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A STUDY ON CONCANAVALIN A BINDING TO HUMAN ERYTHROCYTES

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Interactions of concanavalin A with human erythrocytes were studied using ^{125}I-labelled concanavalin A and a centrifugal technique with dibutyl phthalate which permitted complete separation of bound and free concanavalin A. Binding of ^{125}I-labelled concanavalin A to human erythrocytes was dependent on cell concentration, pH and temperature. Specificity of binding was confirmed by inhibition and dissociation studies with sugars and native concanavalin A. Positive cooperative binding of concanavalin A to human erythrocytes was observed at low concanavalin A concentrations (less than 1 μg/ml) in both buffers studied. Positive cooperativity at higher concanavalin A concentrations (more than 100 μg/ml) was seen in Tris-Hepes buffer but not in phosphate-buffered saline. Consistent with this cooperative effect was the observation that although dissociation of ^{125}I-labelled concanavalin A from the erythrocytes was complete in the presence of 1 mg/ml of the native lectin, release was inhibited by low concentrations (1 μg/ml). A comparison of concanavalin A binding with hemagglutination studies suggests that the amount of concanavalin A bound determines the rate of erythrocyte agglutination and the size of the aggregates formed.

Introduction

The interactions of lectins and cells can be considered a model for various biological phenomena such as the binding of molecules to specific cell surface receptors, cell-cell adhesion and antigen-antibody combination. For the present investigation of cell-lectin interactions, a well-characterized plant lectin, concanavalin A, and a cell with an extensively studied surface membrane, the human erythrocyte, were chosen.

Although the reaction of lectins with cells has been studied mainly by agglutination techniques [1], it has been demonstrated that there is no direct correlation between the amount of concanavalin A bound and the agglutinability of normal cells or their transformed counterparts [2,3]. The enhanced agglutinability observed for the transformed cell was explained by a redistribution of concanavalin A binding sites on the cell surface. On the other hand, Rando et al. [4] recently reported threshold effects of concanavalin A on the lectin-mediated agglutination of modified bovine erythrocytes.

Concanavalin A has been shown to react with human erythrocytes [5–7] and the minor glycoprotein, i.e. band 3 [8], is considered to be the binding site [9]. Although radioactively labelled concanavalin A has also been demonstrated to bind to human erythrocytes [10,11], the detailed properties of this reaction have not been described. In the present study, we used ^{125}I-labelled concanavalin A and a direct binding technique to elucidate the characteristics of the interaction of this lectin with human

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Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; PBS-CM, 10 mM phosphate-buffered saline, pH 7.0 containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$.

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erythrocytes and to compare concanavalin A binding with lectin-induced hemagglutination.

Materials and Methods

Preparation of erythrocyte suspension. Human erythrocytes were prepared from freshly drawn heparinized blood (group O, Rh positive). After separation from the buffy coat, the erythrocytes were washed with 5 vol. 10 mM phosphate-buffered saline (pH 7.4) and then with buffer 1 (75 mM Hepes/25 mM Tris/10 mM $MgCl_2$/10 mM $CaCl_2$/5 mM KCl/ 2 mM EDTA/55 mM NaCl/0.5% bovine serum albumin, pH 7.0). The cell pellet was finally adjusted to 10^8 erythrocytes/ml with the same buffer.

Iodination of concanavalin A. Concanavalin A was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). It was iodinated with carrier-free $Na^{125}I$ (The Radiochemical Centre, Amersham, Bucks, U.K.) in the presence of chloramine-T by the modified method of Hunter and Greenwood [12,13]. Briefly, 2 nmol concanavalin A, calculated as molecular weight 100000 [14], 0.4 nmol $Na^{125}I$ and 250 nmol chloramine-T were reacted in 0.14 ml 0.6 M sodium phosphate buffer (pH 7.4) containing 1 mM $CaCl_2$ and $MgCl_2$ at room temperature for 30 s. The reaction was terminated by an addition of 1 μmol sodium metabisulfite. Radioactively labelled concanavalin A was purified by absorption to and elution from a Sephadex G-100 column (0.8 × 2.5 cm) with 0.3 M methyl α-D-mannoside. After extensive dialysis against 50 mM Tris-HCl (pH 7.5) at 4°C for 3 days to remove the sugar, the purified ^{125}I-labelled concanavalin A was adjusted to a final concentration of 1 mM $CaCl_2$ and $MgCl_2$ and stored at −20°C in small aliquots. The lectin solution was thawed on the day of use and could be stored without loss of binding activity for at least a month. The specific activity of the ^{125}I-labelled concanavalin A obtained in this manner was about 5 mCi/mg.

