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INHIBITION OF LECTIN AGGLUTINABILITY BY FIXATION OF THE CELL SURFACE MEMBRANE

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SUMMARY

Formation of clusters of Concanavalin A binding sites on the surface membrane of lymphoma cells is inhibited by aldehyde fixation of the fluid state of the membrane. Fixation also inhibits cell agglutination by soluble Concanavalin A and binding of cells to Sepharose-conjugated Concanavalin A beads, although there is a similar binding of radioactively labeled concanavalin A molecules to fixed and unfixed cells. Movement of concanavalin A sites on the surface membrane to form clusters is therefore required for cell agglutination. Different degrees of membrane fixation by aldehydes inhibit agglutination by the lectins from wheat germ and soybean.

INTRODUCTION

Lectins can bind specifically to carbohydrate-containing sites on the surface membrane of normal and malignant transformed cells. Among such molecules are Concanavalin A[1] and the lectins from wheat germ[2–5] and soybean[6]. Interaction of these lectins with specific sites can be used as a probe to study structure and function of the cell membrane. Using this probe, differences between normal and malignant transformed cells have been shown in lectin agglutinability[1–10], the number and distribution of Concanavalin A binding sites[11–15], the location of amino acid and carbohydrate transport sites[16] and Concanavalin A-induced cell toxicity[17,18]. Changes in the distribution of Concanavalin A sites as a result of movement of sites and the formation of Concanavalin A site complexes[19–21], and the movement of antigens[22–25], have indicated that receptors can be mobile in the fluid surface membrane[26]. The present experiments were undertaken to determine whether mobility of carbohydrate binding sites on the surface membrane is required for lectin-induced cell agglutination.

MATERIALS AND METHODS

The cells used in the present experiments were derived from the ascites form of a Moloney virus-induced lymphoma[27] grown *in vivo*. This is a T cell lymphoma.

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10^5 lymphoma cells were inoculated intraperitoneally into adult A mice, and the cells used for experiments 10 days after cell inoculation. Cells obtained from animals were washed three times with phosphate buffered-saline (pH 7.2). For aldehyde fixation, 1 vol. of cell suspension diluted in buffer at a concentration of 10^7 cells/ml was mixed with one volume of either 5% glutaraldehyde or 20% formaldehyde at 4 °C. After the time for fixation, cells were centrifuged and resuspended in a solution of 0.2 M glycine at a concentration of 10^7 cells/ml and incubated for 10 min at 4 °C. The cells were washed 3 times with phosphate-buffered saline and then incubated with Concanavalin A. For fixation with $LaCl_3$, cells were suspended in 10^{-2} M $LaCl_3$ in Tris buffer (pH 7) for 30 to 60 min. In contrast to fixation by aldehyde, fixation by $LaCl_3$ was reversible by washing with Tris buffer. To test for agglutination, 0.5 ml of lectin at different concentrations diluted in phosphate-buffered saline was mixed with 0.5 ml cell suspension, at a concentration of 10^7 cells/ml in a 35-mm petri dish. The density and size of aggregates was scored in a scale from − to ++++ after 30 min incubation with shaking at 24 °C[1].

Purified soluble Concanavalin A, Sepharose-conjugeted Concanavalin A (2.6 mg Concanavalin A per 1 ml beads), fluorescein isothiocyanate-conjugated Concanavalin A, and the purified lectin from wheat germ were obtained from Miles-Yeda. The lectin from soybean was prepared as described[28]. Soluble Concanavalin A was radioactively labeled with [^{3}H]acetic anhydride by the method of Miller and Great[29] to give a specific activity of 10^6 cpm/mg. For binding of radioactive Concanavalin A to cells, 0.5 ml labeled Concanavalin A diluted at different concentrations in either phosphate-buffered saline or in the same buffer containing 0.1 M α-methyl-D-mannopyranoside was mixed with 0.5 ml cell suspension ($2 \cdot 10^7$ cells/ml) in a centrifuge tube and incubated for 30 min at 24 °C. The cells were then washed 3 times with phosphate-buffered saline, the pellet dissolved and the radioactivity counted in Triton scintillation fluid. Similar results were obtained in the presence or absence of 10^{-3} M sodium azide. To calculate the amount of labeled Concanavalin A bound specifically, the amount bound in the presence of α-methyl-D-mannopyranoside was subtracted from the amount bound in the absence of this carbohydrate[10]. The results on binding in the present experiments are given as specific binding in cpm/10^7 cells. Soluble Concanavalin A was conjugated to fluorescein isothyocyanate at a ratio of 1.86 fluorescein to protein, 0.5 ml cell suspension was mixed with 0.5 ml fluorescent Concanavalin A, incubated for 15 min at 24 °C or 37 °C, washed and tested for fluorescent with a Leitz Ortholux microscope with a transmitted ultraviolet light[19]. To test for binding of the lymphoma cells to Sepharose-conjugated concanavalin A beads, 0.5 ml cell suspension was mixed with 0.5 ml of the bead suspension. The number of cells bound to the beads and bead aggregation was scored after 30 min incubation at 24 °C[19].

