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INTERACTIONS OF CONCANAVALIN A WITH CHICK EMBRYO FIBROBLASTS TRANSFORMED BY ROUS SARCOMA VIRUS

STUDY WITH AN RSV MUTANT THERMOSENSITIVE FOR TRANSFORMATION

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SUMMARY

The interactions between concanavalin A and chick embryo fibroblasts, normal and infected with Rous sarcoma virus (RSV-BH) or its thermosensitive mutant RSV-BH-Ta, have been studied. Normal chick embryo cells and RSV-BH transformed cells showed at 4 and 25 °C a similar number of concanavalin A receptors per cell. Analysis of the binding data by the Scatchard relation showed that apparent changes in binding as a function of temperature are due to the thermodynamic properties of the process and and not to endocytosis The lectin receptors on the cell surface of normal and RSV-BH infected cells showed homogeneity in their binding properties. Chick cells infected with RSV-BH-Ta showed a lectin binding behavior that was dependent on the temperature at which the cells were grown. At the permissive temperature for transformation (37 °C), the binding process was similar to that observed for normal and RSV-BH infected cells. At the nonpermissive temperature (41 °C), the cells showed at least two sets of concanavalin A receptors. The new set of receptors on the cell surface had a lower lectin affinity than those observed in the same cells at 37 °C.

Chick cells infected with RSV-BH showed an enhanced agglutinability by concanavalin A, as compared with normal cells. Cells infected with RSV-BH-Ta showed a reversal of the correlation between increased concanavalin A agglutinability and the transformed state. At the permissive temperature for transformation, the cells were not agglutinable, whereas at the nonpermissive temperature they presented agglutinability indexes as high as those observed with RSV-BH infected cells. This enhanced agglutinability observed with cells maintained at the nonpermissive temperature for transformation may be related to the new set of low affinity receptors present at 41 °C.

INTRODUCTION

The plant lectin concanavalin A has been used to investigate the architecture

of cell surfaces and its changes upon transformation. In general, there is a good correlation between the transformed state of the cells and their enhanced agglutinability by the lectin [1, 2]. The introduction of thermosensitive mutants of oncogenic viruses has allowed a further confirmation of the relation between increased agglutinability of cells and neoplastic transformation [3–5] However, recently some exceptions have been found. Salzberg and Green [6, 7] have reported that nonvirus-producing hamster cells transformed by MSV are nonagglutinable by concanavalin A. Guerin et al. [8] have shown that a cell line isolated from a murine plasmacytoma, which is susceptible to density inhibition of growth and is not transplantable to mice, presents enhanced agglutinability by this lectin. The mechanism of cell agglutination by lectins is still unclear. Talmadge et al. [9] have selected two surface alterations as the most conspicuous candidates that could influence agglutination. They are small differences in the number of available receptors [10] and differences in their degree of mobility in the surface membrane [11, 12]. Studies from several laboratories [13–18] have shown that there is no difference in the number of concanavalin A receptors present in normal and transformed cells. However, Noonan et al. [19] have presented evidence that a temperature-sensitive cellular mutant infected by SV40 virus showed an increase in lectin binding which paralleled the process of morphological transformation.

In this study, we compare the concanavalin A binding and induced agglutinability of chick embryo cells transformed by infection with RSV-BH and with a mutant RSV-BH-Ta, temperature sensitive for transformation [20]. In cells transformed with this mutant, the morphological changes can occur within one to two hours after shifting from the permissive (37 °C) to the nonpermissive (41 °C) temperature and vice versa. The reversibility of these changes is unaffected by inhibition of DNA, RNA or protein synthesis, suggesting that a temperature-sensitive molecule participates in the morphological transformation [20, 21]. The results obtained with this system are compared to those observed with normal and RSV-BH transformed chick cells. Some of the thermodynamic properties of the concanavalin A binding are discussed and compared to those from previous reports obtained with different cells and with some simple sugars and polysaccharides.

MATERIALS AND METHODS

Cells and viruses

Cells of ten-day chick embryos were cultured in Eagle's minimal essential medium containing sodium pyruvate (5 mM) and additional glucose to give 2 g/l. This medium was supplemented with 5 % fetal bovine serum, 10 % tryptose phosphate broth (Difco), penicillin (50 units/ml), streptomycin (50 μg/ml) and tylosine (50 μg/ml). Secondary chick cell cultures were infected at 37 or 41 °C with RSV-BH or its thermosensitive mutant RSV-BH-Ta (0.1 focus forming units per cell) within 8 h after trypsinization. The efficiency of the infection was not temperature dependent. Cultures subsequently were transferred at 2–3 days intervals. Morphological transformation was apparent within 24 h after infection and virtually all the cells were transformed within 10 days after infection. Cultures were maintained at 37 or 41 °C in humidified 5 % CO_2-atmosphere incubators. Cells were used until 6–8 weeks after infection and then discarded.

The Bryan "high-titer" strain of Rous sarcoma virus (RSV-BH) is mixed with Rous-associated virus (RAV_1, a subgroup A nontransforming avian leukosis virus), and probably a second nontransforming virus, RAVO. A mutant of RSV-BH, RSV-BH-Ta [20] also called tdBE1BH [22], transforms infected cells at 37 °C, but when placed at 41 °C, these infected cells revert to normal phenotype. RSV-BH-Ta cells also contain RAV_1 and RAVO, but the presence of these viruses has no effect on the transformation process.