Binding of ^{125}I-labelled concanavalin A to erythrocytes. For the standard binding procedure, ^{125}I-labelled concanavalin A (100 ng/ml) was incubated with $8 \cdot 10^7$ erythrocytes/ml in 0.5 ml of buffer 1 at 24°C for 3 h. At the end of the incubation, 1.0 ml of cold buffer 1 was added. Duplicate 0.6-ml aliquots were layered onto 0.3 ml dibutyl phthalate in prechilled polystyrene tubes (Fisher Scientific) [15] and centrifuged at 5000 × *g* for 3 min at 4°C in a Fisher Microcentrifuge. After counting total radioactivity, the buffer and the dibutyl phthalate layers were aspirated. The cell pellets were excised and radioactivity in the cell pellets were counted in a gamma well counter.

Nonspecific binding, defined as radioactivity bound in the presence of 0.1 M methyl α-D-mannoside, was usually less than 0.5% of available radioactivity.

In some experiments, separation of bound and free ^{125}I-labelled concanavalin A was performed using the modified microfuge technique [10] in which reaction buffer containing 5% bovine serum albumin was used instead of dibutyl phthalate.

Effects of various sugars on concanavalin A binding. Concanavalin A binding studies were conducted in the presence of various sugars (methyl α-D-mannoside, methyl α- or β-D-glucoside and methyl α- or β-D-galactoside) at concentrations ranging from 0 to 100 mM in the standard binding procedure.

Effects of native concanavalin A on ^{125}I-labelled concanavalin A binding. Binding studies were conducted as described for the standard binding procedure except that the incubation mixture contained native concanavalin A in amounts ranging from 0 to 2 mg/ml. Similar experiments were performed in PBS-CM.

Dissociation of ^{125}I-labelled concanavalin A bound to erythrocytes. ^{125}I-labelled concanavalin A (100 ng/ml) was incubated with erythrocytes ($6 \cdot 10^8$ cells/ml) at 24°C for 60 min. After aliquots were taken for determination of the percentage of concanavalin A bound, the reaction mixture was centrifuged at 0°C, the supernatant was aspirated and the volume restored with ice-cold buffer 1. The same washing procedure was repeated and aliquots were then transferred to tubes containing 19-fold volumes of fresh buffer 1 without or with native concanavalin A (1 mg/ml) or 50 mM methyl α-D-mannoside for further incubation at 24°C. At timed intervals, aliquots were centrifuged through dibutyl phthalate to separate bound and free radioactively labelled concanavalin A.

In another experiment, aliquots were transferred to tubes containing fresh medium with varying amounts of native concanavalin A (0–2 mg/ml) and incubated for 60 min at 24°C to see the effects of the

concentration of native concanavalin A on dissociation.

Results

Procedures

Self-aggregation of concanavalin A occurred during incubation and its degree was dependent on the incubation buffer, being less in PBS-CM than in buffer 1. In the absence of erythrocytes, about 30% of ^{125}I-labelled concanavalin A incubated with 0.5 mg/ml native concanavalin A was aggregated in buffer 1 as measured by the centrifugation technique with bovine serum albumin (Table I). The presence of 100 mM methyl α-D-mannoside in the reaction mixture prevented this self-aggregation. When dibutyl phthalate was employed for separation of bound and free lectin, self-aggregated concanavalin A could be separated completely from the erythrocyte-bound lectin.

