MOBILITY OF CARBOHYDRATE CONTAINING SITES ON THE SURFACE MEMBRANE IN RELATION TO THE CONTROL OF CELL GROWTH

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1. Introduction

Molecules that bind specifically to carbohydrate containing sites on the surface membrane can be used to elucidate changes in the surface membrane associated with changes in the regulation of cell growth. Using as a probe the carbohydrate-binding protein concanavalin A (Con.A) differences between normal and malignant transformed cells have been shown in Con.A-induced cell agglutinability [1–5], the number and distribution of Con.A binding sites [6–9], the location of amino acid and carbohydrate transport sites [10], Con.A induced cell toxicity [11–13], the mobility of Con.A binding sites [14, 15] and membrane stability and the level of cellular ATP [16]. Changes in the distribution of Con.A binding sites [7, 14, 15] and the movement of antigens on the cell surface [17–20], have indicated that receptors can be mobile in a fluid surface membrane [21]. The present studies were undertaken to determine the mobility of Con.A binding sites on the surface membrane in relation to the control of growth, in cells that form a solid tissue and in cells that are in suspension *in vivo*. We have included in these studies normal and transformed fibroblasts as examples of normal and malignant cells that form a solid tissue, and normal lymphocytes and lymphoma cells as examples of normal and malignant cells that are in suspension.

Our results indicate that binding sites for Con.A are floating in the surface membrane in a random distribution. Given the appropriate membrane fluidity, the distribution of sites can be changed by binding of Con.A, followed by movement of the Con.A membrane site complexes on the surface membrane to form a new distribution. The final distribution after Con.A binding was clusters of sites in transformed fibroblasts and lymphoma cells, a concentration of most of the sites on one pole of the membrane to form a cap in normal lymphocytes and what appeared to be no or almost no change from the original random distribution in normal fibroblasts. The order of site mobility was therefore normal fibroblasts < transformed fibroblasts and lymphoma cells < normal lymphocytes. The mobility of Con.A sites was increased by increasing cross-linking with anti-Con.A antibodies or glycogen and by trypsin treatment of the cells before the binding of Con.A. The results suggest, that the mobility of Con.A binding sites can be used as a probe for the fluid state of this specific carbohydrate structure on the surface membrane. Using this proble, we suggest that in cells that form a solid tissue, the transformation of normal into malignant cells is associated with an increase in membrane fluidity of the carbohydrate structure where the Con.A sites are located. However, in cells that are in suspension *in vivo*, the malignant transformation is associated with a decrease in fluidity of this carbohydrate structure on the cell surface. This increased fluidity of the membrane in transformed fibroblasts can explain their lack of contact inhibition, their ability to grow in soft agar and their malignancy in a solid tissue.

Abbreviations:
Con.A: concanavalin A; F-Con.A: fluorescent Con.A; PBS: phosphate-buffered saline.

2. Materials and methods

The transformed fibroblasts used were a line of golden hamster embryo cells transformed *in vitro* after treatment with the chemical carcinogen dimethyl-

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nitrosamine [1], and lines derived from a Simian virus 40 and Rous sarcoma virus induced hamster tumors (Flow Laboratories, Bethesda, Md.). The normal fibroblasts were from secondary cultures of golden hamster embryos. Normal and transformed fibroblasts were cultured in Eagle's medium with a 4-fold concentration of amino-acid and vitamins and 10% fetal calf serum. For the experiments, normal and transformed fibroblasts at 3 or 4 days after seeding were dissociated with 0.02% EDTA by incubation for 15–30 min at 37°, and the dissociated cells washed 3 times with phosphate-buffered saline (pH 7.2) (PBS). Normal lymphocytes were obtained from adult rat lymph nodes as described [5]. Lymphoma cells were obtained from an ascites form of a Moloney virus induced lymphoma grown in A strain mice [5]. This is a T cell lymphoma. 10^5 lymphoma cells were inoculated intraperitoneally into mice and the cells were used in experiments after 3 washings with PBS. The mouse myeloid leukemia cells were from a cloned (D^-) line cultured *in vitro* [22, 23]. Con.A, fluorescein isothiocyanate conjugated Con.A (F-Con.A) at a ratio of 1.86 fluorescein to protein, and goat anti-Con.A antibodies (IgG fraction) were obtained from Miles-Yeda. In the experiments with F-Con.A, the binding was carried out at saturation, cells were fixed for 60–120 min with formaldehyde and glutaraldehyde as described [14, 15], and treated with $LaCl_3$, NaN_3 and DNP for 30 min. Experiments with $LaCl_3$ were carried out in Tris buffer.

