A Quantitative Assay for Concanavalin A-mediated Cell Agglutination*

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SUMMARY

A quantitative method was developed for measuring cell agglutination by the plant lectin, concanavalin A. The method, based on an assay for intercellular adhesion, determines the concanavalin A-stimulated rate of attachment of single cells to a cell layer. The rate depended on the number of single cells and the concentration of concanavalin A. Experiments on concanavalin A agglutinability of mouse BALB/c 3T3 cells agreed qualitatively with observations obtained with a visual method presently in use. For example, the concanavalin A-stimulated rate was low when EDTA was used to prepare the single cells, high when trypsin-dispersed cells were employed, and highest when the latter cells were assayed with trypsin-treated cell layers. The latter result was not explicable in terms of concanavalin A binding to the cell layer, since the same quantities of labeled lectin attached to untreated and to trypsin-treated cell layers.

Concanavalin A attached to an untreated cell layer in such a manner that it retained high binding activity toward surface receptors on trypsin-treated single cells. Above a threshold density of concanavalin A bound to the cell layer, the stimulation of single cell attachment to the layer was directly proportional to the amount of concanavalin A bound to the layer.

The concanavalin A-stimulated rate of attachment was specifically inhibited by methyl α-D-mannopyranoside. However, once single cells were attached to the cell layer in the presence of concanavalin A, they were not released by methyl α-mannoside. Agglutination is considered to be more complex than the simple binding of cells together through lectin bridges.

Plant lectins, such as concanavalin A, have been extensively used to probe the molecular structures of animal cell surfaces (1, 2). This approach is possible because lectins bind with a high degree of specificity to sugars of the type found in cell surface macromolecules. Lectins are multivalent and can, under certain conditions, agglutinate cells to which they bind. At certain lectin concentrations some lectins agglutinate normal interphase cells only after brief exposure to proteases (3, 4), whereas transformed malignant cells are agglutinated without prior protease treatment.

One difficulty associated with studies on lectin-mediated agglutination of normal or transformed cells, as emphasized in recent reviews (2, 5), has been the lack of a quantitative, objective assay. This report presents an objective assay, based on a method for determining the rate of intercellular adhesion (6), in which labeled single cells in suspension are exposed to a confluent cell layer, and the attachment of the single cells to the cell layer is determined as a function of time of incubation. The rate is characteristic of the cell types used.1 Addition of concanavalin A considerably enhanced the rate of attachment of single mouse 3T3 cells to 3T3 cell layers; these cells normally show a low rate of intercellular adhesion (adhesive rate constant of 0.22%/per min). In view of current interest in cell agglutination by lectins, this observation was studied in detail, and a quantitative procedure was developed for measuring concanavalin A agglutinin activity.

This paper describes studies on the kinetics of the assay. Investigations on the effects of trypsinization of the cells and of certain glycosides on the agglutination phenomenon are reported. Preliminary results have been presented (7).

EXPERIMENTAL PROCEDURE

Materials

The following media were used unless otherwise indicated.

Growth Media—The growth media contained either BHK 21

1 In this paper the rate at which single cells attach to a cell layer is designated intercellular adhesion, and the stimulation above this rate by concanavalin A is designated agglutination. Rate is expressed as the number of cells that attach to the cell layer per unit time. Under the conditions described here, the rates of adhesion and agglutination are directly proportional to the number of single cells in the suspension. The rate constant for the adhesion process is designated the adhesive rate constant (6).
or Dulbecco's modified Eagle medium (Gibco, Inc.) supplemented with heat-inactivated calf serum (10% final concentration, Flow Laboratories, Rockville, Md.), and with 100 units of penicillin and 100 µg of streptomycin sulfate (Gibco, Inc.) per ml. Cells grown in either medium gave similar results; Dulbecco's modified Eagle medium is considered the medium of choice for 3T3 cells.

