

CONCAVALIN A INCREASES THE STRENGTH OF BABY HAMSTER KIDNEY CELL ATTACHMENT TO SUBSTRATUM

FREDERICK GRINNELL

From the Department of Cell Biology, University of Texas Southwestern Medical School, Dallas,
Texas 75235

ABSTRACT

The strength of attachment of normal and transformed baby hamster kidney cells was markedly increased when attached cells were treated with concanavalin A (Con A). The cells became less sensitive to detachment by physical shear or by treatment with trypsin or EDTA; however, their morphology, as observed by phase contrast microscopy, did not change. The effects of Con A were prevented by the simultaneous addition of either D-glucose or α -methyl-D-glucoside with the Con A. Also addition of these reagents to the attached cells after Con A treatment partially reversed the effects caused by Con A. Pretreatment of the culture flasks with Con A before cell attachment resulted in an increase in the strength of cell attachment to the culture flasks as compared to untreated controls.

INTRODUCTION

The attachment of cells to a substratum requires, first, that the cell surface make close contact with the substratum surface and, second, that there are cell surface components that attach the cell to substratum. We have recently presented evidence suggesting that the specific cell surface components involved in attachment may be proteins (1) and that these components are exposed on the cell surface in such a way that centrifugation of the cells onto a substratum results in immediate cell attachment.¹ Cell surface glycoproteins have been shown to be involved in baby hamster kidney (BHK) cell aggregation (2), and Apfel and Peters (3) have proposed that these compounds might be involved in cell attachment to a substratum.

Plant lectins such as concanavalin A (Con A)

are useful reagents for investigating the glycoproteins of cell surfaces; Sharon and Lis recently reviewed these studies (4). Because glycoproteins may be involved in cell attachment it was of interest to investigate the effect of Con A on cell attachment. In the course of these studies a novel property of Con A was observed. When normal or malignant baby hamster kidney cells were attached to a substratum and subsequently treated with Con A, the strength of cell attachment markedly increased. The results of these experiments suggest that Con A is acting as an adhesive bridge between the cell surface and the substratum. The observations describing this property of Con A are reported herein.

MATERIALS AND METHODS

Baby hamster kidney cells which were suspension culture-adapted (BHK-21-13s) were the gift of Dr. Adrian Chappel, Communicable Disease Center,

¹ Milam, M., F. Grinnell, and P. A. Srere. 1973. The effect of centrifugation on cell adhesion. *Nat. New Biol.* In Press.

Atlanta, Ga., polyoma-transformed baby hamster kidney cells (BHK-py) were the gift of Dr. Walter Eckhart, Salk Institute, San Diego, Calif. Eagle's minimal essential medium (MEM) (spinner modified), MEM amino acids, MEM vitamins, fetal calf serum, phytohemagglutinin, pokeweed mitogen, and 0.25% trypsin were obtained from GIBCO (Grand Island Biological Co., Grand Island, N. Y.). Human serum albumin (25%, salt poor) was obtained from Armour Pharmaceutical Co., Chicago, Ill. Hepes buffer, fetuin, crystalline bovine serum albumin, and α -methyl-D-glucoside were purchased from Sigma Chemical Co., St. Louis, Mo. Other reagent grade chemicals were obtained from Fisher Scientific Co., Houston, Tex. Con A was purchased from Calbiochem, San Diego, Calif., or from Miles Yeda, Kankakee, Ill. Con A (from either source) was purified essentially according to the method of Agrawal and Goldstein (5).

BHK cells were grown in suspension culture in Wistar medium which is Eagle's MEM (spinner modified) with double the concentration of amino acids (except for $\times 1$ glutamine) and vitamins and supplemented with 0.1 mg/liter ferric nitrate, 2.0 g/liter glucose, 10% tryptose phosphate broth, and 10% fetal calf serum. The final sodium bicarbonate concentration in the medium was 0.5 g/liter.

