INHIBITION OF INTERCELLULAR ADHESION BY CONCANAVALIN A IS ASSOCIATED WITH CONCANAVALIN A-MEDIATED REDISTRIBUTION OF SURFACE RECEPTORS

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ABSTRACT

The inhibition of adhesion between aggregates and layers of embryonic retinal cells by concanavalin A (Con A) and Con A-mediated rearrangements of Con A receptors on retinal cells were studied. A short incubation of aggregates and layers with 10 μg/ml Con A substantially reduced aggregate-to-layer adhesion in a subsequent assay without soluble lectin present. This effect of Con A was dose-dependent, temperature-sensitive, involved events subsequent to Con A binding, and was reduced by cytochalasin B. The inhibition produced by succinylated Con A was substantially increased by incubation with antibody to Con A. Visualization of Con A-receptor complexes by fluorescence microscopy revealed that binding of Con A induced clearing of Con A receptors from filopodia, flattened regions of growth cones, and the edges of axons. This clearing reaction was prevented by the same agents that reduced Con A's inhibition of cell adhesion: low temperature, succinylation of Con A, or cytochalasin B. Aggregate-layer adhesion was restored by releasing Con A at 37°C. Inhibitors of protein and ATP synthesis did not prevent recovery of ability to make adhesions. However, release of Con A at lowered temperatures did prevent recovery. The results suggest that intercellular adhesion is inhibited by events associated with redistribution of Con A-receptor complexes on retinal cells.

KEY WORDS intercellular adhesion · concanavalin A · cell surface rearrangements · nerve growth cones · filopodia

Lectins have been investigated because of the interesting activities associated with their binding to cell surface polysaccharides, hemagglutination, mitogenic stimulation of lymphocytes and agglutination of tumor cells (37). Recent studies demonstrate that the binding of lectins leads to redistribution of lectin receptors within the cell surface (2, 7, 11, 12, 39, 45). These events can alter the distribution and mobility of other surface components (36, 46). However, the functional significance of these surface rearrangements is not presently well understood (12).

Current speculations about intercellular adhesion suggest that proteins and carbohydrates participate in adhesive bonds (9, 17, 22, 26, 32, 33), that receptor mobility may be needed, and that...
cytoskeletal structures are involved (8, 27, 30, 41, 43). Because these features are targets of lectin activity, we studied the effects of lectin binding on the formation of adhesions between embryonic cells.

In vitro determinations define adhesion operationally as the fraction of cell contacts which resist an applied force (23). We measured the adhesion of preformed cell aggregates to cell layers (8). Advantages of this assay are that it is fast and quantitative, the cells have recovered for 24 h from damage produced during tissue dissociation with trypsin, and tissue-specific differences in adhesion are demonstrated.

We have shown that short treatment with concanavalin A (Con A) reduces the adhesion of cell aggregates to cell layers. This effect depends upon crosslinking and redistribution of surface Con A-receptor complexes. Recovery of cell adhesion requires release of Con A and is sensitive to lowered temperature. Possible interpretations are that membrane components involved in intercellular adhesion are Con A receptors whose activity is inhibited by Con A-mediated redistribution within the membrane, or that these surface rearrangements interfere with the formation of adhesions by other means, possibly through surface modulation (12).

MATERIALS AND METHODS

Media and Chemicals

Hams F12 (Grand Island Biological Co., Grand Island, N. Y. [GIBCO]) was made in two ways. F12 was buffered at pH 7.4 with 5 mM N-tris-(hydroxymethyl) methyl-2-aminoethanesulfonic acid (TES) and 5 mM N-2-hydroxethylpiperazine-N'-2-ethane sulfonic acid (HEPES), and supplemented with 10% fetal calf serum (designated F12BS10). F12 buffered with sodium bicarbonate to pH 7.4 and supplemented with 10% fetal calf serum is designated F12S10. Calcium- and magnesium-free Hanks' Salt Solution (CMFH), Hanks' Salt Solution, and Dulbecco's phosphate-buffered saline (PBS) were purchased from Gibco. Trypsin (2 x crystalline), deoxyribonuclease (DNase), cytochalasin B, colchicine, and sugars were purchased from Sigma Chemical Co., St. Louis, Mo. Con A (3 x crystallized, Miles Laboratories Inc., Elkhart, Ind.) was dissolved in PBS as a stock solution of 1 mg/ml. Succinylated Con A (Scon A), 125I-labeled Con A, affinity-purified rabbit antibody against Con A, and fluorescein isothiocyanate (FITC) labeled goat antibody against rabbit IgG were provided by Dr. John F. Ash (2). 3H-acetylated Con A was purchased from New England Nuclear, Boston, Mass.

