galactosyltransferase, Mitranic et al. [8,9] established a model system, consisting of pure bovine milk galactosyltransferase and liposomes. This model system was considered to reflect the environmental situation of galactosyltransferase in natural membranes with the advantage to deal with well defined conditions.

Based on the considerations as outlined above this model system was used in the present work to test the influence of retinoids on membrane-bound galactosyltransferase, taking advantage of the modulating effect of phosphatidylserine on the transferase activity [9].

UDP[^{14}C]galactose (spec. act. 309 mCi/mmol) was from Amersham, U.K., non radioactive UDPgalactose, ovalbumin, pure bovine milk galactosyltransferase (EC 2.4.1.22, 4.6 U/mg protein), L-α-phosphatidyl-L-serine (bovine brain), retinol, retinal, retinoic acid and retinylpalmitate were purchased from Sigma Chemical Corporation, Taufkirchen, F.R.G. Retinylphosphate was a generous gift from Dr. L. De Luca (Laboratory of Cellular Carcinogenesis and Tumorpromotion, NIH, Bethesda, MD, U.S.A.). Stock solutions (3 mg/ml) of retinoids and phosphatidylserine were prepared in chloroform/methanol (2 : 1, v/v). Phosphatidylserine vesicles were prepared by sonicating the lipid in 0.1 M Mes buffer (pH 7.4) using a Bransonic bath sonifier according to [9].

For the preparation of mixed vesicles (lipid/retinoid) the lipid and the respective retinoid were added together, the solvent evaporated and proceeded as given above. Aliquots of the liposome suspension containing 0–125 nmol of phospholipid was placed in test tubes (Fisher Sci. Co. Pittsburgh, PA, U.S.A.), the volume was made up to 25 μl with 0.1 M Mes buffer (pH 7.4) and pure bovine milk galactosyltransferase (3.9 μg of protein in 15 μl buffer) was added to the liposomes and mixed. The enzyme was allowed to equilibrate with the lipid for 10 min at 37°C before the transferase assay reagents were added. The assay consisted of: 40 μl of liposome/enzyme mixture, 0.1 M Mes buffer (pH 7.4) 10 mM $MnCl_2$, 10 mg/ml ovalbumin and 30 μM UDP[^{14}C]galactose (diluted to a spec. act. of 4.2 mCi/mmol with non radioactive UDPgalactose) in a total volume of 50 μl. The transfer of [^{14}C]galactose to ovalbumin was estimated as recently reported [10]. After incubation at 37°C for 10 min the reaction was stopped by cooling to 4°C and the addition of EDTA (final concentration 0.1 M). Samples of the reaction mixture were spotted onto 2.5 cm Whatman No. 1 paper disks (Whatman Ltd., Kent, U.K.). The disks were immersed in 10% (w/v) trichloroacetic acid for 45 min, then rinsed with trichloroacetic acid and finally washed with ethanol/diethyl ether (2:1) and diethyl ether. Subsequently the disks were dried under a heat lamp and the radioactivity estimated.

Fig. 1 shows the effect of 0–125 nmol of phosphatidylserine on the activity of bovine milk galactosyltransferase to transfer galactose onto ovalbumin as acceptor. In the presence of phosphatidylserine an inhibition of the transferase an inhibition of the transferase activity was observed. At the highest phospholipid concentration tested (125 nmol) the enzyme activity was reduced to about 10% of the control activity. Similar results have been reported by [9] using *N*-acetylglucosamine as galactose acceptor. To study the effect of retinoids on the membrane form of galactosyltransferase in an enzyme/liposome model system, a phosphatidylserine concentration was chosen which modulated the transferase activity to about the half of the control activity. As seen in Fig. 1 the presence of 100 nmol of phosphatidylserine caused an about 50% inhibition of the enzyme activity. Therefore this enzyme/lipid concentration was used in all further experiments dealing with 'modulated' galactosyltransferase. Next the influence of the retinoids on the activity of the 'unmodulated' galactosyltransferase was tested. The retinoid concentration was chosen with regard to concentration (up to 10 μM) used in in vitro models for studying retinoid action [6,11,12]. When pure galactosyltransferase was preincubated in the presence of 0.5 nmol of retinoid none of these compounds had an effect on the enzyme activity with the exception of retinylphosphate which appeared to be inhibitory (data not shown).

Table I shows the influence of different retinoids on the activity of 'modulated' galactosyltransferase. At the lipid/retinoid ratio of 200:1 (nmol/nmol), retinol, retinal and retinylphosphate (although itself inhibitory) caused an about 2-fold increase in the enzyme activity. Retinoic acid and retinylpalmitate were ineffective in this system; however, higher concentrations of both compounds were not tested. These data indicate that the effect of the retinoids on the activity of 'modulated' galactosyltransferase may be related to the

