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THE CONCANAVALIN A BINDING PROPERTIES OF CONCANAVALIN A-RESISTANT AND -SENSITIVE HAMSTER CELL LINES

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Summary

The binding of labelled concanavalin A to Chinese hamster ovary cells at 4°C exhibits positive cooperativity. Variant cell lines selected for resistance to the cytotoxic effects of the lectin exhibit altered lectin binding properties. Concanavalin A-resistant cell lines do not bind concanavalin A in a cooperative fashion and bind significantly less lectin than concanavalin A-sensitive cells/cell surface area. A revertant cell line selected from a concanavalin A-resistant population exhibited a near wild-type sensitivity to concanavalin A, bound the lectin in a cooperative manner, and bound approximately the same number of concanavalin A molecules/cell surface area as parental wild-type cells. Experiments were also performed with somatic cell hybrids formed from the fusion of two cell lines which exhibited a wild type sensitivity to concanavalin A (A-W) and from the fusion of a cell line selected for resistance to concanavalin A with a concanavalin A-sensitive cell line (A-7). Both A-W and A-7 hybrid lines were about as sensitive to the cytotoxic effects of concanavalin A as pseudodiploid wild-type cells, bound concanavalin A in a cooperative fashion and both hybrid lines bound approximately the same number of lectin molecules/cell surface area as pseudodiploid wild-type cells at saturation binding. These results support the view that concanavalin A is a suitable selection agent for the isolation of variant cell lines with modified lectin binding properties and suggests that a correlation exists between the expression of the concanavalin A-resistant phenotype and the observed alterations in concanavalin A binding.

Introduction

Cell surface saccharides are very important in intercellular communication, in viral oncogenesis, in the regulation of cell growth, and in the immune response [1—4]. Much of the information about the role played by these com-

plex surface molecules in cellular regulation comes from studies with lectins which bind to specific surface receptors. For example concanavalin A, a plant lectin, is known to cause a variety of interesting but complex biological effects upon cultured mammalian cells; this plant protein induces mitosis in lymphocytes [5], preferentially agglutinates many tumor cell lines [6] and enhances the carbohydrate metabolism of polymorphonuclear lymphocytes [7]. Molecules like concanavalin A, which bind to carbohydrate sites at the cell surface, are being used as tools or probes in an attempt to study changes at the surface which are thought to be involved in the regulation of important cellular events [8,9].

Recently it has been reported that lectins interact with the cell surface of some mammalian cells in a cooperative fashion. For example, the binding of low concentrations of soybean agglutinin to erythrocytes exhibits positive cooperativity [10]; concanavalin A, but not its succinyl derivative, binds to rat thymocytes in a cooperative manner [11]; concanavalin A may interact with mouse B lymphocyte receptors and affect the cooperative behaviour of cellular structures involved in receptor mobility and the movement of the cell [12]; concanavalin A also binds to some membrane-bound enzymes in a cooperative fashion [13,14]. A proper understanding of the mechanism involved in the binding of lectins at the cell surface is important since it may lead to a better understanding of some basic principles of membrane function.

We have recently reported that concanavalin A binds to the surface of Chinese hamster ovary (CHO) cells in a cooperative fashion and have shown that this mechanism of lectin binding is altered in a variant CHO cell line selected for resistance to the cytotoxic effects of concanavalin A [15]. Certainly variant mammalian cell lines with altered concanavalin A binding properties would be valuable for investigations concerned with the interaction of concanavalin A with the surface membrane. With this view in mind we have investigated the binding of concanavalin A to various lectin-sensitive or -resistant CHO cell lines in an attempt to determine a possible relationship between resistance to the cytotoxic effects of concanavalin A and the modified binding properties.

Materials and Methods

Growth conditions and cell lines

Growth conditions. Chinese hamster ovary (CHO) cell lines were grown at 34°C in α-minimal essential medium (Flow Laboratories, Inc.) supplemented with antibiotics and 10% (v/v) fetal bovine serum (Reheis Chemical Co.) as previously described [16]. Plating efficiencies were determined by standard techniques with cultures that had been grown for 10—12 days at 34°C [17]. The number of cells/tissue culture plate was estimated by removing the cells with phosphate-buffered saline containing 0.15% trypsin and passing a dilution of the suspension through a cell counter (Coulter Electronics, Co.). The various cell lines gave negative tests for *Mycoplasma* contamination as determined by plating methods [18].

Concanavalin A-resistant variants. C^R-7 is a cloned CHO cell line isolated from an uncloned population of concanavalin A-resistant cells (culture A)

obtained by a selection method described in detail elsewhere [19]. In brief an uncloned population of concanavalin A-resistant cells was obtained after 10 passages of a non-mutagenized wild type population at 34° C in growth medium containing 40 μg/ml concanavalin A. C^R-7 is a subclone selected from the mixed population of concanavalin A-resistant cells (culture A) and many of the properties of the uncloned population [19] and the C^R-7 variant [20] have been described in detail.