3. Results and discussion

3.1. Final distribution of Con.A binding sites on the surface membrane of lymphoma cells and normal lymphocytes after binding of Con.A

In order to determine the final distribution of Con.A binding sites on the surface membrane, we have examined the interaction of F-Con.A with normal and malignant cells. In agreement with previous results [14], the surface binding of F-Con.A in 99% of the stained lymphoma cells was in small or large clusters of fluorescence that formed an incomplete ring on the cell periphery (fig. 1C). However, about 30% of the stained normal lymphocytes gave a polar fluorescence cap, covering about half of the cell surface area (fig. 1D). Pre-incubation of cells with NaN_3 and DNP inhibited cap formation in normal lymphocytes, but did not inhibit cluster formation in normal lymphocytes and lymphoma cells (table 1). Cap but not cluster formation, therefore requires energy. Formation of caps was also inhibited by low temperature (0°). Addition of NaN_3 or DNP to cells after binding of Con.A resulted in dissociation of the caps but not of the clusters.

Table 1
Inhibition of movement of Con.A binding sites induced by Con.A in normal lymphocytes.

Treatment	Inhibition of cluster formation*	Inhibition of cap formation
Formaldehyde 10%	+	+
Glutaraldehyde 2.5%	+	+
$LaCl_3$ 10^{-3} M	+	+
NaN_3 10^{-2} M	–	+
DNP 10^{-3} M	–	+

+ = Inhibition; – = not inhibited.

* Formaldehyde, glutaraldehyde and $LaCl_3$ also inhibited cluster formation in transformed fibroblasts and lymphoma cells.

3.2. Final distribution of Con.A binding sites on the surface membrane of transformed and normal fibroblasts after binding of Con.A

Experiments on binding of F-Con.A to the surface membrane of fibroblasts have indicated that in 99% of the transformed fibroblasts from the 3 cell lines tested, the surface binding of F-Con.A was in clusters of fluorescence over the cell surface as shown in fig. 1C. Pre-incubation with NaN_3 or DNP did not inhibit the cluster formation. However, most of the normal fibroblasts gave a diffuse random fluorescence covering the surface membrane (fig. 1B). Binding of F-Con.A after fixation of the fluid surface membrane of normal and transformed fibroblasts, normal lymphocytes and lymphoma cells, with aldehyde or $LaCl_3$, resulted in an apparently complete random distribution of Con.A binding sites (fig. 1A, table 1). Fixation after Con.A binding showed the same distributions as Con.A binding without fixation. With all cell types tested, 95–100% of the cells were stained at concentrations higher than 5 μg F-Con.A/ml. The binding of F-Con.A to the membrane was specific, since it was completely inhibited when F-Con.A was pre-incubated with 0.1 M α-methyl D-mannopyranoside as a hapten inhibitor.

