Biochimica et Biophysica Acta, 339 (1974) 438—441

BBA Report

BBA 71196

CELL PLASMA MEMBRANE EXTERNAL SURFACE GLYCOSYLTRANSFERASES: ACTIVITY IN THE CELL MITOTIC CYCLE

H. BRUCE BOSMANN

Department of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642 (U.S.A.)

(Received December 27th, 1973)

Summary

Activity levels of three cell surface glycoprotein:glycosyltransferases were found to be S-peak enzymes in the cell mitotic cycle of L5178Y cells. These enzymes, which may be important in cell—cell adhesion, had virtually no activity in the M-period of the cell mitotic cycle.

Roseman [1] first suggested that cell external surface glycosyltransferases and acceptors may mediate cell—cell adhesion. Since that suggestion, surface glycosyltransferases and acceptors have been identified in chick embryo neural retinal cells [2], normal and transformed fibroblasts [3,4], and human blood platelets [5—7]. The mechanism postulated is that glycoconjugate acceptors and glycosyltransferases are present on the external cell surface and that formation of the activated intermediate or complex formed between enzyme and the two substrates (acceptor and activated monosaccharide) mediates adhesion. Alternatively, aside from the direct mediation of adhesion, the glycosyl-ectoenzyme system may effect recognition, which in turn may activate an adhesion mechanism.

Cells having large complements of either surface glycosyltransferase or surface acceptor, e.g. metastasizing cells [8], might adhere to nonsimilar cells also having high complements of either surface glycosyltransferase or acceptor. Hence, a cell from a tumor that is metastasizing might be expected to adhere to circulating platelets or other tissue cell sites having high levels of the ectoenzyme systems [8].

Recently we [9] have shown that L5178Y cells, which grow in suspension culture in vitro and ascitically in vivo, probably not making much

cell—cell contact during their lifetime, have cell surface glycoprotein:glycosyltransferases and acceptors. Trypsinization (1%, 10 min, 37 °C) of the cell either before or after the reaction took place essentially resulted in no activity of any of the systems studied, indicating that both the enzyme and the acceptor are located on the cell periphery. The fact that in these suspension culture cells glycosyltransferases and acceptors exist on the cell surface may point to the universality of the adhesion mechanism. An alternative explanation is that the surface glycosyltransferases and acceptors merely reflect Golgi-derived enzymes and acceptors carried to the plasma membrane during plasma membrane biogenesis and have no function for cell—cell adhesion. In this event, the surface glycosyltransferases are still of extreme importance because they could bind and/or glycosylate extracellular soluble proteins or glycoproteins. Because of the interest and the importance of these ectoenzymes, we undertook to determine activity levels in synchronized L5178Y cell populations.

L5178Y cells (mouse lymphoma cell line) were grown in suspension culture in sealed containers in Fischer's medium [11] with 10% horse serum and were used in the exponential growth phase. Cell numbers were determined in a Coulter counter. Cells were synchronized by the method of Doida and Okada (see refs 11—14) by applying one treatment with excess thymidine followed by one treatment with colcemid and deoxycytidine. Each hour after release from the colcemid block, 30 ml of cell suspension were centrifuged out of solution at 2500 × *g* for 5 min and assayed for cell surface ectoenzymes as given in the figure legend. Following the 30-min incubation, 99.4% of the cells were viable by dye adhesion tests; no glycosyltransferases, glycosidases, or intercellular enzymes were detectable in the 2500 × *g* incubation supernatant. No measurements of ectoglycosidase activities were made. Fetuin and its degraded products used as acceptors in the reactions were prepared as described previously [15]. Simultaneously with the sampling for analysis, 3 ml of cells were centrifuged out of solution, resuspended in 0.2 ml of Fischer's medium containing 10 μCi of [^{3}H]thymidine (15 Ci/mmole) and incubated at 37 °C for 5 min. The incubation was terminated with 10% trichloroacetic acid, the solution was centrifuged, and the insoluble pellet was washed twice with 10% trichloroacetic acid and once with ethanol—diethylether (2:1, v/v), dissolved in 1 M NaOH, plated on a glass filter disc, and counted in a liquid scintillation counter. Counts per minute from zero-time incubations which were precipitated immediately were subtracted from these results. This radioactive incorporation of [^{3}H]thymidine is an indication of DNA synthesis in the cell and is a useful measure of synchrony and the S-period. Protein was determined by the method of Lowry et al. [16]. Crystalline bovine serum albumin was used as a standard. Protein per cell increased linearly from about 0.9 mg/10^7 cells to about 1.7 mg/10^7 cells after about 9.5 h and then dropped back to 0.9 mg/10^7 cells. In general, there was a doubling of the cells at 9.5—10 h after release from the M-block. Further details of these methods are given in previous publications [12—14].