**Dispersing Solutions**—Tissue culture cells were dispersed with solutions containing either crude trypsin or EDTA, as specified. The trypsin solution, obtained from Gibco, Inc., contained 0.25% crude trypsin in Gibco Solution A; the latter is a balanced salt solution containing NaCl, KCl, glucose, NaHCO₃, and phenol red indicator. The EDTA dispersing solution contained 0.5 mM EDTA in phosphate-buffered saline. Phosphate-buffered saline contains the following components (in grams per liter): NaCl, 8.00; KCl, 0.200; Na₂HPO₄·1.150; KH₂PO₄·0.200, adjusted to pH 7.3.

**Incubation Medium (Medium B)**—This medium consisted of HEPES-buffered Hanks' balanced salt solutions without glucose. The components of this medium (in grams per liter) are the following: NaCl, 8.0; KCl, 0.4; MgSO₄·7H₂O, 0.2; NaH₂PO₄·2H₂O, 0.06; KH₂PO₄·0.06; CaCl₂·0.14; NaHCO₃·0.35, supplemented with HEPES (Sigma). The medium was adjusted to pH 7.3.

**Concanavalin A and Glycosides**—Concanavalin A purchased from Calbiochem (Lot 210073) was dissolved in Medium B immediately before use. The lectin was not further purified since only one or two trace contaminants (stained with Coomassie brilliant blue) were found when a sample was subjected to sodium dodecyl sulfate disc gel electrophoresis (8), and only a single protein peak was detected by fluorescence when the protein was derivatized with dansyl chloride and subjected to affinity chromatography on Sephadex (9); 5.6 moles of dansyl group were introduced per mole of conconavalin A tetramer.

The following glycosides were obtained from Sigma: methyl α-D-mannopyranoside (Lot 52C8379), methyl α-D-glucofuranoside (Lot 124193410), and methyl β-D-glucopyranoside (Lot 12C9530). The glycosides exhibited the expected melting points; in addition, the purity of the most important glycoside, methyl α-D-mannopyranoside, was established by thin layer chromatography and by optical rotation. Methyl α-D-galactopyranoside was obtained as a gift from Dr. Y. C. Lee of this department. L-[3H]Leucine (specific activity, 5 Ci per mmole) and [14C]acetic anhydride (1 mCi/20.4 mg) were obtained from New England Nuclear. All other reagents were of the highest purity commercially available.

**Growth of Cell Lines**—Two mouse fibroblast 3T3 cell lines were used, BALB/c 3T3 (highly contact-inhibited, clone 3, obtained from Dr. S. Roth of this department (6, 10)), and Swiss 3T3 (obtained from Dr. Howard Green, Massachusetts Institute of Technology (11)). Both cell lines were grown in the growth media described above at 37°C in a water-saturated atmosphere of 95% air-5% CO₂ in Falcon tissue culture dishes. The cells were examined for mycoplasma by Microbiological Associates, Bethesda, Md., and were found free of contamination. Stocks of the two lines were maintained in sealed ampules in liquid nitrogen, and cells were passed no more than 20 times for the experiments described below. The BALB/c 3T3 line was highly contact inhibited, reaching a saturation density of 2.6 × 10⁶ cells per cm², while the Swiss 3T3 cells attained a density of 5 × 10⁶ cells per cm². Cells were transferred before reaching confluency. For passage, the plates were first washed with phosphate-buffered saline, and then treated with the trypsin-dispersing solution for 10 to 15 min at 37°C.

**METHODS**

**Preparation of Cell Layers**—Cell layers were prepared from confluent cultures of cells essentially as described by Walther et al. (6). Each well of multiple well tissue culture dishes (model FB16-24TC, Linbro Chemical Co., New Haven, Conn.) was inoculated with 1 ml of a suspension of trypsin-dispersed single cells (2 × 10⁶ cells per ml of growth medium) 24 hours before use. The high cell density (1 × 10⁵ cells per cm²) ensured complete coverage of the plastic substratum.