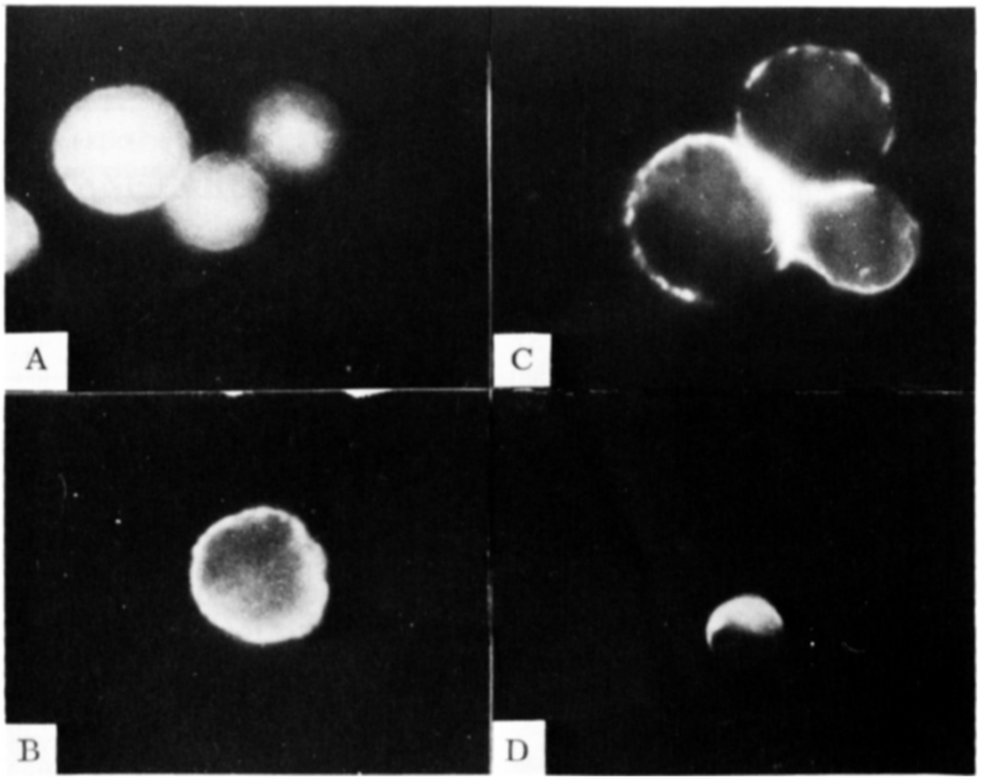


Fig. 1. Distribution of F-Con.A on the surface membrane of normal and transformed cells. A) Binding of F-Con.A to lymphoma cells after fixation with 2.5% glutaraldehyde. Similar results were obtained with normal lymphocytes, normal fibroblasts and transformed fibroblasts. Cells were fixed with 2.5% glutaraldehyde in PBS for 60–120 min, incubated with 0.2 M glycine in PBS for 10 min and then washed 3 times with PBS before incubation with F-Con.A. The binding of F-Con.A shows an apparently complete random distribution. B) Distribution of F-Con.A of the type seen with normal fibroblasts with little or no change from the random distribution. C) Distribution of F-Con.A of the type seen with transformed fibroblasts and lymphoma cells, the formation of clusters. D) Cap formation in normal lymphocytes. Cells were incubated with 100 μg F-Con-A/ml for 15 min at 37°, washed with PBS and the fluorescence determined with a Leitz Ortholux microscope with transmitted UV light × 2500.

3.3. Movement of Con.A binding sites induced by ConA

Differences in the final distribution of F-Con.A on the surface membrane could be due to movement of Con.A molecules, movement of membrane sites in the absence of Con.A, or movement of Con.A membrane site complexes. The experiments were carried out at saturation conditions where all the membrane sites were occupied by Con.A and this excludes the possibility that the differences were only due to movement of Con.A molecules. The second possibility was excluded by the following experiment. Incubation of normal lymphocytes with F-Con.A at 0° or 37° resulted in binding of F-Con.A, but the cells only formed caps at 37°. To determine whether the formation of caps at 37° is a result of movement of membrane sites without Con.A, normal lymphocytes were incubated at 0° and 37° for 30 min, followed by aldehyde fixation at the 2 temperatures and F-Con.A was added after fixation. The results showed that cap formation was completely abolished when Con.A was added after fixation, indicating that caps were not formed as a result of movement of membrane sites without Con.A.

The binding of F-Con.A to the cell membrane suggests that the binding sites for Con.A are floating in a fluid membrane in a random distribution in normal and transformed cells. The results indicate that this random distribution can be changed by interaction with Con.A, followed by movement of the membrane site Con.A complexes in the surface membrane to form a new distribution and that the final distribution of sites was different in the different cell types studied

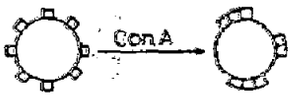
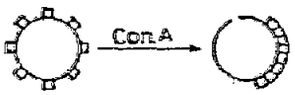
Cell type	Change in distribution of sites induced by Con.A	Final distribution of sites induced by Con.A
Normal fibroblasts		Random
Transformed fibroblasts and lymphoma cells		Clusters
Normal lymphocytes		Caps

Fig. 2. Model of the changes in distribution of sites induced by Con.A in normal fibroblasts (random), transformed fibroblasts and lymphoma cells (clusters) and normal lymphocytes (caps).

