CONCANAVALIN A MEDIATED HEMAGGLUTINATION AND BINDING PROPERTIES OF LM CELLS

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SUMMARY. Changes in the extent of concanavalin A binding and concanavalin A-mediated hemadsorption to mouse LM cells were observed at 5-7° and 15-19°. Incubation of LM cells at 7° or less caused a dramatic inhibition of subsequent concanavalin A-mediated hemagglutination at 22°. Reversal of this inhibition required 20-30 min of subsequent incubation at 22°, indicating that factors other than membrane lipid "fluidity" are determinants of agglutinability.

Oncogenic transformation produces alterations in cell surface membranes (1-3). For example, some plant lectins preferentially agglutinate transformed cells. Transformed and normal cells apparently bind equal amounts of lectins (4-6). This information and the results of some ultrastructural studies have led to the suggestion that the increased agglutinability of transformed cells may result from altered surface distribution of the lectin receptors (7). The inhibition of concanavalin A (Con A) mediated cell agglutination at low temperature indicates that "fluidity" of membrane lipids may influence the availability or organization of Con A receptors (8-9).

Factors other than lipid "fluidity" may influence agglutinability mediated by Con A. Alkaloids that disaggregate microtubules reduce the agglutinability of transformed 3T3 cells (10), and the intracellular concentration of ATP in transformed hamster fibroblasts is a critical determinant for agglutination of these cells (11).

Although selective agglutination of malignant cells by certain plant lectins is used extensively as a criterion for cell surface alteration (1), uncertainties exist in the assay for cell agglutination. We therefore developed a rapid, quantitative assay for Con A-mediated agglutination by

modifying the microhemadsorption method of Furmanski et al. (12). We show that lectin binding and lectin-mediated hemadsorption to LM cells are temperature dependent suggesting a critical role for the physical state of membrane lipids. In addition, we describe evidence which indicates that cold labile substructures, possibly microtubules, play a key role in agglutination.

MATERIALS AND METHODS. Mouse LM cells derived from NCTC clone 929 (L cells) were maintained on Eagle's minimal essential medium with Earle's salts plus 0.5% Difco bacto-peptone (13). All cultures were grown in monolayers in Linbro multi-dish trays (35 mm diameter) to 80-100% confluency.

Con A (3 x crystallized, Miles Labs.) was radioactively labeled with [^{3}H]acetic anhydride (500 mCi/mmole, Amersham/Searle) by the method of Agrawal et al. (14). A specific activity of 5.7 x 10^6 cpm/mg indicated two to three molecules of acetate bound per tetrameric molecule of Con A.

RESULTS. Figure 1 describes the saturation of [^{3}H]Con A binding to LM cells. The concentration dependence for binding at both 0° and 22° was almost linear at Con A concentrations less than 100 μg/ml. Addition of 0.05 M α-methylglucopyranoside (αMeGlu), a specific hapten inhibitor of Con A binding, inhibited subsequent binding to LM cells by 92.5%, demonstrating the binding specificity of the [^{3}H]Con A preparation. Incubation of cells with unlabeled Con A (1,000 μg/ml) for 5 min inhibited subsequent binding of [^{3}H]Con A by greater than 95%. There are marked differences in the kinetics of Con A binding at different temperatures (Figure 2). At 0° or 10°, plots of Con A bound vs time are linear for approximately 8 min and then level off. In marked contrast, Con A binding at 22° increases even after 20 min. To test for specific hapten reversal of bound Con A, 0.05 M αMeGlu was added to cells previously incubated for 5 min with 100 μg/ml of [^{3}H]Con A. This treatment released 55-70% of Con A bound at either 0° or 22°.