For trypsin treatment of cell layers, growth medium was removed by aspiration, the cell layers were washed twice with Medium B, and treated with 0.5 ml of 0.025% trypsin (Gibco 0.25% trypsin diluted 10-fold in Medium B). After incubation for 4 min at 37°C, the trypsin solution was removed by gentle aspiration and the cell layers were washed twice with growth medium. The cell layers were incubated in growth medium for 15 min, and inspected by phase microscopy for complete confluence.

**Preparation of Single Cells for Assay**—Radioactively labeled single cells were prepared essentially as described previously (6). Confluent cultures were trypsin-dispersed as described above and inoculated at a concentration of 1.5 × 10⁴ cells per Falcon tissue culture plate (78.5 cm²). The freshly inoculated cells were maintained in growth medium containing 5 to 10 µCi per ml of L-[3H]leucine (2 Ci per mmole; New England Nuclear) for 12 to 18 hours, followed by a chase period in unlabeled growth medium for 2 hours prior to harvesting. The cells were removed from the culture dish by treatment with either 0.25% trypsin or 0.5 mM EDTA in phosphate-buffered saline (as indicated) for 10 to 15 min at 37°C. The dissociated cells were diluted in 20 volumes of growth medium, collected by centrifugation at 50 × g for 15 min, and resuspended in 20 ml of Medium B, recentrifuged, and finally suspended in Medium B at a concentration of 2.5 × 10⁶ cells per ml. Cells usually incorporated 0.1 to 0.6 cpm of L-[3H]leucine per cell; essentially all of the labeled material was precipitable with cold trichloroacetic acid.

**Assay Procedure**—Immediately prior to the assay, cell layers (untreated or trypsin-treated) were rinsed twice with Medium B, and 1 ml of the labeled single cell suspension was added to each well of the multiple well dish (0.5 ml of suspension per cm² of cell layer). Concanavalin A in Medium B was immediately added with a micropette (routine 50 µl of a 1-mg per ml stock solution) and mixed by swirling. Incubations were conducted at 37°C with gentle agitation (60 strokes per min, 8 cm per stroke) in a Warner-Chilcott reciprocal water bath shaker. After incubation for the required time, the unattached single cells were removed by gentle aspiration. The cell layers were washed three times with 1-ml aliquots of Medium B and lysed with 0.5 × NH₄OH, and radioactivity was determined by standard liquid scintillation techniques as previously described (6).

**Preparation of [3H]Concanavalin A**—Concanavalin A (50 mg) was dissolved in 1.0 ml of a saturated sodium acetate solution at 4°C and permitted to react with [3H]acetic anhydride (1.0 mCi per 20.4 mg) as described by Agrawal et al. (12). After 1 hour the reaction mixture was dialyzed against distilled water and subsequently against a 1.0 M sodium chloride solution. Traces of low molecular weight radioactivity were removed by gel filtration through a Bio-Gel P-10 column equilibrated with 0.9 M sodium acetate. The concanavalin A was concentrated to 1 mg per ml by ultrafiltration (Diaflo) and stored at 4°C in 0.9 M sodium acetate.
FIG. 1. Effect of cell concentration on the rate of cell attachment. Aliquots (1.0 ml) of suspensions containing the indicated concentrations of trypsin-dispersed Swiss 3T3 cells) were exposed to untreated Swiss 3T3 cell layers. Concanavalin A was added as a 50-μl aliquot of a 1-mg per ml solution and mixed by swirling (final concentration, 48 μg per ml). Cell attachment was measured as described under "Experimental Procedure." The rate of cell attachment is expressed as the number of labeled cells attached (X 10^-3) to the cell layer per 10-min incubation (0). The percentage of attachment (right ordinate) represents the percentage of the initial number of cells attached during a 10-min incubation (O).

acetate. Prior to use, aliquots were dialyzed overnight against Medium B. The specific activity of the [14C]concanavalin A was approximately 0.37 μCi per mg (7.4 × 10^6 cpm per mg, as measured in the liquid scintillation system described). The [¹⁴C]-concanavalin A retained activity as demonstrated by glycogen precipitation in an Ouchterlony diffusion plate and migrated identically with the tetrameric form of concanavalin A through a Bio-Gel P-200 column (equilibrated with Medium B).