Preparation of ^{3}H-labeled concanavalin A

Concanavalin A and Sephadex G-50 were obtained from Pharmacia Fine Chemicals. Tritiated concanavalin A was prepared by acetylation with [^{3}H]acetic anhydride (500 Ci/mol, Amersham/Searle) in benzene at 0–4 °C, following the method of Miller and Great [23]. The lyophilized material was dialyzed against water to remove free [^{3}H]acetic acid and further purified by affinity chromatography on Sephadex G-50 (24). The glucose used to elute the ^{3}H-labelled concanavalin A from the Sephadex was removed by extensive dialysis against 0.8 M NaCl/0.025 M sodium phosphate (pH 7.4) containing 0.1 mM $CaCl_2$ and 0.1 mM manganese acetate. The metal ions Ca^{2+} and Mn^{2+} were added in order to avoid demetallization of the lectin, which results in heterogeneity with respect to carbohydrate-binding ability [25]. The lectin solution was concentrated to 3 mg/ml by ultrafiltration in dialysis tubing and stored at −30 °C. Gel chromatography of native and [^{3}H]acetylated concanavalin A on 10 % agarose (Bio Gel A 0.5 m, Bio Rad Lab.) showed similar hydrodynamic properties for both preparations. The ^{3}H-labeled lectin was also identical to native concanavalin A in its hemagglutinating activity toward sheep erythrocytes. Before use, the lectin was diluted to the desired concentrations with phosphate buffered saline.

Cell binding of ^{3}H-labeled concanavalin A

The binding assays were carried out at 4 and 25 °C. Cells were grown in Falcon plastic dishes (35 mm diameter) until they reached near confluency or a cell density of about $5 \cdot 10^5$ cells per plate. The cell monolayers were washed twice with phosphate buffered saline maintained at 4 or 25 °C For tests of specificity, the ^{3}H-labeled concanavalin A was added in the presence of 50 mM methyl-α-D-mannoside (Calbiochem). At the end of the 15-min incubation period at 4 or 25 °C, the liquid was aspirated and the plates were washed four times with cold phosphate buffered saline. The washed cells were solubilized for 1 h with 1 ml of 0.1 M NaOH/5 % Na_2CO_3. Radioactivity measurements were made in a Beckman LS355 liquid scintillation counter using a Triton-toluene scintillation solution [26]. Protein determinations were made by the procedure of Lowry et al. [27], using bovine serum albumin as a standard. The cell number and cell dimensions were calculated from the parameters reported by Bader et al. [28] for similar cells in suspension and nearly spherical in shape.

Agglutination assays

Concanavalin A-mediated cell agglutination was assayed using two different methods: a microhemadsorption method and direct lectin cell agglutination. The modification of the microhemadsorption method of Furmanksi et al. [29], as described by Rittenhouse and Fox [30], was used. The assays were made at 25 °C with

outdated human red blood cells, type O positive. In order to avoid attachment of red blood cells to the plastic substrate due to adsorbed lectin, the cultures were used at high cell density, about $1.5 \cdot 10^6$ cells per dish (35 mm diameter). Specificity tests were made by carrying out the entire procedure in the presence of 50 mM methyl-α-D-mannoside. Cells were solubilized in 0.1 M NaOH, and the solutions centrifuged at $2500 \times g$ for 6 min to remove coarse insoluble material. Hemoglobin content was analyzed spectrophotometrically at 418 nm. Protein content was determined in plates subjected to all the steps of the procedure but omitting the red blood cells. The results are expressed as absorbance units at 418 nm per mg of protein or per 10^6 cells.

Cell agglutination was also assayed following the guidelines of the procedure described by Sela et al. [17]. Subconfluent cells were removed from the plates with $4 \cdot 10^{-4}$ M EDTA in phosphate buffered saline free of Ca^{2+} and Mg^{2+} at 37 or 41 °C. The cells were washed twice with the buffered saline solution and suspended in the same solution. 25 ml of cell suspension, $1 \cdot 10^6$ cells/ml, were mixed with equal volumes of various concentrations of lectin in the cells of Micro Test II plates (Falcon). The plate was kept at room temperature in gentle rotation for 10 min. Agglutination was observed using an inverted microscope and scored on a 0 to 4+ scale. Specificity was determined by carrying out the whole procedure in the presence of 50 mM methyl-α-D-mannoside.

RESULTS

Binding of ^{3}H-labeled concanavalin A to cells

Under the conditions used for the binding studies, it has been assumed that concanavalin A exists mainly as a tetramer of molecular weight 112 000 [1]. The monomeric form contains a binding site which is more complementary to α-D-mannopyranosyl residues but will also bind α-D-glucopyranosyl units [31]. In this report the "concanavalin A specifically bound to cells" is defined as the amount of lectin bound in absence of methyl-α-D-mannopyranoside minus the amount bound in the presence of 50 mM methyl-α-D-mannopyranoside. Binding of the lectin to

TABLE I

EFFECT OF α-METHYLMANNOSIDE ON ^{3}H-LABELED CONCANAVALIN A BINDING TO NORMAL AND RSV-BH INFECTED CHICK EMBRYO CELLS

Cells	T (°C)	cpm/mg		Inhibition %
		Protein*	α-Methylmannoside	
Chick cells	37	26 738	3 011	89
	41	28 000	3 159	89
RSV infected chick cells	37	25 837	3 656	86
	41	26 900	2 713	90
Medium**	37	1 541	1 536	0

* Measurements of bound ^{3}H-labeled concanavalin A were carried out at 25 °C as described in the text. The lectin was used at a concentration of 190 μg/ml.

** Plastic dishes were incubated for several hours with medium plus 10 % calf serum. Dishes were washed twice and the binding assays performed as described for cell monolayers.

serum pretreated plastic dishes was identical in the absence or presence of methyl-α-D-mannoside. However, this carbohydrate inhibits the lectin binding to cell monolayers by 80–90% (Table I), demonstrating that the interactions with the cell surface are mediated by the saccharide binding site of concanavalin A.