The binding of [^{3}H]Con A to LM cells as a function of temperature (0-37°) is plotted in Figure 3. Significant changes in Con A binding oc-

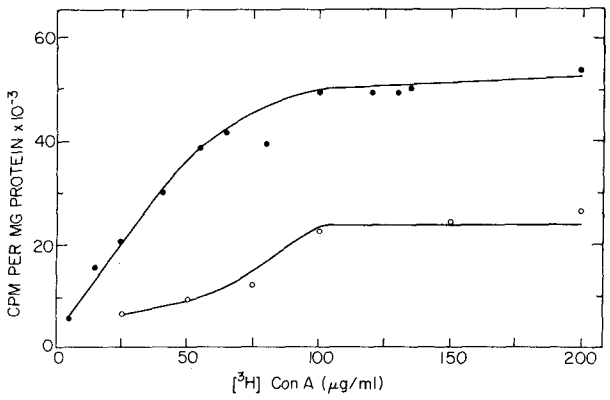

FIGURE 1. Concentration curve for binding of [^{3}H]Con A to mouse LM cells at 22°, ●—●; and 0°, ○—○. Cells in 35 mm wells (Linbro) were washed twice with 0.85% (w/v) sodium chloride solution (saline) and incubated 5 min with 0.5 ml of Con A in a 0.1 M Na_2HPO_4 buffer, pH 7.2 containing 0.85% NaCl and 0.001 M $MgCl_2$ (PBS). After five washes in saline the cells were suspended by incubation in 1 ml of 5% $NaCO_3$-0.1 N NaOH for 1 hr. The samples were divided into equal portions for measurement of tritium content in a solution containing 3:1 toluene-Triton X-100 (15) and protein by the method of Lowry et al. (16). All points in Figures 1-5 are the average of six determinations.

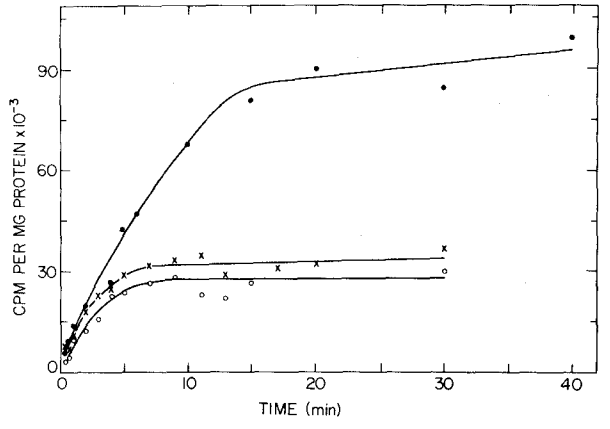

FIGURE 2. Kinetics of [^{3}H]Con A binding to mouse LM cells. The binding assay was performed as described for Figure 1 using 100 μg/ml of [^{3}H]Con A at 22°, ●—●; 10°, X—X; or 0°, ○—○.

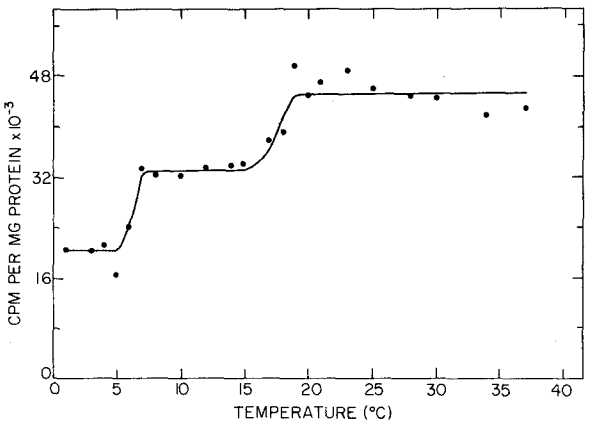

FIGURE 3. Temperature dependence of [^{3}H]Con A binding to LM cells. [^{3}H]Con A (100 μg/ml) binding was performed as described for Figure 1. Cells were incubated in growth medium for 10 min at the test temperature before each 5 min binding assay. All washes were done at the same temperatures as the respective incubation temperatures with Con A.