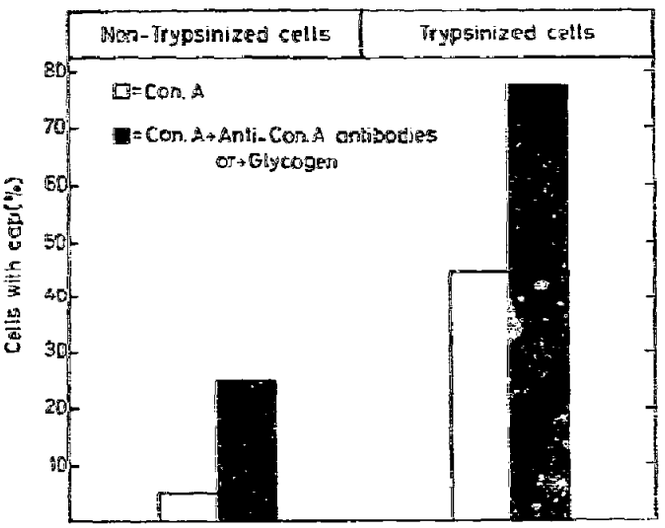


Fig. 3. Percentage of cells with caps in a mouse myeloid leukemic cell line. Cells with and without trypsin treatment (1 μg purified trypsin/ml for 15 min at 37°) were incubated with 100 μg F-Con.A/ml for 15 min at 37° (□) followed by incubation with 100 μg anti-Con.A antibodies or glycogen/ml for 15 min at 37° (■).

(fig. 2). Cells from a mouse myeloid leukemia cell line in which about 5% of the cells formed caps with F-Con.A, were used to obtain further evidence on the induction of movement by Con.A The results show that when a higher cross-linking was induced by adding anti-Con.A antibodies or glycogen after the binding of Con.A, the percent of cells with caps increased from about 5% to 25%. Trypsinization of the cells increased cap formation to about 45% and addition of anti-Con.A antibodies or glycogen increased the percentage of cells with caps in the trypsinized cells to about 75% (fig. 3). These data support the conclusion that the movement of sites is induced by Con.A, and also show that trypsinization of the cells increased the mobility of the sites. Previous results have shown [14] that the about 20% cap formation with F-Con.A in normal lymphocytes increased to about 90% when Sepharose conjugated Con.A with a high local concentration of Con.A was used. Fixation of the surface by formaldehyde, glutaraldehyde or $LaCl_3$ before adding Con.A completely inhibited the mobility of Con.A membrane site complexes to form either clusters or caps (table 1). Binding experiments with radioactive Con.A have indicated, that a similar number of Con.A molecules are bound to the fixed and unfixed cells [15]. The increased mobility of sites after trypsinization of the cells indicates that the increased Con.A agglutinability after trypsinization [1] is due to an increased membrane fluidity.

3.4. Mobility of Con.A binding sites and the differential agglutination of normal and transformed cells

The experiments on binding F-Con.A to normal and transformed cells indicate, that the degree of site mobility increased from no or almost no change in the random distribution in normal fibroblasts to the formation of clusters in the transformed fibroblasts and lymphoma cells, to the formation of caps in normal

Table 2
Final distribution of Con.A binding sites induced by Con.A and the agglutination of normal lymphocytes by Con.A.