Cell Preparation

Cell aggregates and layers were prepared from neural retina of 7-day chick embryos as described previously (8).

Cells were prepared for fluorescence staining and microscopy by adding 1.5 x 10^6 cells in 2 ml F12BS10 to 35-mm culture dishes, each containing a glass coverslip coated with polylysine (20). These dishes were incubated at 37°C in a humidified CO2 incubator.

Aggregate-Layer Adhesion Assay

After 24-h incubation, cell layers were washed twice with PBS. Cell aggregates were collected and washed free from serum, debris, and small cell clumps by repeated sedimentation at 1 g in 15-ml tubes. Aggregates were resuspended in PBS, and the experimental treatments were begun.

After experimental treatments, aggregates were placed in Hanks' Salts at 37°C at a concentration of 160-400 aggregates/ml. To begin an adhesion assay, each cell layer was drained and 0.5 ml of aggregate solution was added with an automatic pipet (Biopette, Becton, Dickinson & Co., Orangeburg, N. Y.). The layers and aggregates were incubated together at 37°C without agitation. The adhesion reaction was stopped by removing the medium and unbound aggregates with a Biopette, and the layers were washed twice with assay medium to remove all free aggregates. The aggregates removed from each layer with the original assay medium plus subsequent washes were pooled in an adjacent empty well. Immediately afterwards, the number of aggregates, bound and unbound, were counted for each layer in a stereomicroscope. The percent of total aggregates bound to each layer was calculated (bound aggregates/bound plus free aggregates).

Con A Binding Assay

Cell layers were prepared by plating 8 x 10^6 neural retina cells in 1 ml of F12BS10 per well of Linbro Dispo Trays. After 24 h, the layers were washed three times with PBS. The binding commenced by adding 1 ml of PBS containing labeled Con A to each layer at 30-second intervals. Binding was stopped by removing the Con A solution and rapidly washing each layer three times with PBS. Cell layers plus bound Con A were then dissolved in a solution of 1% Triton X-100 and 1% sodium dodecyl sulfate (SDS), added to Ready Solv HP (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), and counted in a scintillation counter (Beckman Instruments).

Fluorescence Microscopy of Con A

Binding to Cell Surfaces

After 24 h, the coverslips were rinsed three times with PBS. Then 50 μg/ml Con A or Scon A was added for 15 min at 37°C. Unbound lectin was removed by three
rinses with PBS, and the cells were either fixed or reincubated for 15 min in assay medium to simulate the adhesion assay. The cells were fixed by 15-min incubation in 4% formaldehyde in PBS at room temperature. The coverslips were then rinsed three times, remaining 10 min in the final wash which contained 50 mM NH4Cl to block aldehyde groups. The coverslips were incubated with 50 μg/ml rabbit anti-Con A for 15 min at room temperature. Two rinses followed, and the cells were stained by incubation for 15 min in PBS containing 40 μg/ml FITC-labelled goat antibody against rabbit IgG. The coverslips were rinsed twice and mounted in 50% glycerol in PBS on glass slides.

Cell morphology and fluorescence were examined with a Zeiss Universal microscope equipped with an epifluorescence III RS condenser and a HBO 200 mercury lamp. Kodak Plus-X film was used. Cells were examined with Nomarski differential interference optics using a 63 × planapochromat objective or with phase optics using a 100 × neofluor objective. The focus was adjusted to the upper cell surface, a Nomarski or phase photograph was taken, and then a fluorescence photograph was taken at the same focal plane.

RESULTS

Inhibition of Aggregate Adhesion to Cell Layers by Con A: Effect of Lectin Concentration

The effect of Con A on aggregate-layer adhesion was initially assessed by preincubation with Con A followed by adhesion assays in the presence of the lectin. As seen in Table I, both the duration of Con A treatment and the Con A concentration influenced the reduction of aggregate-layer adhesion. The haptenic sugar for Con A, α-methyl-mannoside, eliminated the lectin’s action while galactose had no effect. This indicates that inhibition of adhesion depends upon sugar-specific binding of Con A.