curred at approximately 5-7° and 15-19°. Approximately twice as much Con A was bound at 22° than at 0°. Figure 4 describes the concentration dependence of Con A-mediated hemadsorption to LM cells. Addition of Con A (100 μg/ml) and 0.05 M αMeGlu together completely abolished hemadsorption (not shown) demonstrating the specificity of the assay. Comparison of data in Figures 2 and 4 shows that at the apparent saturation concentration for Con A binding, hemadsorption is only half maximal, in general agreement with the work of others (10,12). Con A-mediated hemadsorption was studied in the temperature range 0-37° (Figure 5). There was a dramatic quantitative change in hemadsorption from 12 to 17°; a second temperature dependent change occurred at 5-8°.

When LM cells were incubated for 10 min at 7° or less prior to the addition of erythrocytes, Con A-dependent hemadsorption at 22° was inhibited dramatically (Table 1). Reversal of this cold treatment required subsequent incubation at 22° for at least 20 min prior to initiation of the hemadsorption assay. A shift in temperature to 0° does not cause the release of Con A bound at 22°, or vice versa (not shown).

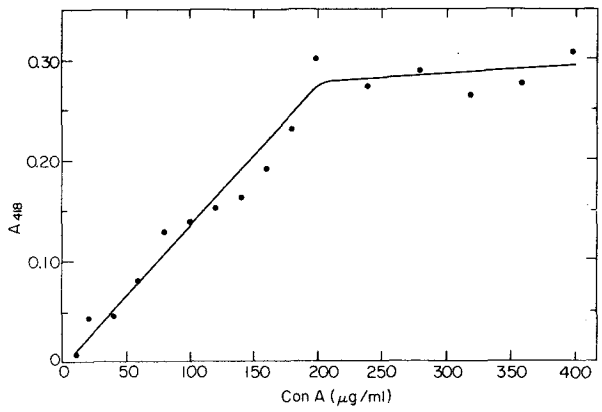

FIGURE 4. Concentration curve for Con A-mediated hemadsorption to LM cells. Cells in 35 mm wells (Linbro) were washed twice with saline and incubated for 5 min with 0.5 ml of Con A in PBS. The cells were then washed five times with saline and incubated for 10 min with rabbit erythrocytes (2% v/v) in PBS. Finally, the cells were washed five times with PBS, solubilized in 5% sodium dodecyl sulfate (w/v) and analyzed spectrophotmetrically for hemoglobin content at 418 nm.

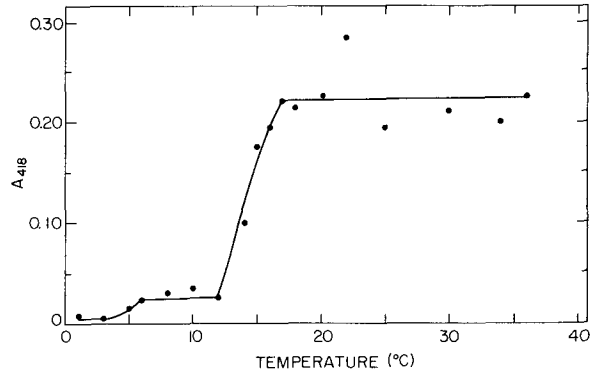

FIGURE 5. Temperature dependence of Con A-mediated hemadsorption to LM cells. The hemadsorption assay was performed with 100 μg/ml of Con A as described for Figure 4. Cells were incubated in growth medium for 10 min at the test temperature before Con A binding and hemadsorption. All washings were done at the same test temperature.