BC^R-2 is a cloned cell line isolated from an uncloned population of concanavalin A-resistant cells (culture B) by a selection procedure similar to the one described for the isolation of C^R-7. However, culture A and culture B were derived from unique non-mutagenized clones of wild-type CHO cells [19]. Also, culture B was maintained in the presence of concanavalin A-containing growth medium 14 times before the subclone named BC^R-2 was isolated.

EC^R-1 was selected from a mutagenized population of CHO cells after one passage in the presence of 40 μg/ml concanavalin A. A recently cloned population of CHO cells was treated with a mutagen by exposing cells growing exponentially on a 15 × 100 cm tissue culture plate at 34° C, at a concentration of $1 \cdot 10^6$ cells, to 300 μg/ml ethylmethansesulfonate for 17 h. The fraction survival of colony-forming ability after mutagen treatment was 0.2. The treated cells were washed with phosphate-buffered saline, resuspended in fresh growth medium, and incubated at 34° C for 10 days to permit regrowth of the surviving cells. The cells were removed with 0.15% trypsin solution and $1 \cdot 10^6$ cells were added to another 15 × 100 mm plate containing 25 ml of growth medium with 40 μg/ml concanavalin A. After incubation in the presence of growth medium with concanavalin A at 34° C for 12 days the plate was observed to have a single colony. The lectin-containing medium was then replaced with fresh growth medium without concanavalin A and the cells were grown to a partial monolayer. The population was cloned [17] and named EC^R-1.

Concanavalin A-resistant revertant. CHO cells selected for resistance to concanavalin A cytoxicity at 34° C are usually temperature sensitive (ts) for growth [17,19]. The ts property of the concanavalin A-resistant line, C^R-7 has been described [20], and was used to isolate a revertant of the C^R-7 population. This was done by incubating $2 \cdot 10^6$ variant cells on 16 oz. Brockway bottles (Brockway Glass Co., Inc.) containing normal growth medium at 39° C (the non-permissive temperature; ref. 20). After approx. 14 days some colonies appeared on the glass surface. A portion of the cells of one of the large colonies was removed with a sterile Pasteur pipette, added to a 15 × 60 mm tissue culture plate and grown to a monolayer at 39° C. These cells were maintained on 16 oz. Brockway bottles at 39° C for about 2 months and then cloned as previously described [17]. The cloned line was continuously cultured at 34° C for 2 months and in subsequent experiments was found to have lost the ts phenotype and to have regained a sensitivity to the cytotoxic effects of concanavalin A. This cell line has been named RC^R-7.

Somatic cell hybrids. A CHO line auxotrophic for glycine, adenosine, and thymidine, AUXB1, has been isolated [21] and was kindly provided for this study by F. Stanley and L. Siminovitch. Preliminary experiments indicated that AUXB1 cells exhibited a wild-type sensitivity to concanavalin A. A ouabain-resistant marker was added to the auxotrophic line by selecting cells capa-

ble of forming colonies in the presence of 2 mM oubain [22]. The hybridization was carried out by incubating $3 \cdot 10^5$ AUXB1 (oubain-resistant) cells with an equal number of either the C^R-7 or wild-type population in a 6 mm diameter well of a 96-well Linbro plastic tray (IS-FS96-TC, Linbro Chemical Co.) containing 0.2 ml of growth medium supplemented with 1.0 mM glycine, 0.04 mM adenosine and 0.04 mM thymidine. The cells were incubated overnight at 34°C, the medium was then replaced with 0.2 ml of phosphate-buffered saline and the cells were placed at 4°C for 10 min. The phosphate-buffered saline was then replaced with 0.2 ml of phosphate-buffered saline containing inactivated Sendai virus (diluted 1 : 10 with buffer; virus was from Lot 134-1, Connaught Medical Research Laboratories) and incubated an additional 10 min at 4°C. Next, the virus suspension was removed and warm growth medium was added. The cells were incubated for another 4 h at 34°C after which the cells were washed with phosphate-buffered saline, removed with trypsin solution and added to a 15 × 60 mm culture plate containing α-medium lacking glycine, adenosine and thymidine but supplemented with 2 mM ouabain. After 10 days a number of colonies appeared on the plates containing the selective medium; these colonies were picked with sterile Pasteur pipettes and independently grown to a partial monolayer on 15 × 60 mm plates in the presence of normal growth medium. The potential hybrids were then cloned [17], retested for the ability to form colonies in the selective medium, and the karyotypes were analysed as previously described [20]. When the AUXB1 (ouabain-resistant) line was hybridized with C^R-7 or with wild-type cells the hybrid clones called A-7 and A-W, respectively, were isolated; they were able to grow in the selective medium and contained modal chromosome numbers per cell approximately twice the value of 21 characteristic of the C^R-7 variant and wild-type lines.