Questions concerning the roles of amount of lectin bound and duration of lectin treatment were addressed by washing out unbound lectin and performing subsequent treatments and adhesion assays without soluble Con A present. Fig. 1 illustrates the effect of Con A concentration on a subsequent adhesion assay. The lower curve indicates that adhesion was reduced to 50% of untreated levels by preincubation in 0.7 μg/ml Con A and near maximal inhibition was achieved at Con A levels of 10 μg/ml. The upper curve in Fig. 1 shows that the inhibition of aggregate-layer adhesion by Scon A was less than that of Con A.

| Treatments | Min added before assay | Aggregate adhesion mean ± SE (n) % |
|------------|------------------------|----------------------------------|
| 10 μg/ml Con A | 0 | 43 ± 2 (6) |
| 10 μg/ml Con A | 2 | 36 ± 3 (3) |
| 10 μg/ml Con A | 5 | 12 ± 2 (3) |
| 10 μg/ml Con A | 10 | 6 ± 1 (6) |
| 10 μg/ml Con A | 10 | 58 ± 4 (3) |
| plus 100 mM α-D-methylmannose | 0.4 μg/ml Con A | 47 ± 3 (3) |
| plus 100 mM galactose | 2 μg/ml Con A | 16 ± 3 (2) |
| 10 μg/ml Con A | 15 | 5 ± 3 (3) |
| 0 | 0 | 62 ± 3 (9) |

Con A was added to aggregates and layers at the concentrations listed in column I. After the times indicated in column II, 15-min adhesion assays were performed with Con A present. The average percent of aggregates which adhered to the layers is listed in column III. (n) is the number of assays performed.

Effect of Con A on Kinetics of Adhesion

In the following text, Con A treatment was carried out for 10 min at 10 μg/ml. This produced slightly less inhibition of adhesion than that shown in Fig. 1. The effect of Con A on kinetics of aggregate-layer adhesion is shown in Fig. 2. Aggregates and layers were incubated with Con A, rinsed, and adhesion assays of various lengths.
Figure 2. Effect of Con A on kinetics of aggregate-layer adhesion. Abscissa: Duration of aggregate-layer adhesion assay. Ordinate: Percent of aggregates adherent to layers. Mean ± SE (n > 3) for each point. —△— Con A treated. —□— untreated.

were performed. Con A reduced the rate of adhesion over the entire assay period.

Binding of Con A to Retinal Layers

Figs. 3 and 4 depict measurements of Con A binding to retinal cell layers. Two labeled forms of Con A were used because of supply limitations. Very little Con A bound to culture wells incubated with F12+BS10 only. Binding was not saturated by the treatments. A portion of the uptake of Con A by the cells might represent endocytosis of membrane-bound lectin; however, binding reactions at 9°C, when endocytosis should be reduced, yielded similar results as at 37°C. Comparison of the effects of Con A on aggregate-layer adhesion with measurements of Con A binding (Figs. 1, 3, 4, and Tables I and II) reveals that occupation of a fraction of the binding sites is sufficient for substantial inhibition of adhesion.

Effect of Duration of Con A Treatment

The significance of the duration of Con A treatment was investigated by incubating aggregates and layers with Con A for 2 min, washing out the lectin, and either immediately beginning an adhesion assay or incubating aggregates and layers for an 8-min chase without soluble Con A before beginning an adhesion assay. Table II shows that the inhibition of adhesion produced by a 2-min treatment with Con A was substantially increased by adding a chase period before beginning the adhesion assay. Apparently, events subsequent to lectin binding contribute to the lectin-induced inhibition of cell adhesion.

Con A Treatment at Low Temperature

The temperature sensitivity of these events was probed by incubating aggregates and layers with Con A at 9°C, then changing to warm medium and beginning an adhesion assay. Assays were not done at 9°C, because low temperatures inhibit aggregate-layer adhesion (8). Although the same amount of Con A was bound at 9°C as at 37°C, the reduction in adhesion following treatment at 9°C was much less than the inhibition produced by Con A treatment at 37°C (Fig. 4, Table II).