Concanavalin A binding to human erythrocytes

Binding of concanavalin A to human erythrocytes was dependent on time and temperature (Fig. 1), erythrocyte concentration (Fig. 2) and pH (Fig. 3). At 24°C, the steady state was attained in 3 h and the equilibrium was maintained at least for 1 h. Binding was directly proportional to the erythrocyte concentration over a 10-fold range. The optimum pH for binding was 7. In the pH range 6–8, nonspecific binding was negligible. In the pH range higher than 8, nonspecific binding increased abruptly and specific binding became negligible.

Specificity of concanavalin A binding

Several monosaccharide derivatives were examined for their inhibitory effects on concanavalin A binding to erythrocytes (Fig. 4). Methyl α-D-mannoside and methyl α-D-glucoside showed almost the same activity and exhibited 50% inhibition at a concentration of 1 mM and complete inhibition at 10 mM. Methyl

TABLE I

COMPARISON OF TWO METHODS FOR THE SEPARATION OF BOUND AND FREE ^{125}I-LABELLED CONCANAVALIN A

The binding studies were performed in buffer 1. At the end of the incubation, portions of the incubation mixture were transferred into prechilled microcentrifuge tubes containing dibutyl phthalate (DBP method) or 5% bovine serum albumin in buffer 1 (BSA method) and centrifuged at 5 000 × *g* for 3 min at 4°C to separate bound and free concanavalin A. Results are the average of two experiments and expressed as percentage of total radioactivity. The binding studies were performed in the absence (–) or presence (+) of 0.5 mg/ml of native concanavalin A in the reaction mixture.

	– Concanavalin A		+ Concanavalin A	
	DBP method	BSA method	DBP method	BSA method
Standard procedure	11.8	12.8	18.1	35.7
Sugar control [a]	0.3	0.9	0.4	0.5
Cell-free blank [b]	0	1.3	0	28.9

[a] Binding studies were performed in the presence of 0.1 M methyl α-D-mannoside.

[b] Erythrocytes were omitted from the reaction mixture.

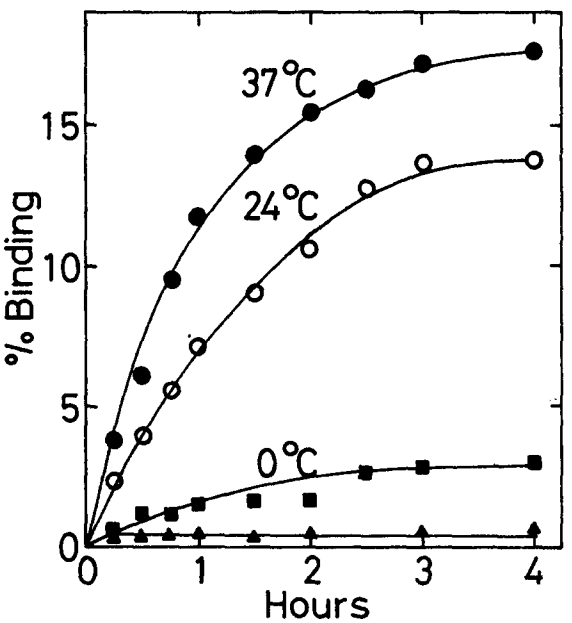

Fig. 1. Effect of incubation time and temperature on the binding of ^{125}I-labelled concanavalin A to human erythrocytes. Erythrocytes ($6 \cdot 10^7$ cells/ml) were incubated at 37°C (●——●), 24°C (○——○) or at 0°C (■——■) with ^{125}I-labelled concanavalin A (100 ng/ml). Percent nonspecific binding at 24°C (▲——▲) was determined in the presence of 0.1 M methyl α-D-mannoside.