Hydroxyurea-resistant variant. H^R-100 is a CHO cell line selected for resistance to hydroxyurea by a previously published method [16,23] and exhibits a plating efficiency of close to 1 in the presence of medium containing 100 μg/ml hydroxyurea. This is four times the concentration required to reduce the plating efficiency of the wild-type population to 10^{-5} [23].

Concanavalin A binding assay

^{3}H-Labelled concanavalin A with a specific activity of 0.5 Ci/g lectin was purchased from New England Nuclear and used in lectin binding experiments [15]. Concanavalin A binding studies were performed with cultures grown on 15 × 60 mm tissue culture plates to approx. $1.8 \cdot 10^6$ cells/plate. The amount of concanavalin A bound was found to be directly proportional to the number of cells plated to approx. $2.5 \cdot 10^6$ cells/plate. Concanavalin A binding experiments performed at room temperature for extended periods of time can lead to problems with endocytosis and non-specific binding of the agglutinin [11,24]; these potential problems, which mask important details in the concanavalin A binding properties were reduced by incubating the cultures at 0°C for 5 min prior to the addition of labelled concanavalin A [25]. The cells were then washed three times with cold 0.154 M NaCl solution and incubated at 4°C for 5 min in phosphate-buffered saline containing the appropriate concentrations of ^{3}H-labelled concanavalin A in a final volume of 2.0 ml. Maximum cell surface binding occurred within the 5 min binding period. The cells were then washed five

times with ice-cold 0.154 M NaCl solution and solubilized in 10% triton X-100 (J.T. Baker Chemical Co.) for 60 min at 37°C. The digested samples were added to an aquasol cocktail (New England Nuclear) and counted in a liquid scintillation counter. There was specificity in the binding of labelled concanavalin A since the amount of binding at all concentrations tested in the presence of 0.2 M methyl α-D-mannoside was 5% or less of the binding which normally occurred in the absence of the inhibitor. The quantity of binding in the presence of the sugar was considered to be non-specific and was routinely monitored and subtracted from the binding data obtained in the absence of the hapten.

Cell surface area determinations

Cells were removed from tissue culture plates with phosphate-buffered saline containing 0.15% trypsin; the cell diameters were determined on at least 200 cells of each type with a light microscope fitted with a micrometer eyepiece. The average cell diameter was used to calculate [26] the cell surface area assuming the cells were smooth spheres.

Results

Lectin sensitivity

The various cell lines used in the lectin binding studies reported in this communication were tested for senstivity to the cytotoxic effects of concanavalin A. A D_{10} value of concanavalin A for a particular cell line was defined as the concentration of lectin which reduced cell survival to 10%. These values were determined from survival curves obtained when the individual cell lines were plated at various cell concentrations in normal growth medium containing different doses of the lectin. Cells which divided to form colonies after 10—12 days incubation were stained with methylene blue and counted [17]. The experiments were performed with cell lines that had been cultured in the absence of the lectin for at least 3 months.

D_{10} values of 18, 45, 48 and 45 were obtained for wild-type, C^R-7, BC^R-2, and EC^R-1 cell lines, respectively. As expected [17,19,20] concanavalin A-resistant cell lines (C^R-7, BC^R-2, EC^R-1) showed less sensitivity to the cytotoxic effects of concanavalin A as compared to wild-type cells; the D_{10} value for the resistant lines was about 2.5 times the value obtained for wild-type cells. Cells selected for resistance to the toxic action of hydroxyurea (H^R100) and somatic cell hybrids (A-W) obtained through the fusion of two concanavalin A-sensitive cell lines (wild-type CHO and AUXB1, ouabain-resistant cells) exhibited the same sensitivity to concanavalin A as the pseudodiploid wild-type population. A revertant concanavalin A-resistant cell line (RC^R-7) selected from the C^R-7 population (see Materials and Methods for the isolation procedure) exhibited a D_{10} value of 25; this cell line was much more sensitive to concanavalin A than the original concanavalin A-resistant variant. However, the RC^R-7 cells were not quite as sensitive to the lectin as the wild-type population and exhibited a D_{10} value which was approx. 1.4 times greater than the wild-type value. The somatic cell hybrids (A-7) formed through the fusion of C^R-7 cells and AUXB1, ouabain-resistant cells also exhibited a far greater sensitivity to the lectin when compared to the C^R-7 population; these hybrid cells gave a D_{10} value of 26

which was approx. 1.4 times the value observed with either the wild-type cells or the A-W hybrid population.

Therefore the cell lines used in the concanavalin A binding studies that are reported in this communication could be broadly classified as either concanavalin A-resistant (C^R-7, BC^R-2 and EC^R-1) or concanavalin A sensitive (wild-type, H^R100, RC^R-7, A-W and A-7).