RESULTS

Agglutinability by soluble Concanavalin A and binding to Sepharose conjugated Concanavalin A beads.

Fixation of lymphoma cells with 2.5% glutaraldehyde or 10% formaldehyde for 4 h and 20 h, respectively, completely abolished agglutination with 10 μg Concanavalin A/ml. With 50 or 100 μg Concanavalin A/ml, the high degree of agglutina-

tion (++++) in the unfixed cells was reduced to fixation to (+) (Fig. 1). This low degree of agglutination of the fixed cells was not increased even with 500 μg Concanavalin A/ml (Fig. 2B). Agglutination was also inhibited by cell fixation with 10^{-2} M $LaCl_3$ for 60 min. This inhibition by $LaCl_3$ was reversed by washing the cells. Unfixed cells with a high degree of agglutinability by soluble Concanavalin A (Fig. 2A) bound to the Sepharose conjugated beads and induced bead aggregation (Fig. 2C). However, most of the fixed cells, with a low degree of agglutinability (Fig. 2B) did not bind to the beads (Fig. 2D). Binding of cells to beads and bead aggregation were specific, since they were completely abolished when the beads were preincubated with 0.1 M α-methyl-D-mannopyranoside.

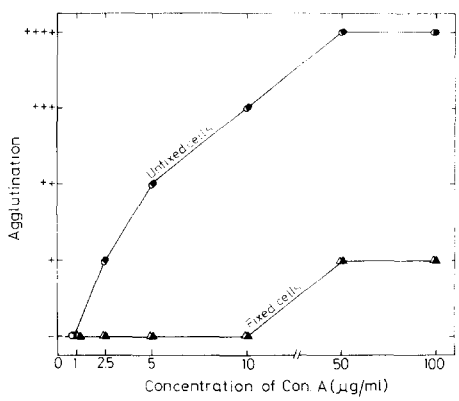

Fig. 1. Cell agglutinability after fixation with aldehydes. Fixation with 2.5% glutaraldehyde for 4 h (△); and with 10% formaldehyde for 20 h (▲). Unfixed cells incubated for 4 h (○) and for 20 h (●). Agglutinability with soluble Concanavalin A after 30 min incubation at 24 °C. Agglutination was also inhibited by cell fixation with 10^{-2} M $LaCl_3$ for 60 min.

Binding of radioactive and fluorescent Concanavalin A

Binding experiments with radioactively labeled soluble concanavalin A have shown, that the same number of molecules were bound to the surface membrane with or without 20 h fixation by 10% formaldehyde (Fig. 3) and that a similar number of molecules were bound with or without 4 h fixation by 2.5% glutaraldehyde (Fig. 4). In order to determine the distribution of Concanavalin A-binding sites on the surface membrane, we examined the interaction of fluorescent Concanavalin A with unfixed and fixed cells. 95–100% of the cells were stained at concentrations of 5 to 100 μg/ml and the staining in both unfixed and fixed cells was completely inhibited when 0.1 M α-methyl-D-mannopyranoside was added as a hapten inhibitor. In 99% of the unfixed cells, the fluorescence was in small or large clusters on the cell surface, whereas the fixed cells gave a diffuse fluorescence without clusters. The diffuse fluorescence obtained after aldehyde fixation was also obtained after fixation with $LaCl_3$. Washing of the $LaCl_3$ fixed cells after binding of fluorescent Concanavalin A, which removed the $LaCl_3$ but not the Concanavalin A, reversed the diffuse to a clustered distribution.