Effect of Anti-Con A on Response to Scon A

Scon A retains the sugar-binding specificity of Con A but has altered effects on membrane receptor mobility, and does not produce rearrangements of Con A receptors (2, 15, 21, 36). These changes are thought to be a result of reduced valency of Scon A, as addition of antibody against Con A to cells with surface-bound Scon A restores the properties of native Con A. As seen in Fig. 1, Scon A has a reduced capacity to inhibit aggregate-layer adhesion. However, as seen in Table III, when binding of Scon A was

Figure 3. Binding of [3H]Con A to neural retinal cell layers at 37°C. Abscissa: Duration of incubation of [3H]Con A with retinal layers. Ordinate: μg [3H]Con A bound per cell layer. Mean ± SE (n = 3) for each point. —△— 5 μg/ml. —□— 20 μg/ml. —□— 40 μg/ml.

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**Effects of Cytochalasin B and Colchicine**

To determine whether microfilaments and microtubules may be involved in the Con A effect on cell adhesion, cytochalasin B (CB) and colchicine were applied to retinal cells. A dose of CB was used which induced rapid "arborization" of cultured heart fibroblasts (34). As seen in Table IV, either drug alone reduced aggregate-layer adhesion.

### Table III

**Effect of Antibody to Con A on Lectin-Mediated Inhibition of Cell Adhesion**

| Lectin | Anti-con A | Aggregate adhesion mean ± SE (n) |
|--------|------------|---------------------------------|
| 30 μg/ml Con A | 0 | 32 ± 3 (5) |
| 30 μg/ml Scam A | 10 μg/ml | 29 ± 1 (2) |
| 30 μg/ml Scam A | 50 μg/ml | 21 ± 3 (4) |
| 30 μg/ml Scam A | 100 μg/ml | 10 ± 2 (4) |
| 30 μg/ml Con A | 0 | 7 ± 2 (2) |
| 30 μg/ml Con A | 100 μg/ml | 1 ± 1 (2) |
| 0 | 0 | 53 ± 2 (2) |
| 0 | 100 μg/ml | 54 ± 1 (2) |

Aggregates and layers were incubated in PBS with Con A or Scam A for 20 min at the concentrations listed in column I. Then unbound lectin was washed out and the cells were incubated for 25 min with rabbit antibody against Con A, as indicated in column II. The percent of aggregate adhesion in a subsequent 15-min assay is indicated in column III. (n) indicates the number of assays done.

### Table IV

**Effects of Cytochalasin B and Colchicine on Aggregate-layer Adhesion**

| Drug | Con A | Aggregate adhesion mean ± SE (n) |
|------|-------|---------------------------------|
| 0 | 0 | 61 ± 3 (8) |
| 0 | 10 μg/ml | 12 ± 3 (3) |
| 5 μg/ml cytochalasin B | 0 | 44 ± 3 (12) |
| 5 μg/ml cytochalasin B | 10 μg/ml | 29 ± 2 (12) |
| 1 μg/ml colchicine | 0 | 16 ± 1 (3) |
| 1 μg/ml colchicine | 10 μg/ml | 2 ± 2 (3) |

Aggregates and layers were incubated 30 min at 37°C in PBS with the drugs listed in column I. Then Con A was added to aggregates and layers, and incubation with the drug was continued. After 15 min, unbound Con A was washed out, and a 15-min adhesion assay was done in the presence of the drugs. (n) indicates number of assays performed.

followed by incubation with anti-Con A, aggregate-layer adhesion was reduced to the levels of Con A-treated cells. These results suggest that the inhibition of cell adhesion by Con A involves crosslinking of lectin-receptor complexes.
adhesion. Neither drug was toxic, as judged by trypan blue exclusion, after a 2-h treatment of cell layers. Interestingly, though CB itself diminished aggregate-layer adhesion, prior treatment of cells with CB reduced the inhibition of adhesion elicited by Con A binding (Table IV). CB did not reduce Con A binding to retinal cells (unpublished observations). Colchicine did not diminish Con A's effect on cell adhesion.

Con A Induced Redistribution of Con A Receptors

The distribution of Con A receptors on retinal cells before and after Con A treatment was assessed by fluorescence microscopy. Retinal cells were plated at low density to examine the cytoplasmic extensions of individual cells. Polylysine-coated coverslips were used because very few cells extended axons on untreated coverslips. Observation and photography of the small spherical somata (4-8 μm) of retinal cells was very difficult, so we primarily examined growth cones, axons, and other flattened regions of the cells. Filopodia and similar cell surface extensions are thought to be important in cell-cell interactions, and are seen on cells at the surfaces of aggregates and layers (4). Because photographs were made at the upper cell surface, the Nomarski and phase photographs may not appear as clear as if focused at the level of the cells' contact with the substratum.