Chick embryo fibroblasts stick tighlty to the dish, making their removal as single cells for binding assays in suspension very difficult. Therefore, their removal was avoided and the assays were performed with cell monolayers, as recommended by Noonan and Burger [32]. The reliability of the binding assays on cells attached to the culture dish has been shown by several authors [13, 15]. In this report the binding studies were carried out at two temperatures, 4 and 25 °C.

The binding of ^{3}H-labeled concanavalin A to normal and RSV-transformed chick cells shows a dependence on the lectin concentration (Fig. 1), similar to that described for normal and transformed mammalian cells [10, 13, 15, 33]. Comparison of the binding curves at 4 and 25 °C indicates an apparent decrease in binding at the lower temperature, 4 °C. The binding profiles for chick cells infected with the mutant RSV-BH-Ta are shown in Fig. 2. The cells maintained at 37 °C for 48 h, the permissive

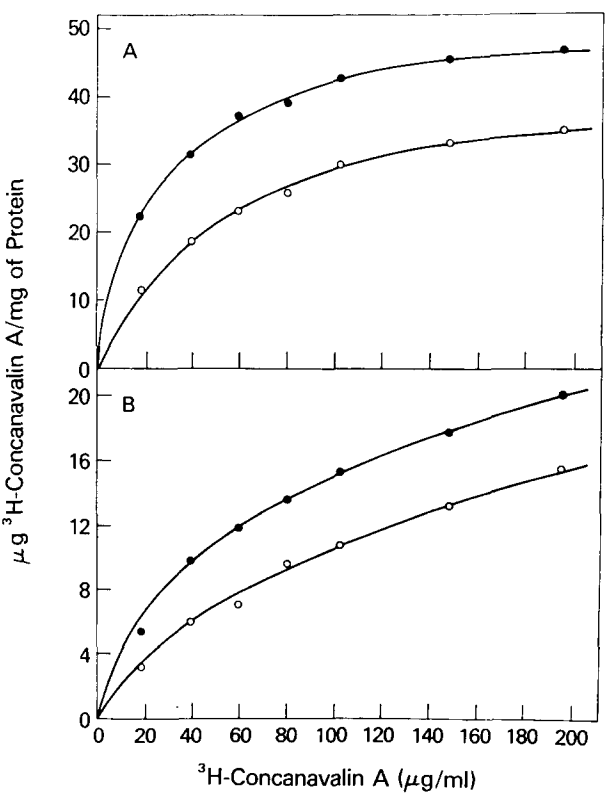

Fig. 1. Specific binding of ^{3}H-labeled concanavalin A to cells grown to near confluency. The binding assays were performed at 4 °C, (○) and 25 °C, (●) as described in the text. (A) Chick cells grown at a density of $4.94 \cdot 10^5$ cells per 35 mm diameter dish; (B) RSV-BH infected cells grown at a density of $4.23 \cdot 10^5$ cells per dish. The amount of lectin bound per mg of total protein was calculated from the ^{3}H-labeled concanavalin A specific acitivity: 1.21 μCi per mg.

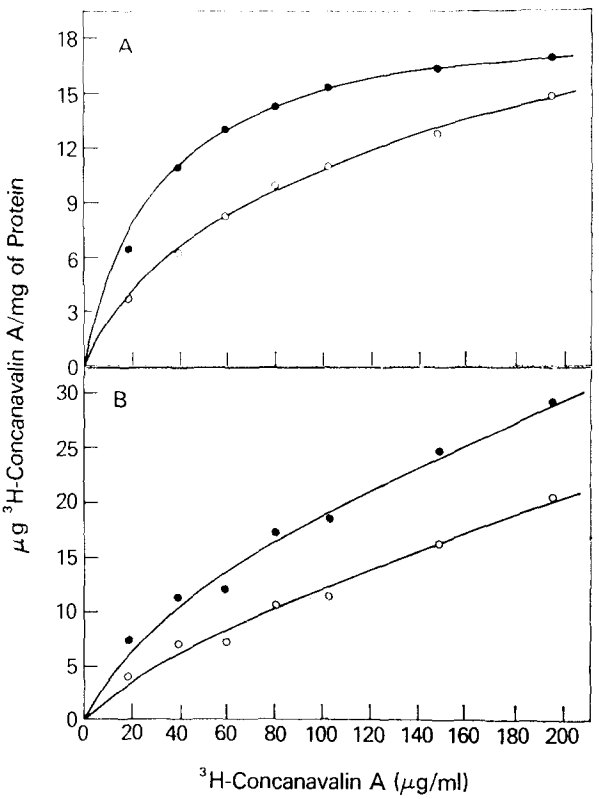

Fig. 2. Specific binding of ^{3}H-labeled concanavalin A to cells infected with RSV-BH-Ta grown to near confluency. The binding assays were performed at 4 °C, (○) and 25 °C, (●) as described in the text. (A) Chick cells infected with RSV-BH-Ta and maintained at the permissive temperature for transformation, 37 °C. Cells were grown to a density of $4.95 \cdot 10^5$ cells per 35 mm diameter. (B) Chick cells infected with RSV-BH-Ta and maintained at the nonpermissive temperature, 41 °C. Cells were grown to a density of $5.5 \cdot 10^5$ cells per dish.

temperature for transformation, presented binding characteristics similar to those observed in normal chick cells (Fig. 2A). The binding curves for cells maintained for 48 h at the non-permissive temperature for transformation, 41 °C (Fig. 2B), showed a pattern which is somewhat similar to that observed in RSV-transformed cells. The binding properties of chick cells, normal and infected by RSV-BH, did not depend on the temperature at which the cells were grown (not shown).

Deranleau [34] has discussed the different types of plots used for binding data presentation and concluded that the direct plot, such as that in Figs. 1 and 2, or the reciprocal and double reciprocal plots, are generally unsatisfactory. These different plots have open upper limits, and in the latter two any portion of the binding curve can be represented as a complete plot. A more rigorous method of analysis will be the use of a system such as the Scatchard plot [35], in which "all the theoretically obtainable data can be plotted, whether experimentally obtainable or not" [34].