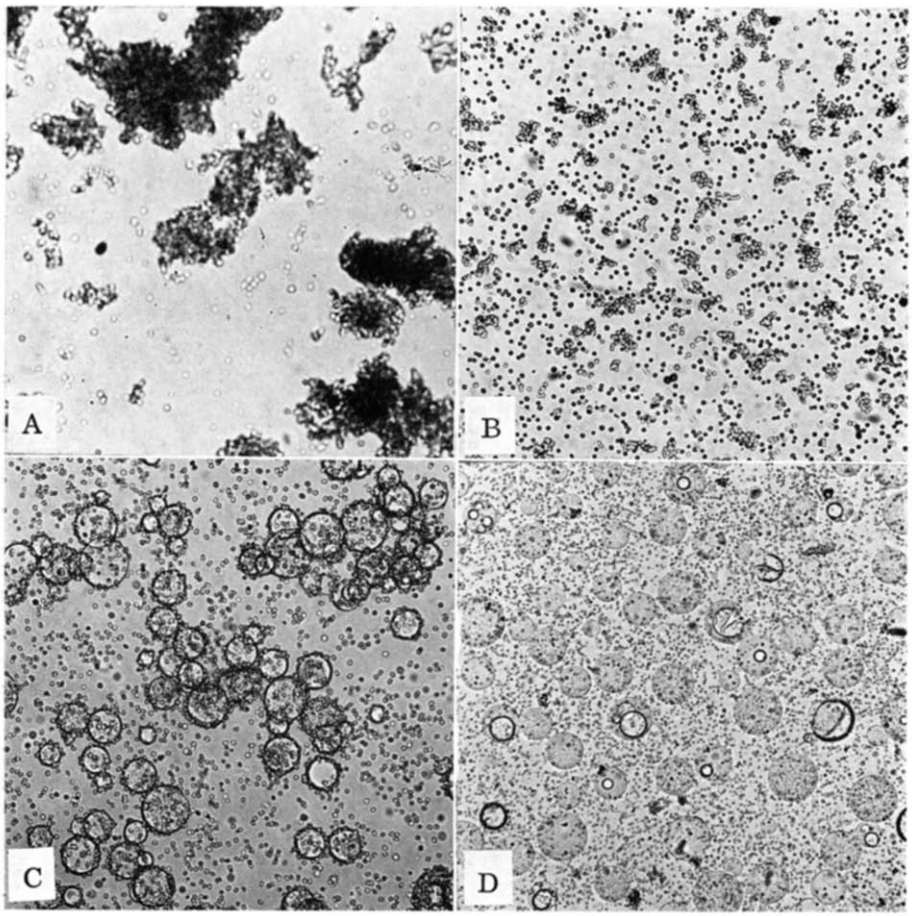

Fig. 2. Binding to Sepharose-conjugated Concanavalin A beads and agglutinability by soluble Concanavalin A of unfixed cells, and after 4 and 20 h fixation with 2.5% glutaraldehyde and 10% formaldehyde, respectively. Agglutination by 500 μg soluble Concanavalin A/ml. (A) Unfixed cells. (B) Fixed cells. Binding of cells to beads (100 μg Concanavalin A/ml). (C) Unfixed cells. (D) Fixed cells. $\times$ 200. Agglutination and binding to beads was scored after 30 min incubation at 24 °C.

Agglutinability by lectins from wheat germ and soybean

The lymphoma cells are highly agglutinable by the lectins from wheat germ and soybean and this agglutination was inhibited by aldehyde fixation. The inhibition of agglutination with 100 μg lectin/ml by fixation with 2.5% glutaraldehyde, required 45 min for wheat germ 3 h for Concanavalin A and 6 h for soybean (Fig. 5).