Binding of concanavalin A to intact cells

We have recently reported [15] that concanavalin A binds to wild-type CHO cells at 4° C with positive cooperativity and that this mechanism of binding is altered in the concanavalin A-resistant variant, C^R-7. Also the variant cells were only capable of binding between 55 and 65% as much lectin/cell as compared to the wild-type population. We have attempted to determine whether there is a relationship between the lectin-resistant phenotype displayed by the three concanavalin A-resistant lines and the altered binding properties previously reported. Concanavalin A binding experiments carried out with various lectin-resistant and -sensitive cell lines are reported in Figs. 1—6 and summarized in Table I. All experiments were performed with cell lines that had been cultured in the absence of a selective agent for at least 3 months.

Concanavalin A-resistant variants. BC^R-2 and EC^R-1 are CHO cell lines independently selected by different procedures for resistance to the cytotoxic effects of concanavalin A. When the binding of labelled concanavalin A to BC^R-2 (Fig. 1) and EC^R-1 (Fig. 2) was examined as a function of lectin concentration saturation binding was observed and the binding curves appeared to be hyperbolic. The data from Figs. 1 and 2 was analyzed by Hill plots [27] and a Hill coefficient of 1.0 was calculated indicating that the labelled concanavalin A was probably binding to non-interacting lectin sites at the surface of these concanavalin A-resistant variants (Figs. 1 and 2, see insets).

The binding data obtained with BC^R-2 and EC^R-1 was further analyzed by the Scatchard method [28] and appeared to give linear plots (Figs. 1 and 2, see insets). By extrapolation to the abscissa the Scatchard representation provides an estimate of the amount of lectin bound/culture at saturation binding. By

TABLE I

SUMMARY OF THE CONCANAVALIN A BINDING EXPERIMENTS

Cell lines	Hill coefficient	Concanavalin A molecules/μm^2 surface area ($\times 10^{-3}$)
(a) Concanavalin A-resistant		
C^R-7	1.0	6.5
BC^R-2	1.0	5.5
EC^R-1	1.0	6.2
(b) Concanavalin A-sensitive		
Wild type	1.8	16
H^R100	1.7	17
RC^R-7	1.4	15
A-W	1.3	15
A-7	1.3	16

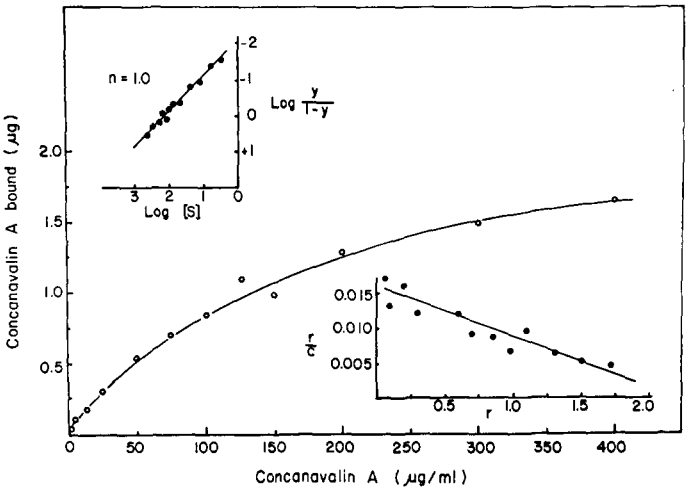

Fig. 1. ^{3}H-Labelled concanavalin A bound/1.8 · 10^6 BC^R-2 cells at various concentrations of lectin. The inset in the upper part shows a Hill plot [27] of the data. The Hill coefficient which represents the slope of this plot was calculated to be 1.0. The inset at the bottom presents the data according to Scatchard's equation [28]: $r/c = nk - rk$, where r represents the amount of lectin bound, c is the free lectin concentration, n is the amount of lectin bound at saturation, and k is the apparent association constant for lectin:receptor site binding. Each point represents the average of duplicate points from a single experiment; similar data has been obtained in three separate experiments.

this method it can be shown that BC^R-2 and EC^R-1 bound approx. 2.1 and 2.3 μg/ml lectin per 1.8 · 10^6 cells, respectively. These values represent 6.3 · 10^6 molecules and 6.9 · 10^6 molecules of concanavalin A bound/variant cell surface (assuming a molecular weight of 110 000 for concanavalin A; ref. 8).

In order to analyze the binding data in relation to cell surface area the average cell diameter was estimated and used to calculate [26] the surface area of BC^R-2 (1145 μm^2) and EC^R-1 (1120 μm^2). At saturation binding the BC^R-2 and

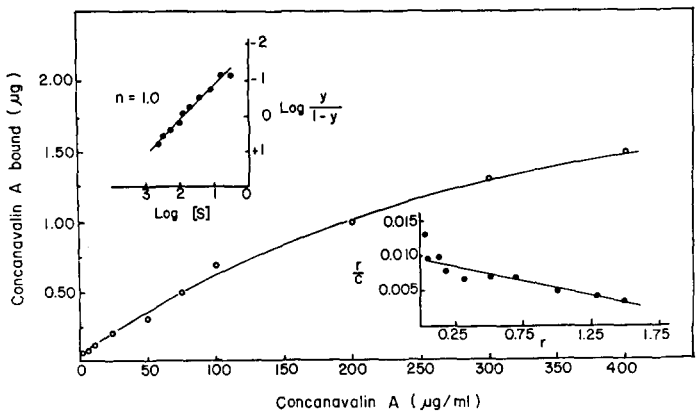

Fig. 2. ^{3}H-Labelled concanavalin A bound/1.8 · 10^6 EC^R-1 cells at various concentrations of lectin. The inset in the upper part shows a Hill plot [27] of the data. The Hill coefficient was calculated to be 1.0. The inset at the bottom presents the data according to Scatchard's equation [28]. Each point represents the average of duplicate points from a single experiment; similar data has been obtained in two separate experiments.