The data given in Fig. 1 indicate that each of the three ectoenzyme

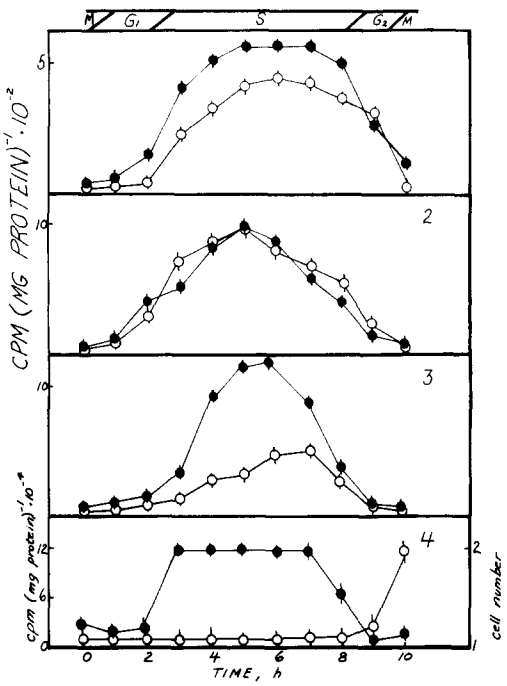

Fig.1. Activity of cell surface glycoprotein:glycosyltransferases and acceptors in synchronous L5178Y cells. Culture and synchrony of cells were as described previously [11—14]. Experiments were performed by harvesting cells in the indicated growth phase, washing them once with 0.1 M Tris (pH 7.6), saline, suspending 0.1 to 0.3 mg (as protein) in 0.1 ml Fischer's medium without serum, and adding the following, to a final volume of 0.180 ml: 10 μl of 0.1 M $MgCl_2$, 10 μl of 0.1 M $MnCl_2$, 50 μl of acceptor protein (0.9 mg of protein) for exogenous activity or 50 μl of water for endogenous activity, and 10 μl of a 3.3 μCi/ml solution of ^{14}C-labeled precursor (nucleotide [^{14}C] monosaccharide; specific activity, 90 Ci/mole). The mixtures were incubated for 30 min at 37 °C (all incorporations of [^{14}C]monosaccharide were linear with respect to time for 1 h), after which 3 volumes of 1% phosphotungstic acid in 0.5 M HCl were added and the precipitate was centrifuged out of solution. The precipitate was washed twice with 10% trichloroacetic acid, once with ethanol—diethylether (2:1, v/v), and dissolved in 1 M NaOH, and the radioactivity was determined in a liquid scintillation counter. Data are cpm per mg protein and means ± 1 S.D. from five independent observations. ○, endogenous activity; ●, exogenous activity. Exogenous activity is total activity minus endogenous activity. Panel 1: fetuin: sialyl surface activity (fetuin minus *N*-acetylneuraminic acid; CMP—^{14}C-labeled *N*-acetylneuraminic acid as substrates). Panel 2: fetuin:galactosyl surface activity (fetuin minus *N*-acetylneuraminic acid, galactose UDP—[^{14}C]galactose as substrates). Panel 3: fetuin:*N*-acetylglucosaminyl activity (fetuin minus *N*-acetylneuraminic acid; galactose, *N*-acetylglucosamine as substrates). Panel 4: DNA synthesis during the cell mitotic cycle as measured by incorporation of [^{3}H]thymidine into acid-insoluble material (●) and cell number (Coulter counter; ○) in arbitrary units. A representation of the cell cycle is given above the four panels [11—14]. Cells were released from mitotic block just before 0 h.