If there are one or more independent sets of concanavalin A binding sites on the cells, and all sites of each set have the same intrinsic affinity for the lectin in the

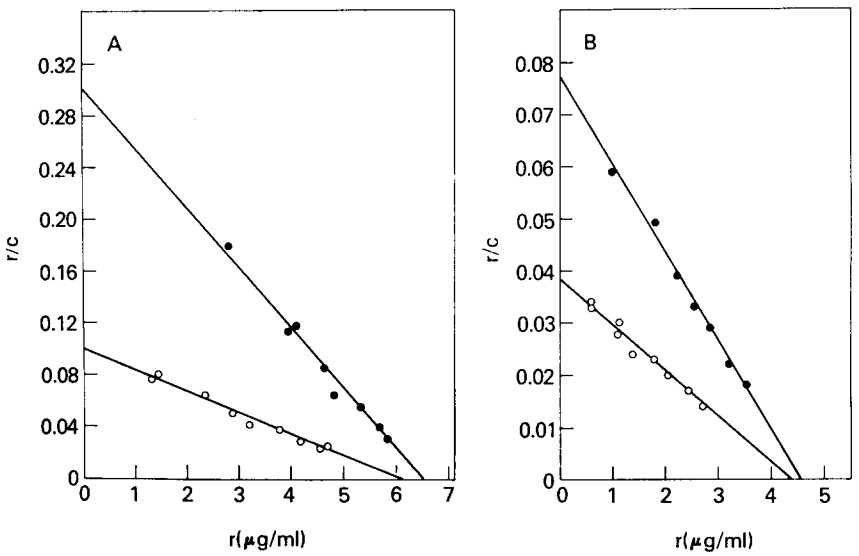

Fig. 3. Binding of ^{3}H-labeled concanavalin A represented according to Scatchard. The conditions of the assay are the same as described in Fig. 1. The data at 4 °C, (○) and 25 °C, (●) are the mean of duplicate experiments. The amount of specifically bound ^{3}H-labeled concanavalin A was determined as described in the text. (A) Chick cells grown to a density of $4.94 \cdot 10^5$ cells per 35 mm dish. (B) RSV-BH infected cells grown to a density of $4.23 \cdot 10^5$ cells per dish.

TABLE II

CELL SIZE AND NUMBER OF ^{3}H-LABELED CONCANAVALIN A MOLECULES BOUND TO NORMAL AND TRANSFORMED CHICK EMBRYO FIBROBLASTS.

The number of bound molecules was determined from the extrapolated n values in the Scatchard plots. The surface areas were determined from the cell dimensions given by Bader et al. [28].

Cells	Surface area per cell (μm^2)	T* (°C)	Molecules of lectin bound		
			per μg protein ($\cdot 10^{-10}$)	per cell ($\cdot 10^{-6}$)	per μm^2 ($\cdot 10^{-3}$)
Chick embryo cells	1195	4	26.0	66	55.2
		25	28.0	70	58.6
RSV-BH infected cells	2360	4	12.8	55	23.0
		25	13.3	57	24.1
RSV-BH-Ta infected cells (37 °C)	2090	4	10.4	36	17.0
		25	10.3	36	17.0
RSV-BH-Ta infected cells (41 °C)	1244	4**	8.4	13	10.8
		25**	9.6	15	12.1
		4***	50.9	82	65.6
		25***	51.5	82	66.3

* Temperature at which the binding assays were carried out.
** Values corresponding to the high affinity concanavalin A receptors.
*** Values corresponding to the low affinity concanavalin A receptors.

absence of cooperativity, the Scatchard relation should apply. At equilibrium, each single set of binding sites gives a straight line following the equation $r/c = Kn - Kr$, where r, lectin bound in μg/ml; c, unbound lectin in μg/ml; n, number of binding sites per cell; and K, intrinsic association constant, which is herein called the "apparent association constant". When there are more than one set of binding sites, a nonlinear relation is obtained. The binding of concanavalin A to normal and to RSV-BH infected chick cells plotted according to Scatchard is shown in Figs. 3A and 3B, respectively. The straight line plots indicate no pronounced heterogeneity in the binding sites on the cell surfaces and a decrease in K values at the lower temperature. The number of lectin receptors per cell, $7 \cdot 10^7$, is higher than that reported by Birdwell and Strauss [36] for normal chick cells, $1.5 \cdot 10^7$, but still in the same order of magnitude. The discrepancy could arise from the differences in experimental conditions and graphical presentation. Our data show that normal and transformed chick cells have nearly the same amount of concanavalin A receptors at both temperatures (Table II), similar to previous results obtained with mammalian cells [13–15]. However, it should be noted that the number of sites per unit of surface area in normal chick cells is at least 2.5-times that present in the RSV-BH transformed cells (Table II). These results are different from those obtained with mammalian cells, where the receptor density in the transformed cells was equal to [37] or higher than [10] in the normal cells. A logical explanation for the differences in the density of