DISCUSSION. Con A mediated agglutinability or hemadsorption of mouse fibroblasts could be affected by any of the following factors: 1) the number of surface receptors accessible to lectin, 2) the spatial distribution in the plane of the membrane of receptors accessible to lectin, 3) cellular

TABLE 1. Effect of temperature on Con A-mediated hemadsorption to LM cells

Incubation 1[a] (Con A binding) Temp. (°C)	Incubation 2[b] Recovery period Temp. (°C)	Time (min)	Incubation 3[c] (Agglutination) Temp. (°C)	A_{418}[d]
22	--	--	22	0.238
0	--	--	0	0.026
22	--	--	0	0.055
0	22	5	22	0.062
0	22	10	22	0.091
0	22	15	22	0.113
0	22	20	22	0.188
0	22	30	22	0.195
2	--	--	22	0.059
3	--	--	22	0.063
5	--	--	22	0.053
6	--	--	22	0.085
7	--	--	22	0.091
8	--	--	22	0.156
10	--	--	22	0.180

(a) Con A binding was performed for 5 min as described in the legend to Figure 1, and unbound Con A was removed before Incubation 2.

(b) Incubation in PBS, pH 7.2 for the indicated time intervals at 22° after the Con A binding step to allow for recovery from the effect of cold temperature incubation.

(c) Hemadsorption with rabbit erythrocytes (2% v/v) was performed as described in the legend to Figure 4 for 10 min.

(d) Values are the average of six determinations.

shape, and 4) repulsive interactions between cells (e.g., electrostatic interactions). For a more thorough consideration, see Nicolson's recent review (17).

We report here new observations on the effects of temperature on lectin binding and lectin-mediated agglutination phenomena. We find that there are not one (8,18), but two critical temperatures at which pleiotropic effects are observed for both Con A binding and Con A-mediated hemadsorption. Below the higher critical temperature (range approximately 14-18°), both hemadsorption and binding are markedly decreased. When cells are chilled below the higher critical temperature, but not below the lower one the inhibitory effect of chilling is rapidly reversed following an upshift

to 22°. It is likely that phenomena at the higher critical temperature occur in response to a change in the physical state of membrane lipids. Critical temperatures for other membrane related phenomena have been observed in eucarotic cells at approximately 20°, correlating with a characteristic temperature in membrane physical state revealed by electron spin resonance spectroscopy (19,20). Below this characteristic temperature, the membrane lipids are in a solid state, and this condition is known to severely restrict the lateral mobility of proteins that penetrate the membrane hydrocarbon phase (21-24). Noonan and Burger have concluded that Con A mediated agglutination requires lateral mobility of Con A receptors in the plane of the membrane (8). We will report elsewhere that the higher critical temperature for Con A binding and hemadsorption is shifted upwards or downwards in LM cells containing more or less saturated fatty acids respectively (20).

Though both Con A binding and Con A-mediated hemadsorption are decreased below the lower critical temperature (range approximately 5-7°), the effects on hemadsorption are not rapidly reversible. This lack of rapid reversibility is not due to a deficiency in Con A binding (Table 1), and if a phase change in membrane lipids were the sole factor involved, rapid reversal of this inhibition (within a few seconds) would be expected after incubation at 22° (25). Our data (Table 1) clearly show that the reversal of inhibition of Con A-mediated hemadsorption of LM cells encountered at 7° or less is a slow, time dependent process (20 min or more). The properties of microtubules might account for this loss of agglutinability below 7°, and for its slow reversibility as well. Incubation in the cold (4° or less, but not 10°) is known to disrupt microtubule structure in the intact cell and prevents polymerization of tubulin *in vitro* (1,26-27). In addition, microtubule disrupting agents such as colchicine inhibit lectin mediated agglutination of viral transformed mouse fibroblasts (SV3T3 cells) (10). We will report elsewhere, however, that colchicine and vinca alkaloids affect

agglutinability of LM cells in a quite different way from that reported for SV3T3 cells (28). Our experiments with colchicine suggest that the slow reversibility of loss of agglutination encountered upon incubation at 7° or less could result from disruption of cytoskeletal components in addition to microtubules. We are therefore led to conclusions similar to those of Nicolson, who has propoased that microfilaments may be structural bridges between microtubules and cell surface receptors (17).

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