Treatment	Final distribution of Con.A binding sites induced by Con.A	Agglutination by 100 μg Con.A/ml
None	Caps	±
NaN_3 10^{-2} M	Clusters	++++
$LaCl_3$ 10^{-3} M	Random	±

lymphocytes (fig. 2). Agglugination experiments with Con.A indicate, that only the cells with the intermediate degree of site mobility that form clusters are highly agglutinating cells. Normal fibroblasts with a low degree of site mobility and normal lymphocytes with a high degree of mobility have a low agglutinability. In each cell system, a similar number of radioactively labeled Con.A molecules were bound to normal and transformed cells per unit cell protein. The results indicate that the formation of a threshold amount of cluster formation of Con.A binding sites is required for agglutination. Further evidence for this assumption was obtained by producing the 3 degrees of site mobility in the same cell type. Normal lymphocytes were treated with $LaCl_3$ or NaN_3, and the untreated and treated cells tested for Con.A agglutinability. The untreated normal lymphocytes with caps and the $LaCl_3$ treated lymphocytes with a random distribution, showed a low degree of agglutination by Con.A. However, the NaN_3 treated lymphocytes which had a clustered distribution, also had a high degree of agglutinability (table 2).

We suggest from these results that in cells that form a solid tissue, the transformation of normal into malignant cells is associated with an increase in membrane fluidity of the carbohydrate structure where the Con.A sites are located. However, in cells that are in suspension *in vivo*, the malignant transformation is associated with a decrease in fluidity of this carbohydrate structure on the surface membrane. This increased fluidity of the membrane in transformed fibroblasts can explain their lack of contact inhibition, their ability to grow in soft agar and their malignancy in a solid tissue. Differences in membrane fluidity may also explain differences in cellular response to hormones, the ability of cells to respond to differentiation inducing stimuli, and the behavior of cells in mitosis [8].

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References

[1] M. Inbar and L. Sachs, Proc. Natl. Acad. Sci. U.S. 63 (1969) 1418.
[2] H. Ben-Bassat, M. Inbar and L. Sachs, Virology 40 (1970) 854.
[3] M. Inbar, H. Ben-Bassat and L. Sachs, Proc. Natl. Acad. Sci. U.S. 68 (1971) 2748.
[4] M. Inbar, H. Ben-Bassat and L. Sachs, Nature New Biology 236 (1972) 3.
[5] M. Inbar, H. Ben-Bassat and L. Sachs, Exper. Cell. Res. 76 (1973) 143.
[6] M. Inbar and L. Sachs, Nature 233 (1969) 710.
[7] H. Ben-Bassat, M. Inbar and L. Sachs, J. Membrane Biol. 6 (1971) 183.
[8] J. Shoham and L. Sachs, Proc. Natl. Sci. U.S. 69 (1972) 2479.
[9] G.L. Nicolson, Nature New Biology 239 (1972) 193.
[10] M. Inbar, H. Ben-Bassat and L. Sachs, J. Membrane Biol. 6 (1971) 195.
[11] J. Shoham, M. Inbar and L. Sachs, Nature 227 (1970) 1244.
[12] M. Inbar, H. Ben-Bassat and L. Sachs, Int. J. Cancer 9 (1972) 143.
[13] Y. Wollman and L. Sachs, J. Membrane Biol. 10 (1972) 1.
[14] M. Inbar, H. Ben-Bassat and L. Sachs, Int. J. Cancer (1973) in press.
[15] M. Inbar, H. Ben-Bassat, L. Sachs, C. Huet and A.R. Oseroff, Biochim. Biophys. Acta (1973) in press.
[16] I. Vlodavsky, M. Inbar and L. Sachs, submitted for publication.
[17] R.B. Taylor, W.P.H. Duffus, M.C. Raff and S. de Petris, Nature New Biology 233 (1971) 225.
[18] I. Yahara and G.M. Edelman, Proc. Natl. Acad. Sci. U.S. 69 (1972) 608.
[19] M. Edidin and A. Weiss, Proc. Natl. Acad. Sci. U.S. 69 (1972) 2456.
[20] F. Loor, L. Forni and B. Pernis, Europ. J. Immunol. 2 (1972) 203.
[21] S.J. Singer and G.L. Nicolson, Science 175 (1972) 720.
[22] E. Fibach, T. Landau and L. Sachs, Nature New Biology 237 (1972) 276.
[23] E. Fibach, M. Hayashi and L. Sachs, Proc. Natl. Acad. Sci. U.S. 70 (1973) 343.