If α-M-methyl mannoside was present during Con A binding, the fluorescence micrographs taken with the same exposure as the others were black (not shown). Therefore, these fluorescence images represent sugar-specific Con A binding. When the cells were fixed before adding Con A (Fig. 5a and b), the fluorescence image had the same outline as the Nomarski image, indicating that Con A receptors were initially distributed over the entire cell surface. This confirms previous observations of Con A receptors on 7-day retinal cells (3). When Con A was bound to living cells, the fluorescence and Nomarski or phase images were quite dissimilar (Fig. 6a and b). Con A-receptor complexes were not distributed across the whole cell surface as they were initially; rather, filopodial extensions of growth cones and the edges of axons lacked fluorescence. If unbound Con A was removed and incubation continued for 15 min, this configuration was retained (Fig. 7a and b). If Con A was added to cells at 9°C, followed by fixation, the entire cell surface was fluorescent (Fig. 8a and b). Apparently, redistribution of Con A receptor complexes does not occur at 9°C.

In a variation of the procedure, cells were incubated with Con A at 37°C, fixed, washed, and reincubated with Con A, followed by staining. As on cells incubated with Con A only before fixation, fluorescence was not seen on filopodia and at the edges of growth cones and nerve fibers (Fig. 12a and b). Apparently, the absence of fluorescence after treatment of living cells is not because Con A was released from receptors within these areas, but rather because receptors that bind Con A at this dose are not present in these regions.

On the other hand, Con A receptors were not redistributed after the binding of Scon A, even with a chase period (Figs. 9a, b and 10a, b). But when Scon A was bound by incubation with anti-Con A, Con A-receptor complexes were cleared from filopodia and other flattened regions of growth cones (Fig. 11a and b).

Effect of CB on Redistribution of Con A-Receptor Complexes

Since a CB-sensitive activity may participate in the inhibition of adhesion by Con A, fluorescence staining was used to determine whether CB alters the redistribution of Con A receptors after Con A binding. As seen in Fig. 13a and b, patches of fluorescence were seen on CB-treated cells, suggesting clustering of Con A receptors; however, fluorescence was also observed on microspikes and at the edges of nerve fibers (Fig. 13a and b). These observations resemble those of Brown and Revel (7) who found that CB prevented the clearing of Con A-receptor complexes from the edges of LA-9 cells.

The movements of lectin-receptor complexes and particles backwards on the surfaces of growth cones have been interpreted as indicating that membrane for axonal growth is inserted at the tips of filopodia and the front of growth cones (6, 19, 28). However, this hypothesis is not consistent with our observations that Con A receptors are cleared from filopodia in spite of the continued presence of Con A (Fig. 6a and b) or that Con A receptors are not cleared when Scon A is bound, even with a chase period, unless anti-Con A is added (Figs. 9a, b, 10a, b, and 11a, b). Rather, our results and others suggest that movements of markers and on the cell surface reflect redistribution of a limited subset of crosslinked surface components through possible cytoskeletal involvement (7, 39, 42). Out data also indicate that

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FIGURE 5 Nomarski image (a) and distribution of Con A-receptor complexes (b) on retinal cell fixed with 4% formaldehyde before incubation with Con A. Con A receptors are distributed over entire cell surface. × 1,800.

FIGURE 6 Phase contrast image (a) and distribution of Con A-receptor complexes (b) on retinal axon incubated with 50 μg/ml Con A for 15 min at 37°C. Note the absence of fluorescence on filopodia extended from the growth cone (gc) and on filopodia extended along the edge of the nerve fiber (arrowheads). × 3,500.

FIGURE 7 Nomarski image (a) and distribution of Con A-receptor complexes (b) on retinal cell incubated with 50 μg/ml Con A for 15 min at 37°C, washed and reincubated without soluble Con A 15 min at 37°C before fixation. Absence of fluorescence on filopodia (arrowheads in a) is maintained after incubation without soluble Con A. × 2,500.

FIGURE 8 Phase contrast image (a) and distribution of Con A-receptor complexes (b) on retinal axon and growth cone incubated with 50 μg/ml Con A for 15 min at 9°C. Con A binding at 9°C does not alter the initial distribution of Con A receptors. × 3,500.
clearing of Con A-receptor complexes from growth cones and the edges of nerve fibers requires crosslinking of receptors and can be thought of as the neural equivalent of capping or transmembrane linkage (2, 5). Although the cells are very much spread out on these polylysine-coated surfaces, our observations of the clearing of receptors from filopodia and cellular extensions are similar to results with cells cultured on untreated coverslips (7, 39, 40, 42).