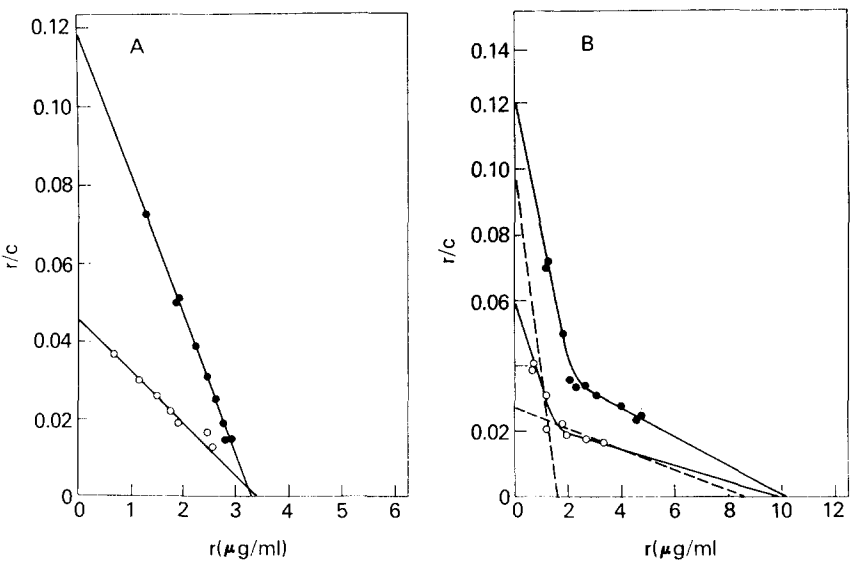

Fig. 4. Binding of ^{3}H-labelled concanavalin A represented according to Scatchard. The conditions of the assay are the same as those described in Fig. 2. The data at 4 °C, (○) and 25 °C, (●) are the mean of duplicate experiments. (A) Chick cells infected with RSV-BH-Ta and maintained at the permissive temperature for transformation, 37 °C, grown to a cell density of $4.95 \cdot 10^5$ cells per 35 mm dish. (B) Chick cells infected with RSV-BH-Ta and maintained at the nonpermissive temperature, 41 °C. Cells were grown to a density of $5.5 \cdot 10^5$ cells per dish. The dashed lines (– – –), represent the approximated graphic parameter fitting of the Scatchard plot for the data obtained at 25 °C. This approximation was made assuming two independent sets of concanavalin A receptors. For clarity purposes the graphic parameter fitting of the data at 4 °C is not shown.

receptors is the increase of cell dimensions in transformed chick cells. Recently, several authors have presented evidence that transformed cells show extensive coverage by microvilli, whereas normal cells present smoother surfaces [38, 39]. This surface feature of transformed cells may cause a significant increase in the total surface area of the cell. As a consequence, our density values and those reported in the literature for concanavalin A receptors in transformed cells are maximal, whereas those given for cells with normal phenotype can be considered close to the true values.

Examination of the binding data obtained with RSV-BH-Ta infected chick cells maintained at 37 °C, the permissive temperature for transformation, revealed a linear plot (Fig. 4A) similar to that observed with normal and RSV-BH infected cells. In contrast, the Scatchard plots obtained with RSV-BH-Ta infected cells maintained at 41 °C showed no linearity (Fig. 4B). From the analysis of the plot it is feasible to postulate at least two different sets of binding sites which can or cannot interact between themselves. From graphic parameter fitting (Fig. 4B), it has been possible to calculate the number of low and high affinity sites (Table II) and their K values (Table III). The total number of receptors on the cell surface of RSV-BH-Ta

TABLE III

THERMODYNAMIC FUNCTIONS FOR THE INTERACTIONS OF CONCANAVALIN A WITH CELL RECEPTORS AND SIMPLE SUGARS

System	T (°C)	$10^{-5} \times K$ (M^{-1})	$-\Delta G^\circ$ (kcal/mol)	ΔH° (kcal/mol)	ΔS° (cal · deg^{-1} · mol^{-1})
Chick embryo cells	4	18.0	7.93	8.32	58.6
	25	52.0	9.16		
RSV-BH infected cells	4	10.0	7.60	5.29	46.5
	25	19.5	8.58		
RSV-BH-Ta infected cells (37 °C)	4	15.0	7.83	7.67	55.9
	25	40.0	9.00		
RSV-BH-Ta infected cells (41 °C)	4[a]	36.0	8.30	5.19	48.7
	25	70.0	9.34		
	4[b]	1.60	7.00	6.10	46.6
	25	3.50	7.56		
Lymphocytes[c]	0	30.0	8.10	6.30	52.6
	37	120.0	10.00		
Rodopsin[d]	2	11.0	8.90	2.90	42.8
	27	17.0	9.90		
p-Nitrophenyl mannoside[e]	4	0.34	5.74	−27.19	−77.4
	27	0.15	5.73		−71.5

[a] Values corresponding to the set of high affinity lectin receptors.
[b] Values corresponding to the set of low affinity receptors.
[c] Thermodynamic functions were calculated from the equilibrium constants given by Betel and Van den Berg [33].
[d] Thermodynamic functions were calculated from the association constants given by Steinemann and Stryer [49].
[e] Thermodynamic functions were calculated from the equilibrium constants reported by Bessler et al. [43].

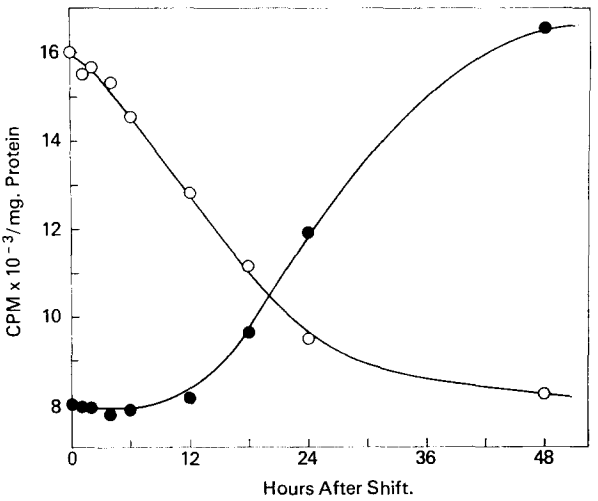

Fig. 5. Binding of ^{3}H-labeled concanavalin A to cells as a function of time after reciprocal temperature shifts. Chick embryo cells infected with RSV-BH-Ta were grown at 37 or 41 °C. Cell cultures grown to near confluency were shifted to the desired temperature and the binding assays performed at selected time intervals at 25 °C. ^{3}H-labeled concanavalin A was present at a concentration of 190 μg per ml. Differences in cell density due to growth time were avoided by making the total of time before shifting plus time after shifting equal for all the cells. Binding of radioactive lectin is expressed as cpm per mg of protein. ^{3}H-labeled concanavalin A binding after shift from 41 to 37 °C, (○—○) and from 37 to 41 °C, (●—●).

infected cells maintained at 41 °C is $9.6 \cdot 10^7$, a number which is approx. 2.5-times higher than that observed in the RSV-BH-Ta infected cells maintained at 37 °C, the permissive temperature for transformation.