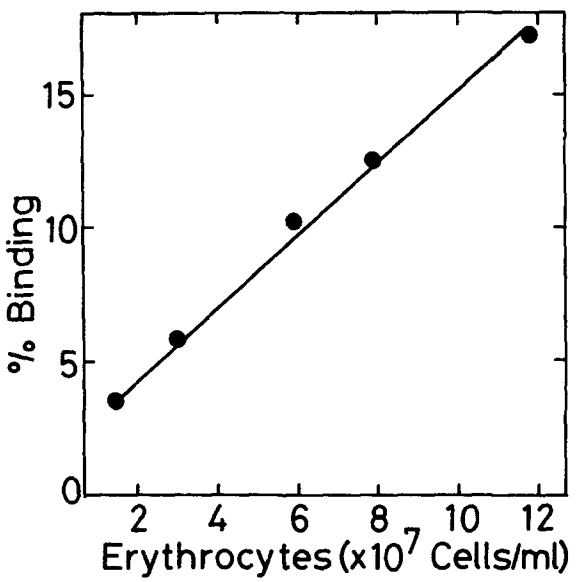

Fig. 2. Effect of erythrocyte concentration on the binding of ^{125}I-labelled concanavalin A. Cells ranging from $6 \cdot 10^6$–$1.2 \cdot 10^8$ cells/ml were incubated with 100 ng/ml of radioactively labelled concanavalin A at 24°C for 3 h.

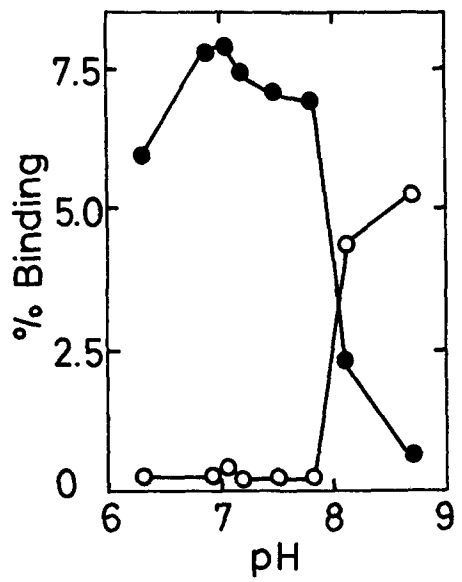

Fig. 3. Effect of pH of the incubation buffer on the binding of ^{125}I-labelled concanavalin A to human erythrocytes. The pH of the buffer was adjusted by changing the molar ratio of Hepes and Tris in buffer 1 and was measured at room temperature. ^{125}I-labelled concanavalin A binding studies were performed as described in Materials and Methods. ●———●, percent specific ^{125}I-labelled concanavalin A binding; ○———○, percent nonspecific binding.

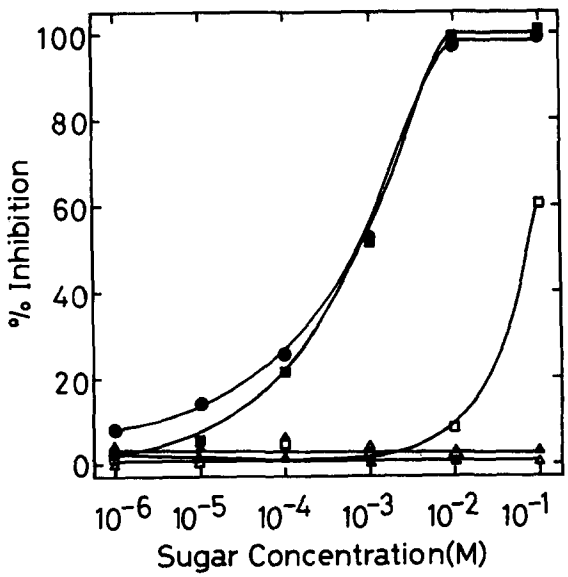

Fig. 4. Inhibitory effects of sugars on ^{125}I-labelled concanavalin A binding to human erythrocytes. ^{125}I-labelled concanavalin A binding studies were performed in the absence or presence of methyl α-D-mannoside (●———●), methyl α- or β-D-glucoside (■———■ or □———□), or methyl α- or β-D-galactoside (▲———▲ or △———△). Inhibition observed at 0.1 M methyl α-D-mannoside was assumed as 100% inhibition.

β-D-glucoside had a weaker inhibitory effect. Methyl α- and β-D-galactoside were not inhibitory.