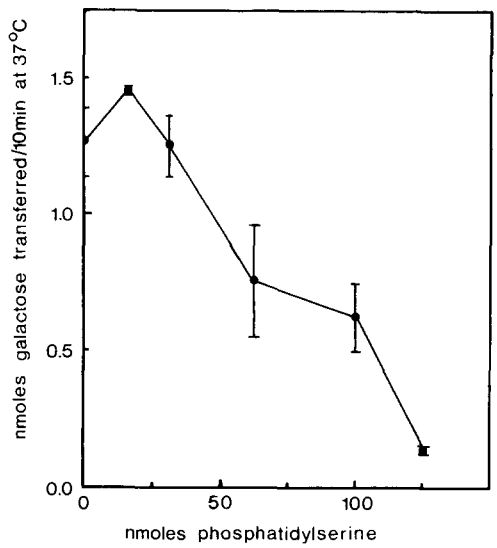

Fig. 1. The effect of phosphatidylserine on the activity of galactosyltransferase. The pure bovine milk enzyme (3.9 μg of protein) was preincubated for 10 min at 37°C with lipid vesicles (0–125 nmol). Subsequently the transferase assay reagents were added and after incubation for 10 min at 37°C the transfer of [^{14}C]galactose to ovalbumin was estimated [10]. Results are the mean of duplicate experiments.

TABLE I

THE INFLUENCE OF RETINOIDS ON THE ACTIVITY OF PHOSPHATIDYLSERINE-MODULATED GALACTOSYLTRANSFERASE

The pure bovine milk enzyme (3.9 μg of protein) was preincubated for 10 min at 37°C with phosphatidylserine vesicles (100 nmol of lipid) in the absence or presence of retinoid (0.5 nmol). Subsequently the transferase assay reagents were added and after incubation for 10 min at 37°C the transfer of [^{14}C]galactose to ovalbumin was estimated [10]. Results are the mean of triplicate experiments.

Retinoid added	nmol galactose transferred per 10 min at 37°C
None	0.56 ± 0.10
Retinol	1.08 ± 0.11
Retinal	0.99 ± 0.07
Retinoic acid	0.53 ± 0.04
Retinylphosphate	1.08 ± 0.05
Retinylpalmitate	0.40 ± 0.01

nature of the endgroup of the molecule, because all retinoids tested in this system had an identical side chain structure.

The structure of retinoids is reminescent to that of detergents and they can exert a membrane destabilizing effect [6,7]. Meeks et al. [6] have reported that both retinoids and detergents, like Triton X-100 can reduce membrane microviscosity from erythrocytes, but their results suggest that the action of retinoids is unlike that of simple detergents. With a view of this report the effect of Triton X-100 on the 'modulated' galactosyltransferase activity was tested. When Triton X-100 (final concentration was 0.05%) was included in the transferase assay done with the 'modulated' enzyme, the addition of the detergent resulted in a 2-fold increase in enzyme activity. This effect was comparable to that of retinol, retinal and retinylphosphate. In contrast, the activity of the 'unmodulated' enzyme was not effected by the detergent. With regards to these results it should be emphasized that membrane-bound galactosyltransferase i.e. from rat liver Golgi membranes [13,14] can be activated by Triton X-100, but the detergent did not activate the soluble enzyme form present in serum [14].

Using an enzyme/phospholipid system as a model for the membrane form of galactosyltransferase, the present results show that some retinoids are able to activate the phospholipid modulated enzyme. The mechanism involved in glycosyltransferase modulation by phospholipids [15,16,17] and how retinoids can interfere with this modulation is not yet known. The results presented here suggest that retinoids beside their function as intermediates in glycosylation reactions may effect the activity of certain glycosylating enzymes by influencing the microenvironment of the enzymes.

The author wishes to thank Professor Dr. G. Wolf (M.I.T., Cambridge, MA, U.S.A.) for his hospitality and Mrs. Evelyn Kainzbauer for her technical assistance.

References

1 Wolf, G. (1984) Physiol. Rev. 64, 873–937
2 De Luca, L.M. (1977) Vitam. Horm. 35, 1–57
2 Plotkin, G.M. and Wolf, G. (1980) Biochim. Biophys. Acta 615, 94–102
4 Fraser, I.H. and Mookerjea, S. (1977) Biochem. J. 164, 541–547
5 Coleman, R. (1983) Biochim. Biophys. Acta 300, 1–30
6 Meeks, R.G., Zaharevitz, D. and Chen, R.F. (1981) Arch. Biochem. Biophys. 207, 141–147
7 Dingle, J.T. and Lucy, J.A. (1965) Biol. Rev. 40, 422–461
8 Mitranic, M.M. and Moscarello, M.A. (1980) Can. J. Biochem. 58, 809–814
9 Mitranic, M.M., Boggs, J.M. and Moscarello, M.A. (1983) J. Biol. Chem. 258, 8630–8636
10 Gmeiner, B.M. (1985) Biochem. Biophys. Acta 829, 76–82
11 Kozirowska, J., Paczek, K. and Tautt, J. (1984) Exp. Cell Res. 150, 97–103
12 Rogelj, S., Loewy, B. and Niles, R.M. (1984) Eur. J. Biochem. 139, 351–357
13 Fleischer, B. and Smigel, M. (1978) J. Biol. Chem. 253, 1632–1638
14 Fraser, I.H. and Mookerjea, S. (1976) Biochem. J. 156, 347–355
15 Singh, O.M.P., Graham, A.B. and Wood, G.C. (1981) Eur. J. Biochem. 116, 311–316
16 Serres-Guillaumond, M., Broquet, P. and Louisot, P. (1984) Biochim. Biophys. Acta 794, 104–109
17 Serres-Guillaumond, M. and Louisot, P. (1983) Biochim. Biophys. Acta 751, 247–253