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QUANTITATION OF *N*-ACETYL-D-GALACTOSAMINE-LIKE SITES ON THE SURFACE MEMBRANE OF NORMAL AND TRANSFORMED MAMMALIAN CELLS

BEN-AMI SELA, HALINA LIS, NATHAN SHARON AND LEO SACHS
Departments of Genetics and Biophysics, The Weizmann Institute of Science, Rehovot (Israel)
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SUMMARY

Measurements of the binding of ^{125}I-labeled soybean agglutinin to cells cultured with fetal calf serum have shown, that there can be a similar number of D-GalNAc-like sites exposed on normal and transformed mouse and rat cells; that there were only 10 % of such sites on transformed hamster cells; and that treatment with pronase can render normal cells agglutinable by soybean agglutinin without increasing the total number of exposed D-GalNAc-like sites.

INTRODUCTION

Studies with the carbohydrate-binding protein Concanavalin A[1,2], and glycoproteins from wheat germ[3], and soybean[4,5], have shown a difference in the structural organisation of the surface membrane in normal and malignant transformed cells. The soybean agglutinin that interacts with *N*-acetyl-D-galactosamine (D-GalNAc)-like sites on the surface membrane[4] can agglutinate transformed mouse, rat and human cells, but does not agglutinate the parental normal cells unless they have been treated for a short time with a proteolytic enzyme[5]. Transformed hamster cells are not agglutinated by soybean agglutinin unless they have been treated for a prolonged period with pronase[5].

Measurements of the binding of labeled Concanavalin A molecules to cells cultured under different conditions have shown that the change in the structure of the surface membrane in cell transformation can be explained by three types of changes in binding sites. There can be an exposure of cryptic sites, a concentration of exposed sites by a decrease in cell size, and a re-arrangement of exposed sites without a decrease in cell size resulting in a clustering of sites[2,6,7].

The present experiments were undertaken to quantitate the D-GalNAc-like sites on the surface membrane of normal and transformed cells by using soybean agglutinin labeled with ^{125}I. Cells were grown under conditions in which a similar number of Concanavalin A molecules were bound to normal and transformed cells[6,7].

MATERIALS AND METHODS

The cell types used in the present experiments were lines of golden hamster cells transformed by polyoma virus and simian virus 40, or by the chemical carcinogen

dimethylnitrosamine[8]; normal hamster cells derived from secondary cultures of 13–14-day old embryos; 3T3 cells transformed by polyoma virus, by simian virus 40 (SV40), and doubly transformed by polyoma and SV40; untransformed 3T3 cells; rat cells transformed by polyoma virus, and normal cells from secondary cultures of rat embryos. Cells were grown as described with fetal calf serum[6,7], and used for experiments 3–4 days after seeding.

The soybean agglutinin was isolated and purified as previously described[9]. The labeling was performed using the chloramine-T method of GREENWOOD *et al.*[10]. 5 mg of the protein were dissolved in 0.7 ml of Ca^{2+} and Mg^{2+} free phosphate-buffered saline (which will be referred to as saline) and 0.05 ml of a $Na^{125}I$ solution (Radiochemical Centre, Amersham, carrier free $Na^{125}I$, 20 mC/ml) were added, followed by 0.1 ml of 0.1 % chloramine-T solution in saline. The mixture was stirred in an ice bath for 5 min, 0.1 ml of 0.24 % sodium metabisulphite in saline was added and the mixture was stirred for another 2 min. The labeled protein was separated from the low-molecular-weight radioactive material by gel filtration on a Sephadex G-150 column (27 cm × 0.7 cm) in saline. The fractions containing the labeled protein were combined and dialysed for 2 h against saline.