EC^R-1 cells bound $5.5 \cdot 10^3$ and $6.2 \cdot 10^3$ molecules of concanavalin A/μm^2 surface area, respectively. These results are close to the previous observation that C^R-7 cells bind approx. 2.5 μg/ml concanavalin A/$1.8 \cdot 10^6$ cells at saturation binding [15]; since the surface area of the C^R-7 cell was estimated at 1150 μm^2 it can be calculated that $6.5 \cdot 10^3$ molecules of lectin was bound/μm^2 surface area of C^R-7 cells at saturating binding.

It is clear that the three independently isolated concanavalin A-resistant cell lines bind concanavalin A in a non-cooperative manner and bind a maximum of $5.5 \cdot 10^3$ to $6.5 \cdot 10^3$ molecules of lectin/μm^2 cell surface area.

Wild-type cells. Recently we reported [15] that the parental wild-type CHO cells used in the selection of the C^R-7 variant line exhibited positive cooperativity in the binding of concanavalin A (Hill coefficient of 1.8). Similar results have been obtained with the independent wild-type populations that were used for the selection of BC^R-2 and EC^R-1 (data not shown). However, as an additional control for these studies, binding experiments were also carried out with another drug-resistant CHO cell line. These cells (H^R100) were selected for resistance to hydroxyurea [16,23] approx. 2 years ago and have been in continuous culture from the time they were isolated. The H^R100 cell line is highly resistant to the toxic effects of hydroxyurea and has a wild-type sensitivity to the cytotoxic effects of concanavalin A.

The binding of labelled concanavalin A to H^R100 cells as a function of lectin concentration is shown in Fig. 3. The binding curve obtained in these studies appeared to be sigmoidal and suggested that concanavalin A was binding to the intact cells with positive cooperativity. When the data in Fig. 3 was examined for possible cooperative effects by means of a Hill plot it became obvious that a

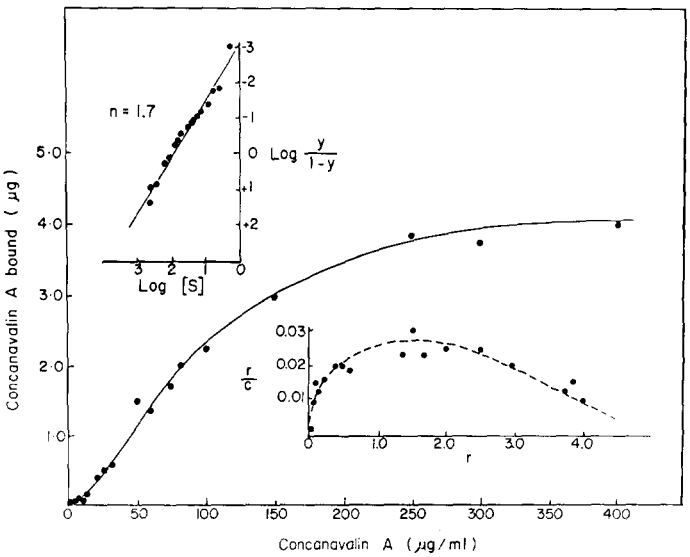

Fig. 3. ^{3}H-Labelled concanavalin A bound/$1.8 \cdot 10^6$ H^R100 cells at various concentrations of lectin. The inset in the upper part shows a Hill plot [27] of the data. The Hill coefficient was calculated to be 1.7. The inset at the bottom presents the data according to Scatchard's equation [28]. Each point represents the average of duplicate points from a single experiment; similar data has been obtained in three separate experiments.

significant amount of lectin was bound to the cells in a cooperative fashion (Fig. 3, see inset). The Hill coefficient was determined to be 1.7.

The non-linearity of the Scatchard plot (Fig. 3, see inset) also indicated that the lectin bound to H^R100 cells in a cooperative manner. Although it is difficult to extrapolate a non-linear Scatchard plot to the abscissa it was still possible to estimate that approx. 5.2 μg/ml concanavalin A was bound per $1.8 \cdot 10^6$ cells at saturation binding. This represents approx. $1.6 \cdot 10^7$ molecules of lectin bound per cell and if the cell surface area (940 μm^2) of H^R100 is taken into consideration it represents $1.7 \cdot 10^4$ molecules of lectin bound/μm^2 surface area. These results can be compared with studies performed with wild-type cells [15] which showed that 4.5 μg/ml concanavalin A was bound/$1.8 \cdot 10^6$ cells at saturation binding. When the surface area (850 μm^2) is taken into account it was estimated that approx. $1.6 \cdot 10^4$ concanavalin A molecules were bound/μm^2 wild-type cell surface area. Therefore the binding data obtained with the wild-type and the H^R100 cells was similar.