The attachment and detachment of cells were measured essentially as described previously (1). For cell attachment, suspension culture cells in the logarithmic growth phase were collected by centrifugation at 1,000 *g* for 5 min, resuspended in 5.0 ml of adhesion salts (0.8 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 116 mM NaCl, 5.4 mM KCl, 20 mM Hepes buffer [pH 7.2]) containing 5% fetal calf serum, and placed in Falcon 3012 polystyrene culture flasks (surface area 25 cm², 30-ml capacity) (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.). The flasks were placed in a 30°C incubator for the time periods designated in the experiments. At the end of the incubations the nonattached cells were resuspended by shaking the bottles at 100 rpm on a R-2 reciprocating shaker (stroke 1 inch) (New Brunswick Scientific Co., New Brunswick, N. J.) for 1 min at room temperature and this suspension was carefully removed with a pipet. For cell detachment, 3.3 ml of detachment medium (composed of 3.0 ml adhesion salts minus $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ plus 1.1 mM EDTA, 0.2 ml 0.25% trypsin [GIBCO], and 0.1 ml 25% human serum albumin [salt poor]) were added to the attached cells which remained in the bottles. The bottles were incubated for 10 min at 30°C and then subjected to shaking on the R-2 shaker for 1 min at 250 rpm. The turbidities of the suspensions of non-attached and attached/detached cells were determined at 640 nm using a Spectronic 70 (Bausch and Lomb Inc., Scientific Instrument Div., Rochester, N. Y.) equipped with digital readout, and cell con-

centrations were calculated from a previously determined relationship between cell number and absorbancy.

RESULTS

Effect of Con A on BHK-21-13s

Cell Detachment

The data in Table I show that incubation of attached BHK-21-13s cells for 10 min with a medium containing trypsin (0.017%) and EDTA (1.1 mM) (total composition of the detachment medium is described in the Materials and Methods section) resulted in detachment of all the cells. On the contrary, when the attached cells were incubated with 40 $\mu\text{g}/\text{ml}$ of Con A for 5 min and then were subjected to detachment medium, very few cells were detached. When the Con A-treated attached cells were incubated with higher levels of trypsin (0.25%), or in detachment medium for longer time periods (30 min), there was only a slight increase in the number of cells that detached. Also, when attached cells were subjected to a shearing force generated by shaking at 330 rpm on a reciprocating shaker, fewer cells detached if the cells were first treated with Con A compared to nontreated controls.

Other plant lectins were tested for their effect on the strength of cell attachment. Phytohemagglutinin, at concentrations above 125 $\mu\text{g}/\text{ml}$, had an effect similar to that of Con A. Pokeweed mitogen had no effect even at a concentration of 250 $\mu\text{g}/\text{ml}$. Nor was there an effect on the strength of cell attachment when the cells were treated with fetuin (0.5 mg/ml), bovine serum albumin (0.5 mg/ml), or fetal calf serum at concentrations of 1%, 10%, or 100% instead of Con A. In fact the addition of fetal calf serum (5%) with Con A inhibited the effect of the latter.

The decrease in cell detachability resulting from Con A treatment was prevented by the simultaneous addition of 0.1 M D-glucose or 0.01 M α -methyl-D-glucoside with the Con A to the cells (Table II, exp. 1). Furthermore, these reagents partially reversed the effects of Con A when added to the cells after the Con A incubation phase of the experiment (Table 2, exp. 2).

After treatment with Con A there were no significant differences observed in the shape or distribution of the cells on the surface. Nor were there any changes in the cells remaining attached to the flasks after treatment with detachment

medium. Furthermore, after all phases of the experiments the attached cells were judged to be intact by their ability to exclude trypan blue.