Effects of native concanavalin A on radioactively labelled concanavalin A binding to human erythrocytes

The percent binding of ^{125}I-labelled concanavalin A increased as the concentration of native concanavalin A in the reaction mixture was increaed from 0 to 1 μg/ml, then declined and attained a minimum value at 50 μg/ml (Fig. 5). Between 50 and 100 μg/ml, the percent binding in buffer 1 jumped up abruptly and attained a maximum value at 500 μg/ml. Agglutination tests performed at the same time showed no agglutination at lectin concentrations lower than 1 μg/ml, hemagglutination without big clusters of erythrocytes at 5–100 μg/ml and hemagglutination with big clusters of erythrocytes at concentrations higher than 500 μg/ml (Table II).

When the binding studies were performed in PBS-CM instead of buffer 1, positive cooperative binding

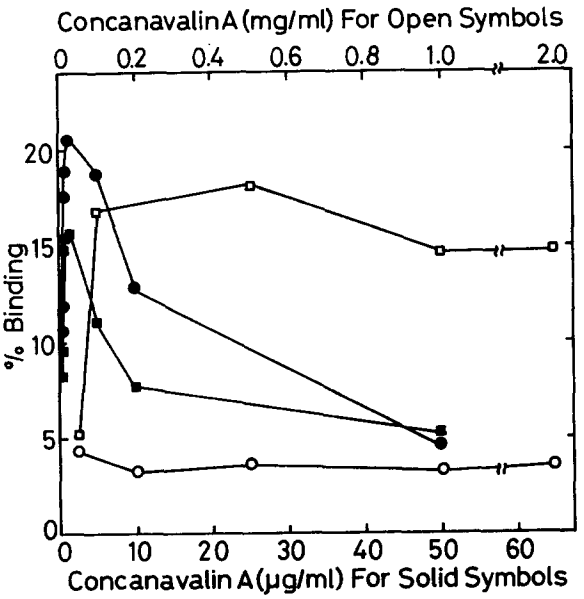

Fig. 5. Effect of concentration of native concanavalin A on ^{125}I-labelled concanavalin A binding to human erythrocytes. 100 ng/ml of ^{125}I-labelled concanavalin A were incubated with $8 \cdot 10^7$ cells/ml of human erythrocytes in the presence of native concanavalin A ranging from 0 to 2 mg/ml at 24°C for 3 h. Binding studies were carried out in buffer 1 (■———■, □———□) or PBS-CM (●———●, ○———○) and bound concanavalin A was separated from the free lectin through the dibutyl phthalate gradient. Nonspecific binding was about 0.3% of available radioactivity at all points. Scales for solid symbols and open symbols are shown at the bottom and at the top of the figure, respectively.

TABLE II

EFFECT OF CONCANAVALIN A CONCENTRATION ON HUMAN ERYTHROCYTE AGGLUTINATION

The agglutination assays were performed in micro V-type plates and scored visually after 3 h incubation at room temperature. –, no hemagglutination; + and ++, hemagglutination without and with large clusters of aggregated erythrocytes, respectively.

	Concanavaling A (μg/ml)						
	0	1	5	10	100	500	1 000
Buffer 1	–	–	+	+	+	++	++
PBS-CM	–	–	–	+	+	+	+

at lower concanavalin A concentration (around 1 μg/ml) was observed, but this phenomenon at higher concanavalin A concentration (more than 100 μg/ml) was not observed. No hemagglutination was observed at concanavalin A concentrations less than 5 μg/ml. Big clusters were not found even at 2 mg/ml concanavalin A concentration in this buffer.