Concanavalin A-resistant revertant. As previously noted the ts properties of the C^R-7 cells permitted us to select a cell line from the C^R-7 population that exhibited an increased sensitivity to concanavalin A. Therefore we were interested in determining if the ability to bind concanavalin A in a cooperative manner had accompanied the partial return to a wild-type sensitivity to the cytotoxic effects of concanavalin A. The binding of labelled concanavalin A to RC^R-7 cells as a function of lectin concentration is given in Fig. 4. The binding curve was not hyperbolic and when the data was analyzed for possible interactions between binding sites by the Hill and Scatchard plots (Fig. 4, see insets) it was clear that the lectin bound to the intact cells in a cooperative manner. The Hill coefficient was calculated to be 1.4 as compared to 1.8 with wild-type cells [15] and 1.7 with H^R100 cells (Fig. 3). This indicates that the interaction

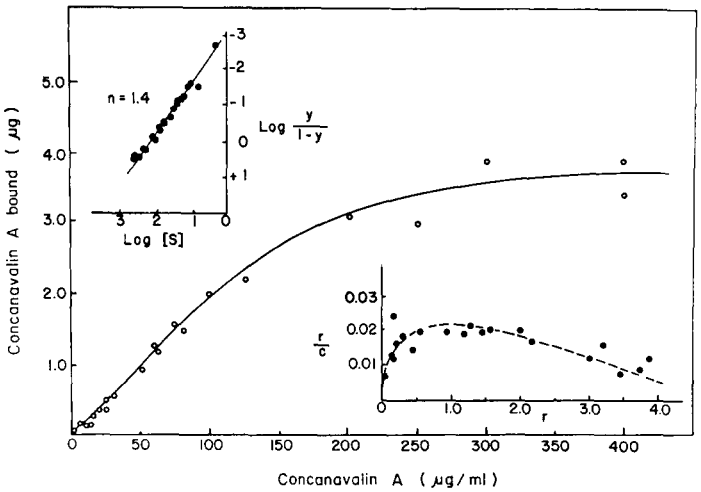

Fig. 4. ^{3}H-Labelled concanavalin A bound/$1.8 \cdot 10^6$ RC^R-7 cells at various concentrations of lectin. The inset in the upper part shows a Hill plot [27] of the data. The Hill coefficient was calculated to be 1.4. The inset at the bottom presents the data according to Scatchard's equation [28]. Each point represents the average of duplicate points from a single experiment; similar data has been obtained in three separate experiments.

between concanavalin A binding sites in the revertant may not be quite as strong as the cooperative interactions observed in the wild-type case.

The non-linear nature of the Scatchard binding curve makes it difficult to determine the amount of lectin bound at saturation but it is possible to estimate that about 5 μg/ml concanavalin A was bound per $1.8 \cdot 10^6$ cells and $1.5 \cdot 10^7$ molecules of lectin per cell. When the cell surface area (1010 μm^2) was considered it was estimated that approx. $1.5 \cdot 10^4$ molecules of lectin was bound/μm^2 surface area. These values are similar to the results obtained in the binding studies with wild-type and H^R100 cells. Therefore it appears that the increase in sensitivity to concanavalin A displayed by RC^R-7 cells is accompanied by a change to a more normal or wild-type lectin binding mechanism.

Somatic cell hybrids. It was shown that the hybrid (A-W) formed between two concanavalin A-sensitive cell lines (AUXB1, ouabain-resistant and wild-type cells) was as sensitive to concanavalin A as pseudodiploid wild-type cells and that a hybrid line (A-7) formed between a concanavalin A-resistant (C^R-7) and a concanavalin A-sensitive line (AUXB1, ouabain-resistant) was almost as sensitive to concanavalin A as the A-W line; although A-7 cells contained a copy of the C^R-7 genome the lectin-resistant property was not expressed. Therefore, if there is a relationship between concanavalin A sensitivity and concanavalin A binding properties we would not expect the altered binding properties observed with C^R-7 cells to be expressed in the A-7 hybrid line; A-W and A-7 cells should exhibit similar lectin binding characteristics.