In the experiments described above approxi-

TABLE I
Detachment of BHK-21-13s Cells Treated with Con A*

| Experi- ment | Detachment medium | Incuba- tion time | Relative detachment | |
|-----------------|--------------------------------|-------------------------|------------------------|-----------|
| | | | -Con A | +Con A |
| | | <i>Min</i> | | |
| 1 | Detachment medium ‡ | 10 | 100 | 12 |
| | Detachment medium less EDTA | 10 | 68 | 8 |
| | Detachment medium less trypsin | 10 | 61 | 8 |
| | 25% trypsin (Gibco) | 10 | 100 | 32 |
| 2 | Detachment medium | 30 | 100 | 37 |
| | Detachment medium less EDTA | 30 | 100 | 17 |
| | Detachment medium less trypsin | 30 | 100 | 24 |
| 3 | Detachment medium | 10 | 100 | — |
| | Adhesion salts, 100 rpm § | 1.5 | <5 | <5 |
| | Adhesion salts, 330 rpm | 1.5 | 42 | 9 |

* For each experiment, 2×10^6 cells were attached for 30 min as described in the Materials and Methods section. After the incubations, the nonattached cells were removed and 2.0 ml of adhesion salts were added to the attached cells. Con A was added at a concentration of 40 $\mu\text{g}/\text{ml}$ where indicated. After 5 min at room temperature, the adhesion salts \pm Con A were removed. 3.0 ml of the specified detachment medium were then added and the flasks placed at 30°C for the indicated time periods. Flasks were then subjected to shaking at 250 rpm for 1 min on the New Brunswick R-2 shaker. The detached cells were recovered and cell concentrations and relative detachment determined as described in the Materials and Methods section. The data are from representative experiments.

‡ Detachment medium is described in the Materials and Methods section.

§ New Brunswick R-2 shaker (1 inch stroke) at room temperature.

TABLE II
Specificity of the Con A Effect on Attached BHK-21-13s Cells*

| Experi- ment | First addition to attached cells | Second addition to attached cells | Relative detach- ment |
|-----------------|-------------------------------------|-----------------------------------------|-----------------------------|
| 1 | None | | 100 |
| | Con A (20) | | 29 |
| | Con A (20), D-glu | | 93 |
| | Con A (20), α MG | | 93 |
| 2 | None | none | 100 |
| | Con A (20) | none | 37 |
| | Con A (20) | D-glu | 76 |
| | Con A (20) | α MG | 73 |

* For each experiment, 2×10^6 cells were attached for 30 min as described in the Materials and Methods section. After the incubations, the nonattached cells were removed and 2.0 ml of adhesion salts were added to the attached cells (first addition). Con A ($\mu\text{g}/\text{ml}$ in parenthesis), D-glucose (D-glu) (final concentration 0.1 M), and α -methyl-D-glucoside (α MG) (final concentration 0.01M) were added as indicated. After 5 min at room temperature, the media were removed. In exp. one the relative detachment was then determined as described in the Materials and Methods section. In exp. 2, 2.0 ml of fresh adhesion salts were added to the attached cells (second addition). D-glu and α MG were added at the same concentration as above. After 10 min at room temperature the various incubation media were removed and relative detachment was then determined as described in the Materials and Methods section. Data are from representative experiments.

mately 50% of the starting cells in the incubations were attached during the 30-min attachment phase. This value, about 10^6 cells, varied less than 10% from day to day.

Effect of Con A on BHK-21-13s Detachment as a Function of Incubation Time

The data in Fig. 1 illustrate the time dependence of Con A inhibition of cell detachability at three different Con A concentrations. The effect of Con A was linear with time; however, no more than 90% of the cells were observed to be affected. At 5 or 15 $\mu\text{g}/\text{ml}$ of Con A there was a 1-min lag before any effect of the Con A was observed.