DISCUSSION

Our results have shown that formaldehyde fixation of lymphoma cells inhibited cell agglutination by soluble concanavalin A and binding to Sepharose conjugated concanavalin A beads, without changing the binding capacity of soluble radioactively labeled Concanavalin A molecules. Similar results were obtained

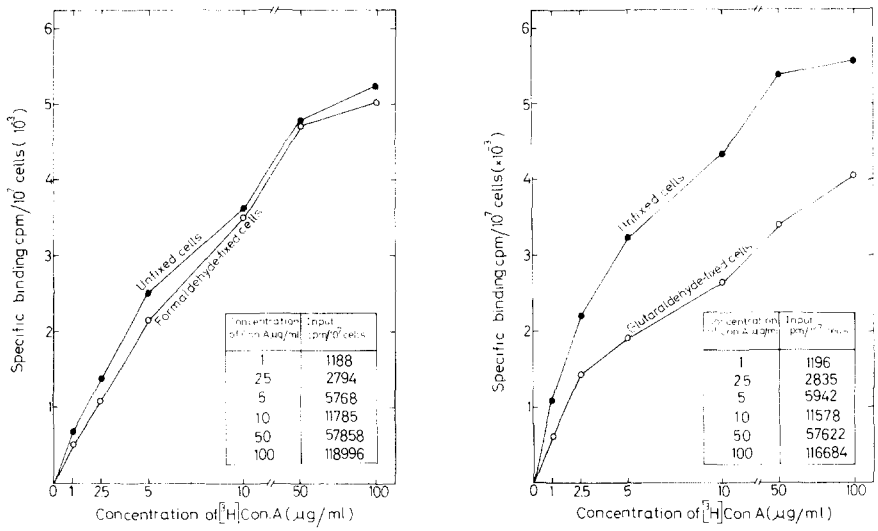

Fig. 3. Specific binding of soluble radioactively labeled Concanavalin A molecules to the surface membrane after formaldehyde fixation. ●, unfixed cells; ○, cells fixed with 10% formaldehyde for 20 h. The specific binding in this figure and in Fig. 4 was 75 to 90% of the total binding in the absence of α-methyl-D-mannopyranoside.

Fig. 4. Specific binding of soluble radioactively labeled Concanavalin A molecules to the surface membrane after glutaraldehyde fixation. ●, unfixed cells; ○, cells fixed with 2.5% glutaraldehyde for 4 h.

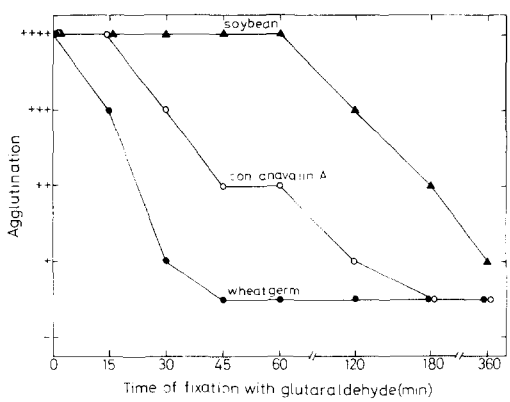

Fig. 5. Agglutinability by different lectins, 100 μg/ml, of cells fixed with 2.5% glutaraldehyde. ●, wheat germ; ○, Concanavalin A; ▲, soybean. Agglutinability was scored after 30 min incubation at 24 °C.

with fixation of cells by glutaraldehyde. Inhibition of agglutination by aldehyde which inhibits membrane fluidity and site mobility[19,20], suggests that movement of concanavalin A binding sites on the surface membrane is required for cell agglutination. Direct evidence for this assumption was obtained from the experiments which showed the fixation that inhibited agglutination also inhibited formation of clusters. These results support our suggestion that binding sites for Concanavalin A are floating in the fluid membrane in a random distribution, and that this distri-

bution is changed by interaction with Concanavalin A molecules to form clusters of Concanavalin A membrane site complexes[19,20].

Aldehyde fixation also inhibited agglutination by the lectins from wheat germ and soybean. Inhibition of agglutination by the three lectins required different degrees of aldehyde fixation in the order: soybean > Concanavalin A > wheat germ. A similar order for these three lectins has been found for the gain of agglutinability of normal hamster cells after trypsin treatment[6].

We have suggested that differences between normal and malignant transformed cells in Concanavalin A agglutinability[1,7–9], the distribution of concanavalin A-binding sites[9–15], the location of amino acid and carbohydrate transport sites[16] and Concanavalin A-induced cell toxicity[17,18] are associated with differences in membrane fluidity[19,20]. It will be of interest to determine the mobility of different lectin-binding sites on the surface membrane, and the association between movement of binding sites and the activation of normal lymphocytes by lectins[30].

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