RESULTS

Effect of 3T3 Cell Number and Concanavalin A Concentration on Rate of Agglutination—In the experiments described in this section, trypsin-dispersed single cells were tested with untreated 3T3 cell layers. Similar kinetic results were obtained with cells pretreated as described in the following section. In the presence of 48 μg of concanavalin A per ml of incubation mixture, the rate of cell attachment was directly proportional to single cell number in the range 5,000 to 50,000 cells per ml (Fig. 1). A single cell concentration of 25,000 cells per ml was selected to minimize intercellular adhesion and agglutination of these cells in suspension above the cell layer. This single cell concentration is approximately 2% of the cell concentration usually employed in the visual assay (3). The interaction between single cells and the cell layer, rather than interactions between single cells per se, was favored both by the large number of cells in the layer, and by the rapid settling and concentration of the single cells above the cell layer. Microscopic and autoradiographic experiments confirmed that the cells which did attach to the cell layer (both in the presence and absence of concanavalin A) attached as single cells, not as aggregates (Fig. 2). Above a concentration of 50,000 cells per ml, small aggregates were observed in the suspension when concanavalin A was present.

The rate of cell attachment was also a function of the concentration of concanavalin A (solid curve, Fig. 3). Below 2 μg per ml of the lectin, there was no detectable stimulation of cell attachment to the cell layer. The concentration used for routine work, 48 μg per ml, gave close to the maximal rate of attachment. While not studied here, the results in Fig. 3 indicate that the assay can be used as the basis for determining the agglutinin activity of crude lectin preparations, thus serving as an aid in purification of agglutinins in general.

Effect of Method of Cell Preparation—Studies from other laboratories, using visual procedures (3, 13), indicated that concanavalin A-agglutinated 3T3 cells dissociated with proteases, but not cells dissociated with EDTA. Therefore, in the present experiments both EDTA and trypsin were used to prepare suspensions of single cells; these were then tested with either
ship between the amount of concanavalin A bound to a cell and concanavalin A binding can be influenced by a number of factors including growth conditions, state of confluency, and others (15). Previous reports (3, 14) indicate that there is neither a direct nor simple relation to the single cells and the cell layer had been treated with trypsin (Fig. 4E). These results therefore provide a quantitative evaluation of the effect of concanavalin A on both EDTA- and trypsin-dispersed single cells to trypsin-treated cell layers. Since the rate of attachment of single cells and the cell layer was only slightly stimulated, about 1.5-fold above the background rate of intercellular adhesion (Fig. 4A).

Binding of Concanavalin A to Cell Layers—Several reports (2, 15) indicate that there is neither a direct nor simple relationship between the amount of concanavalin A bound to a cell and cell agglutinability. Both agglutinability and the extent of concanavalin A binding can be influenced by a number of factors including growth conditions, state of confluency, and others (15).

Radioactively labeled concanavalin A was used in experiments designed to investigate whether there is indeed a correlation between the amount bound to cell layers and the differences in trypsin-induced agglutinability that are as illustrated in Fig. 4. A similar assay for measuring the amount of lectin bound to cell layers has recently been reported (16). The rate of binding of [14C]concanavalin A to 3T3 cell layers was first determined as a function of the concentration of the labeled lectin. As shown in Fig. 5A [14C]concanavalin A binding was observed at all concentrations tested. At the lower concentrations used in these experiments, the rates of binding were approximately proportional to the concentration of concanavalin A. Binding of labeled concanavalin A to trypsin-treated and untreated cell layers indicated that similar quantities of lectin were bound to both types of cell layers over a wide range of concentrations (Fig. 5B).