The Langmuir isotherm and the Scatchard plot

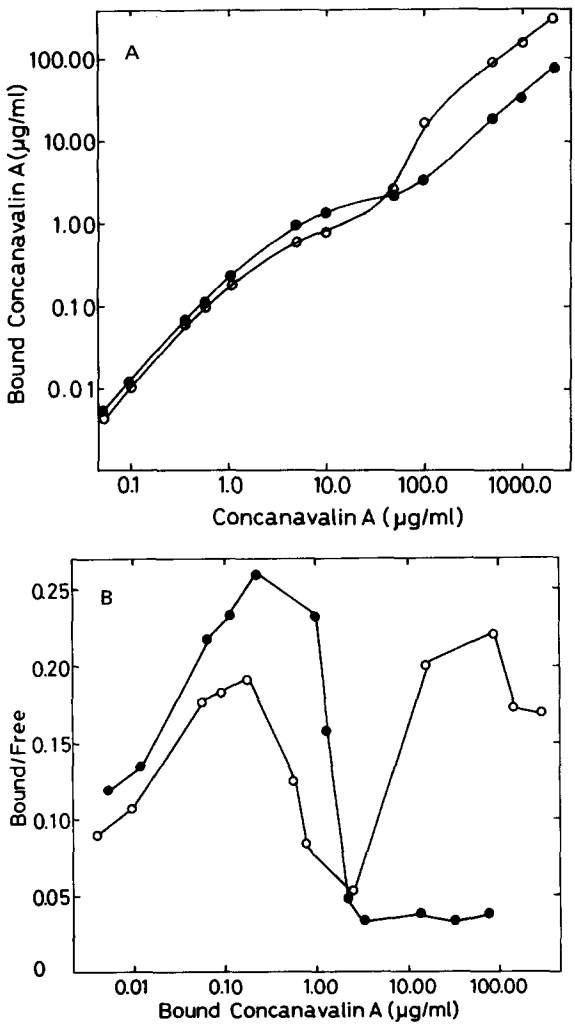

Fig. 6. (A) Langmuir isotherm curves of ^{125}I-labelled concanavalin A binding to human erythrocytes in buffer 1 (○———○) or PBS-CM (●———●). Experimental data are identical to those used in Fig. 5. (B) Scatchard plots of ^{125}I-labelled concanavalin A binding to human erythrocytes in buffer 1 (○———○) or PBS-CM (●———●). Experimental data are identical to those used in Fig. 5.

analysis of the same data are shown in Fig. 6A and B, respectively. In both buffer systems, the amount of bound concanavalin A attained a plateau at about 10 μg/ml lectin concentration (Fig. 6A). The abrupt increase in the amount of bound concanavalin A observed in buffer 1 at about 100 μg/ml lectin concentration (Fig. 6A) corresponds to the sudden change in the ratio of bound to free concanavalin A at about 10 μg/ml of bound concanavalin A in the Scatchard plots.

If it is assumed that the intercept of the steeply descending part of the Scatchard plots with the abscissa indicates the maximal binding capacity, it may be calculated that one erythrocyte has about 150 000 concanavalin A binding sites.

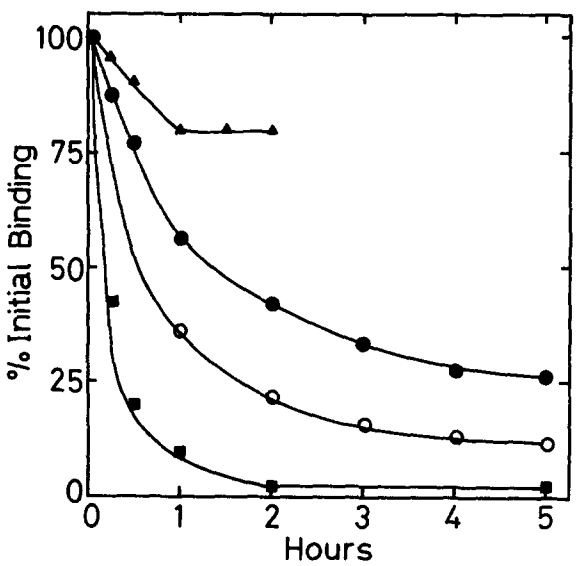

Fig. 7. Time course of dissociation of bound ^{125}I-labelled concanavalin A from human erythrocytes at 24°C. Erythrocytes were pretreated with radioactively labelled concanavalin A at 24°C in buffer 1 and the unbound lectin was washed off; they were then incubated in 20-fold volumes of buffer 1 (▲——▲), buffer 1 + 1 mg/ml native concanavalin A (●——●) or buffer 1 + 50 mM methyl α-D-mannoside (■——■) at 24°C. At timed intervals, dissociated and bound ^{125}I-labelled concanavalin A were separated as described in Materials and Methods. Similar experiments were performed in PBS-CM containing 1 mg/ml native concanavalin A (○——○). The ^{125}I-labelled concanavalin A bound at the end of the incubation for association was considered 100%.