Release of Con A and Recovery of Adhesion

The inhibition of aggregate-layer adhesion by Con A was diminished when Con A treatment was followed by incubation with α-D-methyl-mannoside before an adhesion assay (Table V). This recovery of adhesion did not occur unless α-D-methyl-mannoside was present, indicating that removal of bound Con A is required for recovery.

Because polysaccharides which bind Con A may be deposited by the cells on the dish surface and between the cells, we wished to determine the extent of Con A removal from the retinal cells after incubation with α-D-methyl-mannoside. Retinal cells on coverslips were treated with Con A, washed, incubated with 100 mM α-D-methyl-mannoside for 60 min, and then fixed and processed for fluorescence microscopy. As expected, there was very little fluorescent material on these coverslips. The only fluorescence on the cells consisted of individual submicron spots scattered across the cell surfaces. Large clusters of fluorescence such as seen in Figs. 6 b, 7 b, or 11 b were not present. These patches may represent Con A not removed by α-D-methyl-mannoside. These observations confirm our finding that recovery of adhesion is associated with removal of most of the Con A from the retinal cells.

Effect of Inhibitors on Recovery

Metabolic inhibitors were added during the recovery period. Neither 10 μg/ml cycloheximide, which inhibits protein synthesis by more than 90% (unpublished data), nor 10 mm azide substantially reduced the recovery of adhesion produced by α-D-methyl-mannoside compared to cells treated with the inhibitors but not Con A (Table VI). Azide treatment alone seemed to slightly inhibit adhesion (8). This recovery process does not seem to require normal synthesis of protein or ATP.

Effect of Lowered Temperature on Recovery

One treatment which severely limited the restoration of adhesion induced by α-D-methyl-mannoside was incubation at temperatures below 37°C. As seen in Fig. 14, aggregates and layers incubated with α-D-methyl-mannoside at 23°C or less after Con A binding at 37°C showed no recovery of adhesion. At 30°C the recovery was slight. This effect of lower temperatures may occur because recovery involves a temperature-sensitive process beyond removal of Con A or because the removal of Con A is temperature sensitive.

The removal of [125I]Con A from retinal cells in the presence of α-D-methyl-mannoside was measured. Retinal cell layers were reacted with 10 μg/ml of [125I]Con A for 10 min at 37°C, rinsed, and reincubated with α-D-methyl-mannoside for various periods at 37°C and 23°C. Then, the layers were rinsed, dissolved, and the 125I remaining with the layers was counted.

Fig. 15 illustrates the release of Con A and recovery of aggregate-layer adhesion as a function of length of incubation with the sugar at 23°C and at 37°C. Con A removal at 37°C paralleled the recovery of adhesion at 37°C. However, Con A removal and the recovery of adhesion at 23°C did not follow similar curves. This indicates that recovery from Con A-induced inhibition of cell adhesion requires release of the lectin, plus another process(es) which is blocked at lower temperatures.

DISCUSSION

Lectins have been reported to inhibit myoblast fusion (10), conjugation of Tetrahymena (14), cell aggregation (13), cell adhesion to artificial substrata (29), and membrane channels (25). In this study the inhibitory effects of Con A on cell adhesion have been characterized and related to the effects of Con A on the distribution of Con A receptors.

There is apparent conflict between Con A’s inhibition of aggregate-layer adhesion and the agglutination by Con A of dissociated retinal cells (24). However, considering the data below, there are great differences between these two cell contact phenomena, especially in terms of cell surface events, and they are not easily compared. Agglutination of cells involves much higher concentrations of lectins than necessary to substantially inhibit aggregate-layer adhesion (18, 24). Glutaraldehyde-treated retinal cells can be agglutinated with lectins (24), whereas glutaraldehyde treatment abolishes aggregate-layer adhesion of retinal
TABLE V