Radioactively labeled concanavalin A was used in experiments to determine the rates of cell attachment. Rates are expressed as the percentage of cells attached in 20 min. Each bar represents the range of three determinations.
Fig. 5. Binding of \[^{14}C\]concanavalin A to BALB/3T3 cell layers. A, time course of binding of labeled concanavalin A. \[^{14}C\]-Labeled concanavalin A was prepared as described under "Experimental Procedure" and dialyzed against Medium B for 20 hours before use. Untreated cell layers were incubated at 37° with 1.0-ml aliquots containing the indicated concentrations (micrograms per ml) of \[^{14}C\]concanavalin A. At the indicated times, the solutions were removed by aspiration, and the cell layer was washed three times with Medium B. The quantity of radioactivity bound to each cell layer was determined as described in the text. B, binding of \[^{14}C\]concanavalin A to untreated

may be defined as specific concanavalin A binding (15). The data in Fig. 5B also illustrate that this amount was similar for both trypsin-treated and untreated cells.

Thus, the results of the concanavalin A binding experiments did not explain the increased aggregatability of 3T3 cells which resulted from trypsin treatment. Explanations for this phenomenon have been offered. One proposal (17) is that trypsin treatment results in clustering of concanavalin A receptor sites on the cell surface, thereby making the cells agglutinable by the lectin. Another idea (18) is that the distribution of the receptor sites is the same on both trypsin-treated and untreated cell surfaces, but that the binding of concanavalin A to the trypsinized cell (but not the untrypsinized cells) results in clustering of binding sites which in turn gives rise to agglutination. Another possibility is that concanavalin A binds to untreated normal cells in such a manner that it cannot interact with the binding sites on neighboring cells; thus no agglutination occurs.

One advantage of the present assay is that it permits independent treatment (with lectins, proteases, or other agents) of one of the two interacting cell populations (i.e. either the cell layer or the single cells). This type of experiment is shown in Fig. 6. Cell layers (not trypsinized) were pretreated with concanavalin A, the unbound lectin removed by washing, and the resulting layer tested with trypsin-treated single cells. The single cells attached at a rate comparable to the rate obtained when the lectin was present during the assay.

The results in Fig. 6A show that the rate of attachment of trypsin-dissociated cells to these layers was stimulated as a function of the length of prior incubation with concanavalin A. These results can be presented in another manner (Fig. 6B), which shows that above a certain threshold level, 0.2 \(\mu\)g of concanavalin A bound per cm\(^2\) of cell layer, the rate of cell attachment was directly proportional to the quantity of concanavalin A which was bound to the cell layer. The data in Fig. 6B were calculated from the results in Fig. 5, where concanavalin A binding was shown to be a function of time. In these experiments (Fig. 6), it was important to ascertain whether the observed activity resulted from the bound lectin, or whether it resulted from lectin which became soluble during the course of the assay (approximately 30%). However, the results in Fig. 5B show that even if all of the bound concanavalin A in the experiment in Fig. 6 was released during the incubation with single cells, the concentration (0.5 \(\mu\)g per ml) would be far below that where agglutination of the cells by the soluble concanavalin A could be detected (Fig. 3). From these considerations we conclude that concanavalin A bound to cell layers which were not treated with trypsin, and that the bound lectin is "fully" active in promoting attachment of trypsin-treated single cells to the layers. At this time, therefore, the results are not in accord with any of the three ideas described above in that concanavalin A does not need to react with trypsin treated cells in order to promote agglutination, i.e. it is active when bound to untreated cells. This result appears to conflict with those in Fig. 4 which show that concanavalin A is more active in promoting cell agglutination to trypsin-treated than with untreated cell layers. Conceivably the explanation is purely quantitative, i.e. that a greater fraction of the concanavalin A bound to trypsin-treated cells can interact with other cells compared with concanavalin A bound to untreated cell surfaces.

**Effect of Methyl \(a\)-d-Manopyranoside on Agglutination**—The binding of concanavalin A to simple sugars and glycosides has been extensively investigated (12, 19, 20) and recently reviewed (1, 5), and it has been shown that binding constants are highest for \(a\)-linked sugars of the \(d\)-gluco or \(d\)-manno configurations. The nature of the group attached to C-2 also influences the binding constant. The most effective of the simple glycosides is methyl \(a\)-d-manopyranoside.