The results of lectin binding studies with A-W and A-7 cells are presented in Figs. 5 and 6. Clearly the binding data obtained with the two hybrid cell lines was similar. An analysis of the binding data indicate that concanavalin A bound to both hybrid lines with positive cooperativity. However, the interaction between concanavalin A binding sites in these pseudotetraploid cells appeared to

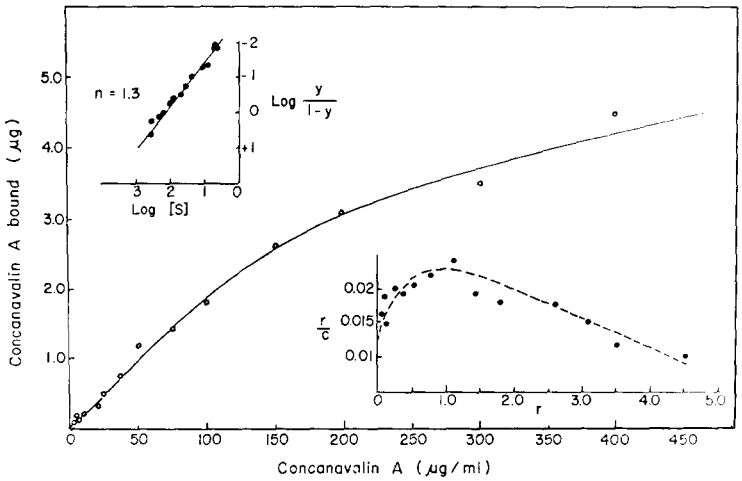

Fig. 5. ^{3}H-Labelled concanavalin A bound/$1.8 \cdot 10^6$ A-W cells at various concentrations of lectin. The inset in the upper part shows a Hill plot [27] of the data. The Hill coefficient was calculated to be 1.3. The inset at the bottom presents the data according to Scatchard's equation [28]. Each point represents the average of duplicate points from a single experiment; similar data has been obtained in three seperate experiments.

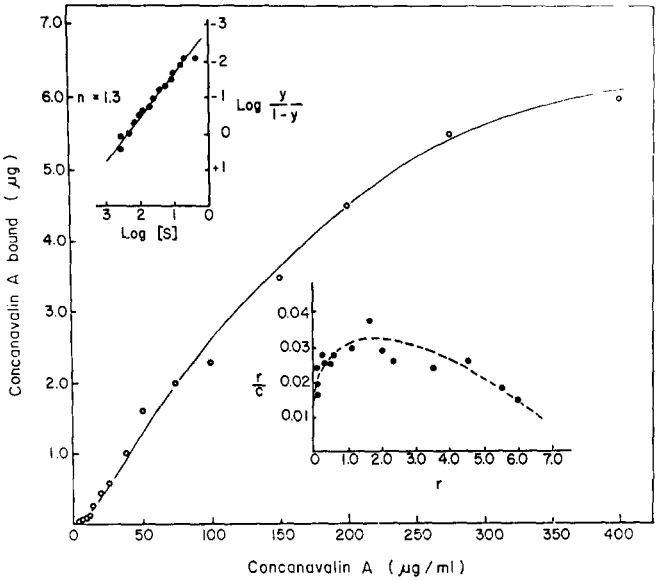

Fig. 6. ^{3}H-Labelled concanavalin A bound/1.8 · 10^6 A-7 cells at various concentrations of lectin. The inset in the upper part shows a Hill plot [27] of the data. The Hill coefficient was calculated to be 1.3. The inset at the bottom presents the data according to Scatchard's equation [28]. Each point represents the average of duplicate points from a single experiment; similar data has been obtained in two separate experiments.

be weaker than in the pseudodiploid wild-type cells. Although the reasons for these weaker interactions is not known a comparison of the cooperative interactions between lectin binding sites in the A-W cells and A-7 cells indicated that the amount of interaction between concanavalin A binding sites was approximately the same in both cell types; the Hill coefficient for the binding of concanavalin A to each cell line was calculated to be 1.3 (Figs. 5 and 6, see insets).

Once again the lack of linearity with Scatchard plots made it difficult to accurately determine the maximum amount of lectin bound per culture. However, it was possible to estimate that approx. 5.5 and 8.0 μg/ml of concanavalin A bound to 1.8 · 10^6 cells of the A-W and A-7 hybrid lines, respectively. These values represent 1.7 · 10^7 and 2.4 · 10^7 molecules of concanavalin A bound/cell surface. When the surface area of A-W (1110 μm^2) and A-7 (1510 μm^2) hybrid cells was used in an analysis of lectin binding it was found that approx. 1.5 · 10^4 and 1.6 · 10^4 molecules of concanavalin A were bound per μm^2 cell surface area of A-W and A-7 cells, respectively. These estimates of concanavalin A bound/unit cell surface area are very close to the values that were obtained with pseudodiploid concanavalin A-sensitive cell lines.