Binding of concanavalin A to RSV-BH-Ta infected cells at different times of temperature shift is shown in Fig. 5. A temperature shift from 41 to 37 °C resulted in a decrease of binding sites at the cell surface, a process that occurred without an appreciable lag period. The increase in binding sites observed in cells shifted up, 37 to 41 °C, occurred after a lag period of about 12 h and was completed in 48 h. Examination of the kinetics of changes in binding sites as a function of the shift time suggests that the products responsible for the increased number of sites at 41 °C are unstable at 37 °C. Their removal or alteration at 37 °C might result in the observed decrease of binding sites. The lag period observed in cells shifted up (Fig. 5) seems to indicate an absence in the cell at 37 °C of the required products, and that in order to observe the changes, DNA or protein synthesis is required. The fact that the alterations responsible for changes in binding took place at a slower rate than those conducing morphological changes [20] or to hemadsorptive properties (Okazaki, T., Marciani, D. J. and Bader, J. P., unpublished), imply that the modifications responsible for binding behavior are different from those involved in morphological transformation and other properties of these cells.

Thermodynamics of concanavalin A-cell receptor interactions

Some thermodynamic functions for the binding process can be calculated from the value of the apparent association constant K [40]. The thermodynamic values of

standard free energy change (ΔG_0) were calculated from K by the relation $\Delta G^\circ = -RT\ln K$. The effect of temperature on the binding process was evaluated from the enthalpy change (ΔH°) using the equation

$$\ln \frac{K_2}{K_1} = \frac{-\Delta H^0}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

where K_1 and K_2 are the apparent association constants obtained at 4 and 25 °C. The standard entropy change (ΔS°) was calculated from the equation $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$. The association constants (Table III) range from $1 \cdot 10^6$ to $5 \cdot 10^6$ M^{-1}, with the exception of the low affinity sites in RSV-BH-Ta infected cells at 41 °C, which presented K values of one order of magnitude lower, i.e., 10^5 M^{-1}. These values are close to those reported for concanavalin A binding to lymphocytes [33, 41] and concanavalin A interactions with glycopeptides from ovalbumin and IgM cryoglobulin, $K = 2.5 \cdot 10^6$ and $9.7 \cdot 10^5$ M^{-1}, respectively [42]. However, these association constants are several orders of magnitude larger than those observed for the lectin interactions and monosaccharides [43]. So and Goldstein [44] have shown that concanavalin A can accommodate structures as large as trisaccharides, and that the affinities increased from the monomer up to the trimer. A comparison of the ΔG° for the concanavalin A-trisaccharide interactions, −7.43 kcal/mol, with that observed for interactions with cell receptors, −8 to −10 kcal/mol, indicates that the mannobiose units in the glycopeptide are not sufficient to explain the degree of affinity. Other interactions with groups such as *N*-acetyl-D-glucosamine and/or amino acids in the glycoproteins may contribute to the total free energy of binding. For comparative purposes, our values for the thermodynamic functions are shown in Table III together with those reported for other concanavalin A binding systems. It is apparent from our data and other reports that the interactions of this lectin with cell surfaces have a positive reaction enthalpy, whereas its interactions with small saccharides present a negative reaction enthalpy. These results are very different from those reported for another lectin, wheat-germ agglutinin, where K slightly decreases when the temperature is raised [45], presenting a negative enthalpy similar to that measured for the formation of antibody-hapten complex [46].

Agglutination of cells

Normal and transformed chick cells were assayed for their ability to be agglutinated by concanavalin A. Using a modification of the microhemadsorption method of Furmanksi et al. [29], RSV-BH infected cells were found to be more agglutinable than normal cells (Fig. 6). Treatment of normal and RSV-BH infected chick cells with trypsin caused an enhancement of the lectin-mediated hemadsorption (not shown). The presence of 50 mM methyl-α-D-mannoside inhibited the hemadsorption process, indicating the specificity of the test. From Fig. 6 it is possible to note that chick cells present some degree of concanavalin A-mediated hemadsorption. Cells infected with the RSV-BH-Ta mutant and maintained at 37 °C showed levels of the lectin-mediated hemadsorption similar to or lower than those observed for normal cells (Fig. 6). The same assay gave very different results when cells infected with RSV-BH-Ta and maintained at 41 °C were tested. The cells which are not transformed at this temperature showed an apparent enhancement of the concanavalin A-mediated hemadsorption, which, however, could not be significantly inhibited by