The agglutinability of 3T3 cells to cell layers was inhibited by...
Fig. 6. Effect of preincubation of cell layers with concanavalin A. A, effect of length of preincubation with concanavalin A. BALB/c 3T3 cell layers were incubated in Medium B for 30 min, and concanavalin A (40 µg per ml) was added (in the absence of single cells) for various lengths of time as indicated. Excess free lectin was removed by aspiration, and the cell layers were incubated with 30,000 labeled single BALB/c 3T3 cells for 20 min in the absence (○) or presence (●) of concanavalin A (40 µg per ml). Cell attachment was measured as described in the text. The bars give the range of triplicate determinations. B, relationship between cell attachment and the amount of concanavalin A bound to the cell layers. The observed cell attachment in A was plotted against the amount of concanavalin A bound during the preincubation. The binding of the unlabeled concanavalin A was estimated from, and assumed to be identical with, the binding of [14C]concanavalin A shown in Fig. 5. Cell layers were 2 cm² in area.

The results shown in Fig. 7 indicate that the simultaneous addition of methyl α-mannoside and concanavalin A to the assay mixture prevents the lectin from exerting its role as an agglutinin. On the other hand, when single cells were permitted to attach to the cell layer in the presence of concanavalin A under standard conditions for 10 min, and the mixture was then treated with 2.8 mM methyl α-mannoside, no further agglutination occurred, but the cells already attached to the cell layer remained attached (Fig. 8). Thus, the agglutination phenomenon was not rapidly reversed. The same results were obtained with higher concentrations of methyl α-mannoside (up to 40 mM). These results are discussed below.

**DISCUSSION**

The present study established conditions for a quantitative assay for measuring concanavalin A-stimulated rate of single cell attachment to a cell layer. The parameters of the assay were established with both BALB/c and Swiss 3T3 cells, which gave similar results in a number of experiments. The assay offers the following advantages over the previously published methods that measure agglutination by visual estimation of the extent of cell aggregation in the presence of lectin (3, 4). (a) The method reported here is an objective, quantitative assay. (b) It permitted measurement of lectin-stimulated agglutination above a background rate of intercellular adhesion. (c) The suspension of single cells and the cell layer could be independently manipulated prior to the measurement of attachment rates. (d) The rate of attachment was dependent on the concanavalin A concentration and the method may therefore prove useful in the isolation of agglutinins and haptens.

There are some aspects of the assay that require further study. (a) Reproducibility was considered acceptable, although occasional variations in absolute rates of agglutination were observed with different cell preparations. The reasons for this variability are not understood at this time. (b) The cell type under investigation must be capable of forming a layer which adheres to a fixed substrate. However, it may be possible to use the assay with cells such as erythrocytes by employing a reference cell layer of a different type, such as 3T3 cells. (c) Cells with high intercellular adhesive rates cannot be used at present. The high background rate impaired the quantitative measurement of the
of the aggregates formed in the presence of concanavalin A, it did not completely dissociate the aggregates to single cells. This resulted in a final concentration of 23,000 cells per ml, 15 µg of the concanavalin A per ml, and the indicated concentration of methyl glycoside. The percentage of cells attached at 10 and 20 min was determined and rates are presented as the percentage of cells attached in 20 min. Each point represents a single determination. □, background adhesion rate (no concanavalin A added). The following represent attachment in the presence of concanavalin A: ■, no methyl glycoside added; ▲, plus methyl α-D-galactopyranoside; Δ, plus methyl β-D-glucopyranoside; ○, plus methyl α-D-glucopyranoside; ●, plus methyl α-L-mannopyranoside. The shaded area is considered to be a range in which the glycosides are ineffective.

stimulated rate of cell attachment in the presence of concanavalin A. An assay similar in principle to the one described here has been applied to leucocytes which have low intercellular adhesive properties but which are agglutinated by concanavalin A (21).