To measure the binding of labeled soybean agglutinin, cells were washed twice with saline and removed from the Petri dish with a 0.02 % disodium versenate solution[1]. 10^7 cells were mixed with 2 ml saline containing varying amounts of the labeled agglutinin and incubated at 24° for 30 min (ref. 2). The cells were then washed 4 times with 12 ml saline. The final pellet was suspended in 1 ml saline, and the radioactivity measured in a Packard γ counter. Triplicate samples were taken for each point. The results were reproducible with a variation of ± 10 %. Adsorbed radioactivity was calculated on the basis of cell surface area, which was determined by measuring cell diameter with a calibrated ocular, and by measuring the volume of a given amount of cells, centrifuged in a graduated capillary tube[2].

RESULTS

The specificity of binding of soybean agglutinin to the cell surface was measured by preincubating the labeled agglutinin with different saccharides for 30 min before

TABLE I

INHIBITION BY VARIOUS SACCHARIDES OF THE BINDING OF ^{125}I-LABELED SOYBEAN AGGLUTININ TO THE CELL SURFACE

Saccharide added (0.5 *M*)	*Counts/min per* 10^7 *cells incubated with 25 µg/ml labeled agglutinin*		
	3T3 polyoma	*Rat polyoma*	*Hamster polyoma*
—	73 962	201 000	16 221
Sialic acid	59 304	171 403	16 307
L-Fucose	67 048	167 891	15 461
α-Methyl-D-mannose	76 420	193 045	16 003
N-Acetyl-D-glucosamine	69 386	178 068	15 798
α-Methyl-D-glucose	70 002	187 186	15 096
D-Galactose	49 385	131 860	13 207
N-Acetyl-D-galactosamine	5 921	13 861	10 628

adding to the cells. Similar results were obtained with 0.05–0.5 M saccharide, and the results with 0.5 M are given in Table I. It is clear that D-GalNAc is a specific inhibitor of the binding of labeled soybean agglutinin to the cell surface. In the presence of D-GalNAc, the adsorption of labeled agglutinin to 3T3 SV40 and rat polyoma transformed cells was reduced by 92 %. There was 30–50 % inhibition with D-galactose, whereas all other saccharides tested showed a weak or no inhibition. The non-specific binding, *i.e.* the amount of radioactivity bound to cells in the presence of D-GalNAc, was similar when the agglutinin was pre-incubated with D-GalNAc before adding to the cells, and when D-GalNAc was added after the cells had been incubated with the agglutinin. Non-specific binding was most pronounced with the transformed hamster cells.

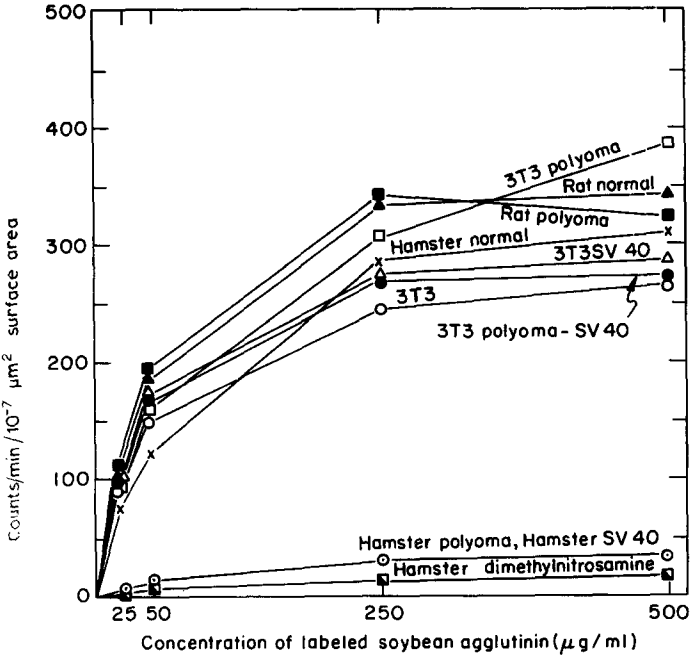

Fig. 1. D-GalNAc-specific binding of labeled soybean agglutinin to normal and transformed cells.