In other experiments attached cells were treated with Con A for 1 min. The Con A was then re-

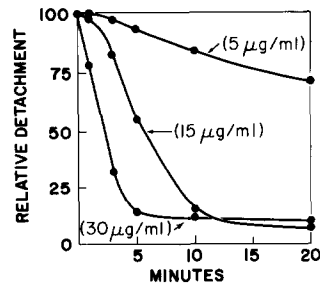


FIGURE 1 Effect of Con A on BHK-21-13s cell detachment as a function of the time of incubation. For each experiment, 2×10^6 cells were attached for 30 min as described in the Materials and Methods section. After the incubations, the nonattached cells were removed. 2.0 ml of adhesion salts, containing Con A at the indicated concentrations, were added to the attached cells for the specified times at room temperature after which the media were removed and relative detachment determined as described in the Materials and Methods section.

moved, and the cells were incubated an additional 4 min with adhesion salts. The detachability of these cells was compared to cells treated with Con A for 1 min and then immediately detached, or, with cells treated with Con A for 5 min, then detached. It was observed that treatment with Con A for 1 min resulted in the same detachability whether the cells were detached immediately or incubated in adhesion salts for an additional time period before detachment.

Effect of Con A on Cell Detachment as a Function of Con A Concentration

The experimental results presented in Fig. 2 show that Con A treatment for 5 min significantly decreased the detachability of BHK-21-13s cells at Con A concentrations above 10 $\mu\text{g/ml}$. In these experiments the starting number of cells during the attachment phase of the incubations was varied and resulted in a different number of attached cells. The effect of Con A was similar whether the experiments contained 7.3×10^5 , 1.0×10^6 , or 1.2×10^6 attached cells.

Con A affects the detachability of BHK-py cells at essentially the same concentrations as were effective with BHK-21-13s cells. BHK-py and BHK-21-31s cells were tested for agglutination by Con A. 10^6 cells in 1.0 ml adhesion salts containing Con A were incubated at room temperature for 30 min while being shaken at 100 rpm on the New Brunswick R-2 reciprocating shaker (1 inch

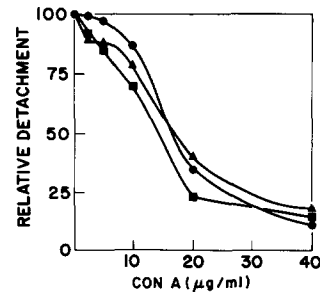


FIGURE 2 Effect of Con A on BHK-21-13s cell detachment as a function of Con A concentration. For each experiment, cells were attached for 30 min as described in the Materials and Methods section. After the incubations the nonattached cells were removed. The number of attached cells at the end of the attachment phase were: (●) 1.2×10^6 , (■) 1.0×10^6 , or (▲) 7.3×10^5 . 2.0 ml of adhesion salts containing Con A at the indicated concentrations were added to the attached cells for 5 min at room temperature, after which the media were removed and relative detachment determined as described in the Materials and Methods section.

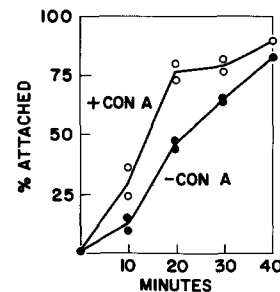


FIGURE 3 Attachment of BHK-21-13s cells after treatment of Falcon flasks with Con A at a final concentration of $120 \mu\text{g/ml}$. Other details are the same as in the *note to Table III.

stroke). BHK-py were agglutinated under these conditions at Con A concentrations as low as $20 \mu\text{g/ml}$. There were few free cells and clumps contained 10 or more cells. On the other hand, BHK-21-13s cells were very poorly agglutinated by Con A even at a Con A concentration of $100 \mu\text{g/ml}$.

Effect of Pretreating Falcon Flasks with Con A on Subsequent BHK-21-13s Cell Attachment and Detachment

Falcon flasks were treated with $40 \mu\text{g/ml}$ of Con A in 2.0 ml adhesion salts for 5 min at room

TABLE III
Attachment of BHK-21-13s Cells after Treatment
of Falcon Flasks with Con A*

| Con A | Cells attached | Calculated relative detachment ‡ |
|------------------|----------------|----------------------------------|
| $\mu\text{g/ml}$ | % | |
| None | 44 | 100 |
| 12 | 49 | 100 |
| 24 | 48 | 100 |
| 48 | 50 | 100 |
| 120 | 71 | 74 |
| 240 | 73 | 52 |

* For each experiment a Falcon flask was treated with 2.0 ml of adhesion salts containing Con A at the concentrations indicated. After 25 min at room temperature, the media were removed and 2×10^6 BHK-21-13s cells were tested for attachment to the flask in a 30-min incubation as described in the Materials and Methods section. Data are from a representative experiment.