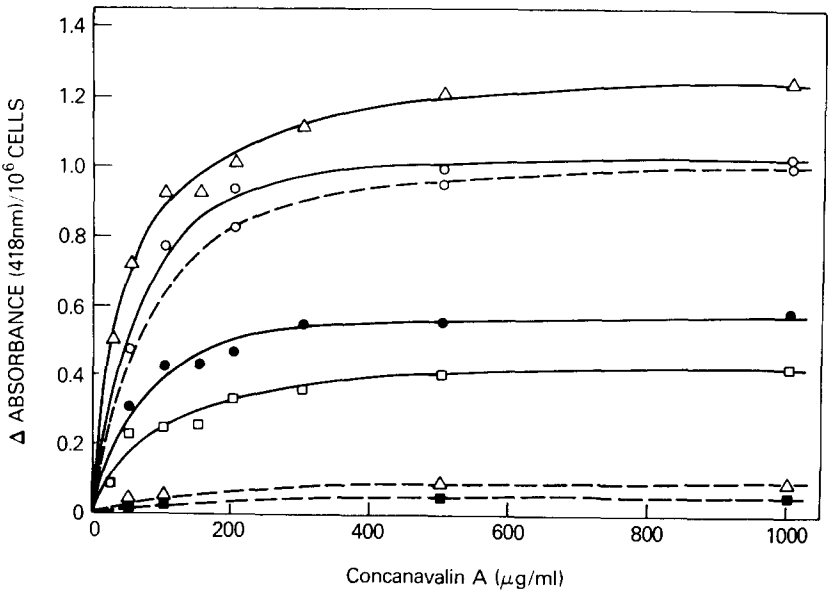

Fig. 6. Concentration curve for concanavalin A-mediated hemadsorption to chick cells, normal and transformed by RSV-BH and its thermosensitive mutant for transformation, RSV-BH-Ta. Cells were grown to a high density, about $1.5 \cdot 10^6$ cells per 35 mm dish, in order to avoid attachment of red cells to the plastic substrate due to adsorbed lectin. Cells were washed twice with Ca^{2+}- and Mg^{2+}-free phosphate buffered saline and incubated for 10 min with 0.5 ml of concanavalin A in saline. The cells were then washed four times with saline solution and incubated for 10 min with 2 % (v/v) washed human erythrocytes type O (+). Finally, the cells were washed five times with saline solution and solubilized in 1 ml of 0.1 M NaOH. The hemoglobin content was analyzed spectrophotometrically at 418 nm. The cell number was calculated from the protein content and/or from cell counts from plates treated in the same form as for hemadsorption but excluding the erythrocytes. (△), RSV-BH infected cells; (●), normal chick cells; (○) RSV-BH-Ta infected cells grown at 41 °C; (□) RSV-BH-Ta infected cells grown at 37 °C. Assays in the absence, (–) and in the presence, (– – –) of methyl-α-D-mannoside.

methyl-α-D-mannoside (Fig. 6). Further investigation of this lack of inhibition by the mannoside with RSV-BH-Ta infected cells maintained at 41 °C revealed a new hemadsorbing surface property, which is non-mediated by concanavalin A and disappears upon temperature shifting to 37 °C (Okazaki, T., Marciani, D. J. and Bader, J. P., unpublished). The possibility of a nonspecific temperature effect on the cell surface properties was investigated in normal and RSV-BH infected cells grown at 37 or 41 °C. The degree of lectin mediated hemadsorption was in each case temperature independent. These results agree with those of Burger and Martin [4], and confirm the role of the viral mutation in the modulation of cell surface properties.

The agglutination by concanavalin A of suspended normal and transformed cells was also tested using a method similar to that described by Sela et al. [17]. Normal cells were not significantly agglutinable by the lectin (Fig. 7), in agreement with previous reports [2, 4, 5]. Cells infected with RSV-BH-Ta and maintained at 37 °C showed very low agglutination by lectin. At this temperature the cells are morphologically transformed and present physiological properties characteristic of the transformed state [20, 21]. Assays carried out with RSVS-BH-Ta infected cells

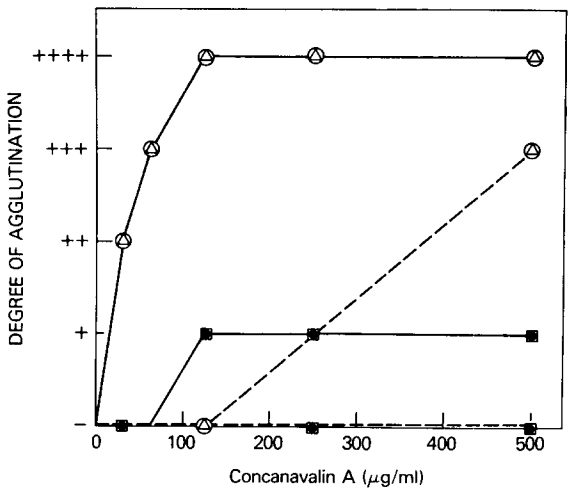

Fig. 7. Concanavalin A-mediated agglutination of chick cells, normal and infected with RSV-BH or its thermosensitive mutant, RSV-BH-Ta. The assays were performed as described in the text. (−), no agglutination; (+), less than 25 % of cells agglutinated; (++), approximately 50 % agglutination; (+++) approximately 75 % of cells agglutinated; (++++), essentially all the cells are agglutinable. The inhibitor, α-D-methylmannoside was used at a concentration of 0.05 M (△), RSV-BH infected cells; (●), normal cells; (□) RSV-BH-Ta infected cells grown at 37 °C; (○), RSV-BH-Ta infected cells grown at 41 °C. Agglutination in the absence, (−) and in the presence of inhibitor, (- - -).

maintained at 41 °C, the nonpermissive temperature for transformation, indicated a significant increase in agglutinatibily (Fig. 7). Addition of 50 mM methyl-α-D-mannoside inhibited the agglutination process, proving the specificity of the reaction. Our results are an exception to the generally accepted correlation between transformation and concanavalin A-induced agglutinability [1, 2].

DISCUSSION

There is a general consensus that normal and transformed cells do not differ in the number of concanavalin A receptors per cell [13–16, 18]. However, Noonan et al. [19] and Noonan and Burger [10] have reported that under certain conditions, i.e., short exposure to the lectin at low temperature, transformed cells present a higher number of receptors than normal cells. In order to elucidate this issue, we have examined the binding of concanavalin A to chick cells, normal and infected with RSV-BH or one of its mutants thermosensitive for transformation, RSV-BH-Ta [20].