The results with the present quantitative procedure generally agree with those reported using the visual method (3). For example, EDTA-dispersed 3T3 cells were agglutinated at a low rate compared to trypsin-dispersed preparations. Trypsin treatment of either set of cells was sufficient to elicit a marked concanavalin A effect (Fig. 4), but maximal agglutination rates were attained with prior trypsin treatment of both 3T3 cell layer and single cells. While differential effects at low temperatures have been reported (22), other workers have shown that trypsin-treated and untrypsinized 3T3 cells bind similar amounts of concanavalin A at temperatures above 20° (13, 14, 22). This agrees with our results, where the rates of binding at 37° were also similar (Fig. 5). Furthermore, concanavalin A which bound to an untrypsinized intact layer of cells did so in a manner which allowed the lectin to retain its agglutinating capacity (Fig. 6). These results are not in accord with present theories which require that concanavalin A promotes agglutination by a specific interaction only with trypsin-treated normal cells.

The concanavalin A-mediated attachment of single cells to cell layers was specifically inhibited with methyl α-mannoside (Fig. 7), but single cells which had become attached were not rapidly released (Fig. 8). We have studied the irreversibility of agglutination with the visual assay (3) and have observed (data not shown) that while methyl α-mannoside decreased the size of the aggregates formed in the presence of concanavalin A, it did not completely dissociate the aggregates to single cells. This observation is in agreement with the original observations of others (3). The absence of total reversibility of concanavalin A-mediated agglutination as observed with the visual assay may account for the apparent irreversibility shown in Fig. 8. Thus, in our hands, the qualitative results obtained with the two assays are in general agreement. We conclude, therefore, that the assay reported here may be used for the quantitative determination of concanavalin A-mediated agglutination.

A number of explanations have been offered for the inability either to reverse the binding of concanavalin A to cell surfaces or to reverse concanavalin A-mediated cell agglutination with methyl α-mannoside (2). We suggest that the observed agglutination of fibroblasts by concanavalin A is complex, involving first the formation of cell-cell bonds through concanavalin A bridges (reversed by methyl α-mannoside). Subsequent to this agglutination, adhesive bonds may be formed between the agglutinated cells. These bonds are stable (6) and methyl α-mannoside-resistant. It has been reported that concanavalin A receptor sites are distinct from and independent of membrane sites involved with cell adhesion phenomena (23). According to our interpretation, intercellular adhesion sites between cells would interact with a higher frequency and form stable cell-cell adhesions more rapidly because the cells are juxtaposed via concanavalin A bridges. Comparison of lectin agglutinability among various cell types therefore may reflect a composite of cell surface phenomena, not merely the binding of cells through lectin bridges. The assay reported here should be applicable both to investigate the involvement of these cell surface phenomena and to investigate the basis for the differences in agglutinability between normal and malignant cells.

Fig. 7. Effect of methyl glycoside concentration on concanavalin A-mediated cell attachment. BALB/c 3T3 cell layers were prepared as described in the text. Equal volumes of a suspension of labeled BALB/c 3T3 trypsin-disassociated cells (50,000 cells per ml) and a 2-fold concentrated solution of glycoside plus 30 µg per ml of concanavalin A in Medium B were rapidly mixed, and a 1.0-ml aliquot of the mixture was added to each cell layer. Standard assays were conducted with trypsin-dissociated BALB/c 3T3 single cells (1.0 ml, 25,000 cells per ml) and untrypsinized cell layers. The assay was conducted with and without concanavalin A (15 µg per ml) and methyl α-mannoside (2.8 mM) as indicated below. The percentage of cells attached was determined as described in the text. Each point represents a single determination. The background attachment rate was determined in the absence of concanavalin A (○—○); the rate of attachment was determined in the presence of concanavalin A alone (●—●) and in the presence of concanavalin A plus 2.8 mM methyl α-mannoside added at time zero (▲—▲). In one set of experiments, concanavalin A was added at zero time in the absence of methyl α-mannoside. After 10 min (arrow) methyl α-mannoside (2.8 mM) was added and the incubation continued for an additional 10 min (●—●).
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