| I       | II                   | III          | IV            |
|---------|----------------------|--------------|--------------|
| Con A   | Recovery treatment   | Recovery time (min) | Aggregate adhesion mean ± SE (n) |
| 10 µg/ml| 0                    | 0            | 10 ± 2 (3)   |
| 10 µg/ml| 0                    | 20           | 10 ± 4 (3)   |
| 10 µg/ml| 0                    | 40           | 12 ± 2 (6)   |
| 10 µg/ml| 0                    | 70           | 12 ± 2 (3)   |
| 10 µg/ml| 100 mM α-D-Me-mannose| 10           | 34 ± 2 (9)   |
| 10 µg/ml| 100 mM α-D-Me-mannose| 20           | 39 ± 3 (5)   |
| 10 µg/ml| 100 mM α-D-Me-mannose| 40           | 45 ± 2 (11)  |
| 10 µg/ml| 100 mM α-D-Me-mannose| 70           | 51 ± 3 (3)   |
| 10 µg/ml| 100 mM sucrose       | 40           | 15 ± 3 (3)   |
| 10 µg/ml| 100 mM galactose     | 40           | 15 ± 2 (3)   |
| 0       | 100 mM α-D-Me-mannose| 40           | 52 ± 3 (8)   |
| 0       | 100 mM galactose     | 40           | 61 ± 2 (3)   |
| 0       | 0                    | 40           | 55 ± 3 (9)   |

Aggregates and layers were treated for 10 min with Con A as listed in column I. Then unbound Con A was washed out and the aggregates and layers were incubated in Hanks' salts plus the sugars indicated in column II for the time periods in column III at 37°C before an adhesion assay. The percent adhesion in a subsequent 15-min assay is listed in column IV. (n) indicates the number of assays.

Cells (8). Cells can be agglutinated with lectins immediately after treatment with trypsin or EDTA (EGTA), two agents which severely impair intercellular adhesion (8, 18, 22, 24, 26, 27).

Concentration Dependence of Con A Effect

We have found that inhibition of aggregate-layer adhesion depends upon the concentration of Con A and the duration of Con A treatment (Fig. 1, Tables I and II). Substantial inhibition of adhesion was achieved with occupation of a fraction of the available Con A binding sites (Figs. 3 and 4). The number of Con A molecules bound per cell was not determined because of possible unequal binding of Con A to different cell types, to cells at different positions in a layer, or to polysaccharides deposited extracellularly. The number of Con A receptors on a variety of cells ranges from $6 \times 10^5$ to $10 \times 10^7$ (18).

The lectin binding properties of a polysacchar-
Table VI
Effect of Inhibitors on Recovery from Con A Treatment

| I | II | III Aggregate adhesion mean ± SE (n) |
|---|----|---------------------------------|
| Con A | Inhibitor during recovery | % |
| 0 | 10 µg/ml cycloheximide | 56 ± 3 (6) |
| 10 µg/ml | 10 µg/ml cycloheximide | 43 ± 4 (6) |
| 0 | 10 mM azide | 49 ± 4 (6) |
| 10 µg/ml | 10 mM azide | 37 ± 5 (6) |
| 0 | – | 56 ± 2 (9) |
| 10 µg/ml | – | 44 ± 3 (9) |

Cell aggregates and layers were incubated for 10 min with Con A as listed in column I. Unbound Con A was washed out and the cells were then incubated for 15 min at 37°C in PBS containing the inhibitors in column II, followed by 40-min incubation at 37°C in Hanks' Salts containing 100 mM methyl-mannose plus the same inhibitors. Aggregates and layers were then rinsed into Hanks' Salts, and a 15-min adhesion assay was done. (n) indicates the number of assays.

Figure 14 Abscissa: Temperature of 40 min incubation in Hanks' Salts with 100 mM α-D-methyl-mannoside after initial 10-min binding of Con A at 37°C. Ordinate: Percent of aggregate-layer adhesion in a subsequent assay. Mean ± SE (n ≥ 3) for each point. ---untreated. ---Con A treated.

Figure 15 Recovery of adhesion and release of Con A from retinal cells incubated with α-D-methyl-mannoside at 37°C and at 23°C. Abscissa: Duration of incubation in Hanks' Salts containing 100 mM α-D-methyl-mannoside. Left ordinate: Percent of aggregate-layer adhesion assayed at 37°C after recovery at 23°C or 37°C. Right ordinate: Percent of originally bound Con A released from cells. Mean (n = 3) for each point.

without soluble lectin before an adhesion assay (Table II). The inhibition of adhesion by Scon A was increased when Scon A binding was followed by incubation of retinal cells with antibody to Con A (Table III). And finally, CB reduced the extent to which Con A inhibited adhesion (Table IV).