systems studied was essentially an S-peak enzyme in the L5178Y mitotic cell cycle (that is, the enzyme had peak activity in the S-period of the cell cycle and was not a 'continuous' or 'step' enzyme). Each enzyme showed a rather distinct pattern: the sialyl-enzyme system, a broad peak of activity; the galactosyl-enzyme system, a less broad peak of activity; and the *N*-acetylglucosaminyl-enzyme system, a fairly sharp peak of activity in the S-phase. Both endogenous (measurement of monosaccharide transfer onto the same

or an adjacent cell glycoconjugate) and exogenous (measurement of monosaccharide transfer onto exogenously added glycoprotein, e.g. fetuin minus *N*-acetylneuraminic acid) followed rather similar patterns of activity during the cell mitotic cycle. There was virtually no activity of these ectoenzymes during the M-periods.

It has previously been demonstrated that glycoprotein synthesis occurs primarily in the S-phase of L5178Y cells and certain lipid/glycolipid synthesis occurs specifically in non-S periods of the cell cycle [17]; these results have recently been confirmed in KB cells [18]. Furthermore, total cell glycoprotein:glycosyltransferases have been shown [19] to be cell mitotic cycle S-peak enzymes, although activity distribution was somewhat different and considerable activity was present in the M-phase [19]. Therefore, the present results may merely reflect whole total (primarily Golgi) cell activity patterns. However, consistent with the hypothesis that the ectoenzymes mediate cell—cell adhesion, adhesion would be expected to be maximal during the S-phase and minimal during the M-phase when indeed (a) cell adhesion to glass or to other cells is negligible, (b) normal cell surfaces resemble "malignant" cell surfaces, and (c) cells grown in monolayer round up [20—22]. Thus in the M-phase, when cell adhesion is minimal, activity of the presently studied surface-active glycosyltransferases is also minimal.

This work was supported in part by grant CA-13220 from the National Institutes of Health. The author is a Research Career Development Awardee of NIGMS. I thank K.R. Case, R. Ball, and D. DeHond for excellent technical assistance.

References

1 Roseman, S. (1970) Chem. Phys. Lipids 5, 270—297
2 Roth, S., McGuire, E.J. and Roseman, S. (1971) J. Biol. Chem. 251, 536—540
3 Roth, S. and White, D. (1972) Proc. Natl. Acad. Sci. U.S. 69, 485—489
4 Bosmann, H.B. (1972) Biochem. Biophys. Res.Commun. 48, 523—529
5 Jamieson, G.A., Urban, C.L. and Barber, A.J. (1971) Nature New Biol. 234, 5—7
6 Bosmann, H.B. (1972) Biochim. Biophys. Acta 279, 436—442
7 Bosmann, H.B. (1971) Biochem. Biophys. Res. Commun. 43, 1118—1123
8 Bosmann, H.B., Bieber, G.F., Brown, A.E., Case, K.R., Gersten, D.M., Kimmerer, T.W. and Lione, A. Nature 246, 487—489
9 Bosmann, H.B., Bernacki, R.J. and Kirschbaum, B.B. (1974) Proc. Colloq. Int. sur les Glycoconjugates (Montrueil, J., ed.) CNRS, Paris, in press
10 Fischer, G.A. and Sartorelli, A.C. (1964) Methods Med. Res. 10, 246—262
11 Doida, Y. and Okada, S. (1967) Exp. Cell. Res. 48, 540—548
12 Bosmann, H.B. (1970) Biochim. Biophys. Acta 203, 256—260
13 Bosmann, H.B. and Bernacki, R.J. (1970) Exp. Cell Res. 61, 479—486
14 Bosmann, H.B. (1971) J. Biol. Chem. 246, 3817—3823
15 Bosmann, H.B. (1970) Eur. J. Biochem. 14, 33—40
16 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
17 Bosmann, H.B. and Winston, R.A. (1970) J. Cell Biol. 45, 23—33
18 Chatterjee, S., Sweeley, C.C. and Velicci, L.F. (1973) Biochem. Biophys. Res. Commun. 54, 585—590
19 Bosmann, H.B. (1971) Arch. Biochem. Biophys. 145, 310—318
20 Poste, G. (1972) Exp. Cell Res. 73, 319—328
21 Rubin, H. (1972) Science 167, 1271—1272
22 Burger, M.M. (1971) Growth Control in Cell Cultures (Wolstenholme, G.E.W. and Knight, J., eds) Churchill and Livingstone, London