To calculate the specific binding of labeled agglutinin, the non-specific counts were subtracted from the total number of counts adsorbed. The number of agglutinin molecules specifically bound per μm^2 cell surface was calculated from the molecular weight of soybean agglutinin (100 000; ref. 9), and the specific labeling of the agglutinin ($5 \cdot 10^7$ counts/min per mg). The results (Fig. 1, Table II) show that in the normal and transformed rat cells, there was no difference in the number of agglutinin molecules bound per μm^2 surface area. A similar result was found with the untransformed 3T3 cell line and its SV40 and doubly transformed lines, whereas a small increase (30 %) was found in the 3T3 polyoma transformed cell line. The specific binding of agglutinin per μm^2 in the transformed hamster cells was, however, only 5–10 % of the binding on normal hamster cells. The drastic decrease in the number of D-GalNAc-like sites

TABLE II

D-GalNAc-SPECIFIC BINDING OF SOYBEAN AGGLUTININ PER μm^2 CELL SURFACE

The cells were incubated with 500 μg/ml labeled agglutinin.

Cells	*D-GalNAc-specific adsorption per 10^7 cells (counts/min)*	*Number of molecules adsorbed per cell ($\times 10^{-6}$)*	*Surface area per cell (μm^2)*	*Number of molecules per μm^2 ($\times 10^{-3}$)*
Hamster normal	329 098	4.00	1070	3.7
Hamster polyoma	29 054	0.35	870	0.4
Hamster dimethylnitrosamine	13 747	0.16	840	0.2
Hamster SV40	31 013	0.37	920	0.4
Rat normal	427 983	5.1	1260	4.1
Rat polyoma	531 019	6.4	1630	3.9
3T3	601 427	7.3	2270	3.2
3T3 SV 40	227 086	2.7	810	3.3
3T3 polyoma	288 312	3.4	760	4.5
3T3 polyoma-SV40	201 343	2.4	730	3.3

on transformed hamster lines can probably explain the non-agglutinability of these cells.

Normal hamster and rat cells, and untransformed 3T3 cells, are rendered agglutinable by soybean agglutinin after incubation with 1 μg pronase per ml for 10 min. There was no difference or a small decrease in the number of labeled molecules adsorbed to cells treated with pronase.

DISCUSSION

The present results indicate that under the culture conditions used, there was generally no exposure of D-GalNAc-like sites on the surface membrane by cell transformation or treatment with a proteolytic enzyme, as was also found with Concanavalin A binding sites on cells grown under the same conditions[6,7]. In addition to the mechanism of the re-arrangement of sites to produce clustering[6] that can explain agglutinability by agglutinins, the removal of interfering structures close to the binding site by transformation or treatment with the enzyme may also play a role in rendering the cells agglutinable.

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REFERENCES

1 M. INBAR AND L. SACHS, *Proc. Natl. Acad. Sci. U.S.*, 63 (1969) 1418.
2 M. INBAR AND L. SACHS, *Nature*, 223 (1969) 710.
3 M. M. BURGER, *Proc. Natl. Acad. Sci. U.S.*, 62 (1969) 994.

4 H. LIS, B. SELA, L. SACHS AND N. SHARON, *Biochim. Biophys. Acta*, 211 (1970) 582.
5 B. SELA, H. LIS, N. SHARON AND L. SACHS, *J. Membrane Biol.*, 3 (1970) 267.
6 H. BEN-BASSAT, M. INBAR AND L. SACHS, *J. Membrane Biol.*, in the press.
7 M. INBAR, H. BEN-BASSAT AND L. SACHS, *J. Membrane Biol.*, in the press.
8 E. HUBERMAN, S. SALZBERG AND L. SACHS, *Proc. Natl. Acad. Sci. U.S.*, 59 (1968) 77.
9 H. LIS, N. SHARON AND E. KATCHALSKI, *J. Biol. Chem.*, 241 (1966) 684.
10 F. C. GREENWOOD, W. H. HUNTER AND J. S. GLOVER, *Biochem. J.*, 89 (1965) 114.