Examination of the lectin concentration curves for binding with normal or RSV-BH infected chick cells at 4 and 25 °C indicates at the lower temperature an apparent decrease of concanavalin A bound per mg of protein. Noonan and Burger [10] have proposed that the binding at 0 °C is a measure of the true number of receptors on the cell surface, whereas the large binding at 22 °C is the result of nonspecific interactions and endocytosis. Huet et al. [47] have interpreted the differences in binding caused by temperature as a result of the dimer-tetramer transition in

concanavalin A. At low temperatures (0–4 °C), the dimeric form of concanavalin A is predominant; therefore most of the cell-bound lectin may be dimers, which have a significantly different binding affinity from that of the tetramers [48]. Analysis of our data by the Scatchard plot showed that the differences in binding at 4 and 25 °C are neither the result of new receptors nor of nonspecific adsorption or endocytosis, but are caused by a decrease in affinity reflected by a decrease in the apparent association constant K (Table III). This positive reaction enthalpy is in agreement with the results reported for other concanavalin A binding systems [33, 49]. Our data does not exclude the possibility of a differential binding of dimers and tetramers [47]. Nicolson [1] suggested that Noonan and Burger [10] selectively measured a set of high affinity concanavalin A receptors, analogous to those measured by Cuatrecasas [50] in fat cells. In the selected range of lectin concentrations used in the present study, no significant heterogeneity of the receptors with respect to their binding capacities was observed. However, it is possible that at lower or higher concentrations than those reported here, a population of very high affinity receptors analogous to those found in some cells [10, 50, 51] and perhaps also low affinity receptors can be present on the cell surface.

Studies of ^{3}H-labeled concanavalin A binding to cells infected with the mutant RSV-BH-Ta yielded unexpected results, which depended on the state of morphological transformation of the cells. The binding properties of RSV-BH-Ta infected cells maintained at the permissive temperature for transformation (37 °C) were somewhat similar to those observed with normal and RSV-BH infected cells. However, the number of receptors per cell and unit surface area were lower than the corresponding values observed in normal and RSV-BH infected cells. Cells infected with RSV-BH-Ta and maintained at the nonpermissive temperature (41 °C) showed a higher number of concanavalin A receptors than that observed in normal cells (Table II). These receptors could be separated into two sets having different affinities for the lectin. This heterogeneity of the receptors must originate from some membrane alteration that is not present in normal or RSV-BH infected cells.

Kinetic analysis of the changes in concanavalin A binding after thermal shift up or down indicates that the process is completed in about 48 h, suggesting that these cell surface alterations involve synthesis of new components. Analysis of the polypeptide composition of cell membranes from chick embryo fibroblasts infected by RSV-BH-Ta showed no differences at 37 or 41 °C, with the exception of a large glycoprotein(s), mol. wt. 250 000, that was decreased at the permissive temperature for transformation [53]. It seems feasible to postulate, in this specific case, that changes in the levels of this glycoprotein may be partially responsible for the alterations in concanavalin A receptors.

Evaluation of the capacities of normal and RSV-BH infected cells to be agglutinated by concanavalin A gave results similar to those reported previously by several authors for different cell systems [1, 2]; i.e., that transformed cells present an enhanced concanavalin A-induced agglutinability. In this report, the same results were obtained from direct concanavalin A agglutination [17] and from concanavalin A-mediated hemadsorption assays [29, 30]. Porter et al. [53] have suggested that the presence of microvilli would be favorable for concanavalin A agglutination because of the increased contact between neighboring cells [39, 53]. In agreement with that proposal, Hale et al. [38] have recently shown that RSV-BH infected chick cells

present an extensive coverage by microvilli, whereas density-inhibited cells have smooth surfaces. This difference may explain the increased agglutinability by concanavalin A of RSV-BH infected cells in spite of their low lectin-bound density.

Concanavalin A-mediated agglutination of cells infected by RSV-BH-Ta revealed that they were not agglutinable at the permissive temperature (37 °C), whereas at the nonpermissive temperature (41 °C) the cells showed enhanced lectin agglutinability and also a new hemadsorbing surface property (Okazaki, T., Marciani, D. J. and Bader, J. P., unpublished). Burger and Martin [4] have reported that increased hyaluronic acid synthesis in RSV infected chick cells might interfere with lectin induced agglutinability, and recommended a preliminary treatment of the cells with hyaluronidase. The lack of agglutinability of RSV-BH-Ta infected cells at 37 °C could arise from hyaluronic acid interference. However, several groups using RSV infected chick cells without hyaluronidase treatment have been able to detect differences in concanavalin induced agglutinability [5, 54]. Moreover, in spite of the different agglutinabilities for RSV-BH or RSV-BH-Ta infected cells (37 °C), both cells showed similar amounts of hyaluronic acid: 0.86 and 0.90 pg per cell respectively [20]. Therefore, the inhibition of agglutination by hyaluronic acid in our system, becomes rather questionable. The enhanced concanavalin A-induced agglutinability observed in these cells expressing the normal phenotype can be related to a significant increase in the total number of the lectin binding sites on the cell surface. It is feasible that in this particular case the increase of lectin receptors may play a role in the agglutination process. The lack of correlation between concanavalin A agglutinability and transformed state, is the opposite of that observed with cells infected with other thermosensitive mutants of RSV [4, 5]. As has been suggested by Salzberg and Green [6, 7] and Biquard [55], the possibility exists that cell transformation and the surface alterations associated with concanavalin A interactions are controlled by the expression of different sarcoma virus genes. The manner in which this mutant, RSV-BH-Ta, alters the behavior of the cells is not yet understood. It is quite possible that alterations of some proteins and/or lipids in the cell membrane may result in changes of membrane architecture, conducing to the expression of new properties such as those presented here.

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