(Nonattached cells after 30-min attachment phase) + (detached cells) $\times 100$

‡ $\frac{\text{total number of cells in experiment}}$

temperature. This medium was then removed and the flasks used to test cell attachment. There was no difference in the attachment or detachment of BHK-21-13s with pretreated or nontreated flasks. However, when the flasks were incubated with higher concentrations of Con A (120 $\mu\text{g/ml}$) for a longer incubation period (25 min) there was a significant increase in the rate of cell attachment to the treated flasks, as shown by the results presented in Fig. 3.

The results in Table III show the effect of pretreating the flasks with various concentrations of Con A on the subsequent attachment of cells to the flasks. Not only was there an increase in attachment with an increase in Con A concentration, but, at higher concentrations of Con A, there was an increase in the strength of cell attachment to the flasks.

DISCUSSION

We have observed that Con A treatment of BHK cells previously attached to Falcon flasks results in an increase in the strength of attachment of the cells. This reaction can be quantitatively and simply measured and thus has the potential to be a biological assay for Con A activity. The increase in strength of attachment is reflected in the decreased detachability of the cells by physical shear

or by treatment with trypsin or EDTA. The effect of Con A is specific since it is prevented or reversed by either D-glucose or α -methyl-D-glucoside.

The concentrations of Con A that were observed to cause an increase in strength of cell attachment are similar for BHK-py and BHK-21-13s cells; however, only BHK-py cells are significantly agglutinated by Con A, even at very high Con A concentrations. Therefore, under these conditions, the effect of Con A on the strength of cell attachment is apparently independent of the arrangement of Con A binding sites of the cell surface. Differences in this arrangement have been proposed to account for differences in agglutinability of normal and transformed cells (6).

An increase in strength of cell attachment might be accounted for by the flattening of the cells (i.e., an increase in the area of contact between cell and substratum) or by the agglutination of the cells on the surface of the Falcon flasks. However, observations of the cells by phase contrast microscopy before and after Con A treatment revealed that there were no changes in cell shape or the distribution of cells on the surfaces of the Falcon flasks.

Another possible explanation for the increase in strength of cell attachment was the direct bridging of Con A between the cell surface and the surface of the Falcon flask. If this were the case, we anticipated that pretreatment of flasks with Con A might result in an increase in the strength of cell attachment and an increase in the rate of cell attachment. In fact, we observed both effects when the flasks were pretreated with Con A. In these experiments, higher Con A concentrations and longer incubation times were required than in experiments where Con A was added to already attached cells. This probably reflects the inefficiency of randomly binding Con A to the surfaces of the flasks and then to the cells, as compared to having the surfaces of the cells and the flask simultaneously in apposition.

Thus, treatment of attached cells with Con A probably results in formation of bridges between adjacent areas of the cell surface and the Falcon flask surface. It is likely that Con A binds to specific carbohydrate portions of glycoproteins on the cell surface and binds to the Falcon flask by the mechanism of nonspecific adsorption (7).

The increase in strength of cell attachment caused by Con A treatment may account for the effect of Con A on cell mobility described by Friberg et al. (8). They reported that Con A treatment of cells resulted in inhibition of cell migra-

tion, whether or not the cells were agglutinable by Con A. The concentrations of Con A which they observed to inhibit cell migration are similar to those we observed to cause an increase in strength of cell attachment. The inhibition of macrophage mobility by Con A has also been described (9). Since cell movement requires both making new adhesions and breaking old ones (10), an increase in the strength of a cell's attachment would retard cell mobility.

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