Discussion

The results of concanavalin A binding experiments at 4°C with three independent concanavalin A-resistant cell lines (ref. 15, Figs. 1 and 2) indicates that concanavalin A binds to independent, non-interacting lectin receptor sites at the variant cell surface. These observations are in marked contrast to an interpretation of the binding data obtained with concanavalin A-sensitive CHO cells

(ref. 15, Figs. 3—6) which indicated that a cooperative mechanism of concanavalin A binding was involved. Some recent binding studies from other laboratories [10—12] have also suggested that membrane conformational changes can occur during the binding of lectins. Furthermore a comparison of the number of molecules of lectin bound to the cell surface of concanavalin A-sensitive and -resistant cells show that between $1.5 \cdot 10^4$ and $1.7 \cdot 10^4$ molecules of lectin and between $6.2 \cdot 10^3$ and $6.5 \cdot 10^3$ molecules of lectin were bound per μm^2 cell surface area of concanavalin A-sensitive and concanavalin A-resistant cells, respectively. The number of concanavalin A molecules bound/cell ($6.3 \cdot 10^6$—$2.4 \cdot 10^7$) observed in these studies was within the orders of magnitude reported for the binding of lectins with other cell types (10^6—10^8). Also, it should be noted that the binding technique determines the number of concanavalin A molecules bound/cell surface but due to the multivalent nature of the concanavalin A molecule the actual number of cell receptors involved in the binding of this lectin is actually greater than the number of concanavalin A molecules bound. However, it can be clearly stated from the concanavalin A binding experiments (Figs. 1—6 and Table I) that concanavalin A-sensitive cells were capable of binding approx. 2.5 times more concanavalin A per unit surface area than the concanavalin A-resistant cells. This difference in lectin binding ability probably plays an important role in determining a sensitive or resistant phenotype especially when it is recognized that the D_{10} value exhibited by concanavalin A-resistant cells is also about 2.5 times greater than the value observed with the concanavalin A-sensitive cells.

The results presented in this communication support the view that concanavalin A is a suitable selection agent for the isolation of variant cell lines with altered lectin binding properties and suggest that a correlation exists between the concanavalin A-resistant phenotype exhibited by the three independently isolated lectin-resistant variants and the alterations in concanavalin A binding described in this report. The following observations support this statement: (1) CHO cells with a wild-type sensitivity to concanavalin A bind the lectin at 4°C with positive cooperative; (2) three independently isolated concanacalin A-resistant cell lines were found to bind concanavalin A at 4°C in a non-cooperative fashion; (3) selection of a revertant line (RC^R-7) exhibited a near wild-type sensitivity to concanavalin A and bound a significant amount of lectin in a cooperative manner; (4) hybrid cells (A-7) containing the concanavalin A-sensitive and concanavalin A-resistant genomes exhibited a near wild-type sensitivity to concanavalin A and bound the lectin with positive cooperativity; and (5) concanavalin A-resistant cells were found to bind significantly less lectin when compared to concanavalin A-sensitive cells. Of course these studies do not rule out the possibility of selecting a class of concanavalin A-resistant variant which does not exhibit an altered lectin binding pattern [17,29] because it is very likely that more than one type of cellular change can lead to a concanavalin A-resistant phenotype [19].

In theory the cooperative binding effects observed with concanavalin A-sensitive cells could be explained by the presence of an equilibrium between dimer and tetramer species of concanavalin A which occurs at about pH 6.0 and above [8]. Certainly the binding of ligands to a protein composed of an equilibrium mixture of different molecular weight species can lead to complex

cooperative effects [30]. However, in this case, it is highly unlikely that the above explanation is applicable since the binding of the lectin with concanavalin A-resistant cell lines did not appear to involve cooperative effects. The presence of classes of receptors with widely differing affinities for concanavalin A at the surface of concanavalin A-sensitive cells could also contribute to the non-hyperbolic nature of the saturation binding curve. Although we do not know the precise mechanism that is involved in the interaction of concanavalin A with concanavalin A-sensitive cells the simplest and most likely model is that the binding of the first lectin molecules to the surface membrane modifies the cell surface in such a way as to expose new lectin receptor sites.

The lack of cooperativity in lectin binding with concanavalin A-resistant cells may be due to alterations in cell surface structures which prevent or modify the ability of the variant cell membrane to undergo the conformational changes detected with concanavalin A-sensitive cells. A reduction in the amount of lectin bound to the cell surface of concanavalin A-resistant as compared to concanavalin A-sensitive cells suggest that the lectin-resistant cell surface either contains carbohydrate modifications or that some potential receptor sites exposed in concanavalin A-sensitive cells are present but are not available for concanavalin A binding with lectin-resistant cells due to the alterations at the variant cell surface. In fact some recent experiments in our laboratory with several surface labelling techniques have indicated that concanavalin A-resistant cells contain alterations in cell surface proteins [31]. A prominent difference between concanavalin A-resistant and -sensitive cells may be the presence in resistant cells of a high molecular weight glycoprotein (M_r = 155 000) which appears to be absent in sensitive cells (Ceri, H. and Wright, J.A., unpublished observations).

The properties of the variant cells suggest alternate approaches for studying the cooperative interaction of multivalent ligands with cell surface receptors. For example we are now investigating a possible connection between the membrane changes in the concanavalin A-resistant cells as detected by the absence of cooperativity in lectin binding with the fluidity properties of the surface membrane and the presence or absence of modified cell surface structures.

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