Relationship of Redistribution of Receptors to Inhibition of Adhesion

Fluorescence staining of Con A-receptor complexes revealed that binding of Con A to retinal cells induced clearing of Con A receptors from filopodia, flattened portions of growth cones, and the edges of nerve fibers (Fig. 7a and b). Procedures which reduced the inhibition of cell adhesion by Con A also interfered with redistribution of Con A-receptor complexes. Con A receptors were not redistributed when incubated with Con A at 9°C (Fig. 8a and b). Scon A-receptor complexes were not cleared unless Con A treatment was followed by antibody to Con A (Figs. 9a, b, 10a, b, and 11a, b). Cytochalasin interfered with rearrangements of receptors (Fig. 13a and b). These data suggest that the same activities produce rearrangements of Con A-receptor complexes and inhibition of intercellular adhesion.

Release of Con A and Recovery of Adhesion

Recovery of aggregate-layer adhesion accompanied release of Con A at 37°C (Fig. 14). Neither new proteins nor normal ATP synthesis
seem to be required for recovery (Table VI). However, lowered temperatures prevented the recovery of ability to adhere, even though the release of Con A was not diminished (at 23°C at least, Figs. 14 and 15). This temperature sensitivity of recovery may mean that recovery involves surface movements. The average mobility of Con A receptors exhibits temperature-dependence below 37°C (33).

**How Does Con A Inhibit Adhesion?**

The inhibition of intercellular adhesion by Con A can be explained in several ways. One line of explanation assumes that the retinal Con A receptors include molecules which participate directly in intercellular adhesion. Another line assumes that Con A receptors are not cell ligands, but that rearrangements of Con A-receptor complexes inhibit adhesion by other means.

Initial discussion assumes that surface components involved in cell adhesion are Con A receptors. In this regard, Hausman and Moscona (17) describe an aggregation-promoting factor from retinal cells which contains mannosyl residues.

**If Cell Ligands are Con A Receptors**

If cell ligands are Con A receptors, then we have found that binding of Con A and subsequent redistribution interferes with their function in intercellular adhesion. Con A might interfere with adhesion by steric blocking of molecular interactions. However, we have found that at 23°C Con A could be released from retinal cells, but intercellular adhesion remained reduced. This suggests that Con A-induced rearrangements of cell surface components rather than the actual presence of Con A account for the inhibition of cell adhesion.

Rearrangements of Con A receptors might interfere with their function in intercellular adhesion in several ways. The regions from which Con A receptors are cleared, filopodia and other flattened extensions, are the sites of adhesive contacts of cells with artificial substrata and with other cells (1, 4, 16, 31). Hence, removal from these areas of Con A receptors which are cell ligands should reduce the ability of such structures to initiate adhesive bonds. Alternatively, adhesive contacts may form anywhere on a cell, and Con A inhibits adhesion, by lowering the mobility of Con A receptors, after crosslinking and redistribution (36). Lateral mobility of cell ligands may be important to intercellular adhesion (8, 27, 41).

Finally, redistribution may induce conformational changes in cell ligands which interfere with their ability to form bonds between cells.

**If Cell Ligands Are Not Con A Receptors**

The other explanation supposes that cell ligands are not Con A receptors, but that crosslinking and redistribution of Con A receptors inhibits cell adhesion by other means. Our experiments with cytochalasins implicate cytoskeletal elements in the rearrangement of Con A receptors, and associated inhibition of adhesion. We also found that CB or colchicine treatment alone reduced aggregate-layer adhesion (43). Edelman (12) has postulated that several cell surface functions may be coordinately regulated by alterations in the structure, location, and activity of surface receptors via associations with the submembranous cytoskeleton. Aspects of this phenomenon, termed surface modulation, which might explain Con A's inhibition of intercellular adhesion, are the inhibition of receptor mobility by locally bound Con A (35, 44, 45) or alterations in the functional association of microfilaments and microtubules with the cell surface (2, 5, 11, 38). The cytoskeleton may be involved in moving and positioning individual membrane components or in moving and extending large portions of the cell (43).

These studies indicate that lectin-mediated rearrangements of cell surface components can rapidly and substantially alter cell function. Without identifying the molecules which mediate intercellular adhesion, we cannot say whether Con A acts by directly binding to these molecules or by other means, such as surface modulation. Whatever the case, intercellular adhesion depends upon the dynamic nature of the cell surface. The involvement of polysaccharides in an important cell surface function is also indicated.

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