Dissociation of bound radioactively labelled concanavalin A

Erythrocytes pretreated with ^{125}I-labelled concanavalin A were further incubated at 24°C in the presence of 1 mg/ml native lectin or 50 mM methyl α-D-mannoside (Fig. 7). During procedures to wash off unbound lectin, no dissociation of bound radioactive concanavalin A occurred. A control incubation which was performed in the absence of both the sugar and the native concanavalin A indicated that by dilution alone 20% of bound concanavalin A was dissociated in 1 h. In the presence of 1 mg/ml native concanavalin A, 45% of the bound lectin was released in 1 h and 75% in 4 h. When the same experiments were performed in PBS-CM, about 90% of bound concanavalin A was dissociated in 4 h. Bound concanavalin A was dissociated rapidly in the presence of 50 mM methyl α-D-mannoside with complete release being observed in 90 min.

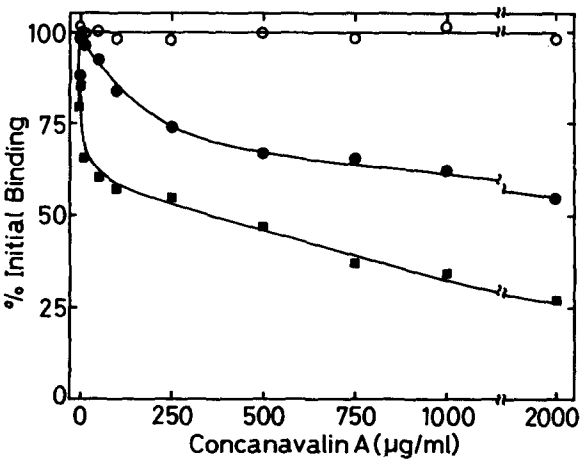

Fig. 8. Effect of the concentration of concanavalin A on the dissociation of bound ^{125}I-labelled concanavalin A from human erythrocytes at 0°C (○——○), 24°C (●——●) or 37°C (■——■). Human erythrocytes pretreated with ^{125}I-labelled concanavalin A and freed of unbound radioactivity were incubated in 20-fold volumes of buffer 1 containing native concanavalin A ranging from 0 to 2 mg/ml at 0, 24 or 37°C for 60 min. At the end of the incubation, aliquots were transferred onto prechilled dibutyl phthalate in microcentrifuge tubes and centrifuged at 5000 × *g* for 3 min at 4°C to separate dissociated and bound ^{125}I-labelled concanavalin A.

Effects of temperature and concanavalin A concentration on dissociation

The dissociation rate was dependent on the incubation temperature (Fig. 8). At 0°C, native concanavalin A at up to 2 mg/ml had no dissociation effect. At higher temperatures, the lower concentrations of native concanavalin A showed an inhibitory effect on dissociation (Fig. 8), i.e., the amount of bound ^{125}I-labelled concanavalin A which disssociated was less than that observed in the absence of native concanavalin A. This cooperative effect was maximum at 1 μg/ml of native concanavalin A, and was observed up to 10 μg/ml at 37°C and up to 50 μg/ml at 24°C. Maximum dissociation observed at 2 mg/ml was 45% at 24°C and 70% at 37°C.

Discussion

The interactions of concanavalin A and human erythrocytes were studied using a direct binding technique employing ^{125}I-labelled concanavalin A with a high specific activity (5 mCi/mg) and the dibutyl phthalate density centrifugation method to separate erythrocyte-bound concanavalin A from free lectin.

The previous observation that concanavalin A undergoes self-aggregation during incubation [16] was confirmed in the present study. It was further shown that the separation technique which employs 5% bovine serum albumin density centrifugation [6] leads to the recovery of a portion of the concanavalin A in the excised pellet even in the absence of erythrocytes, especially when the incubation mixture contains more than 0.5 mg/ml of this lectin (Table I). However, bound lectin could be separated completely from free and self-aggregated concanavalin A with the use of the dibutyl phthalate density centrifugation method (Table I).

The effect of pH on the interaction between concanavalin A and cells had not been previously described. The interaction of concanavalin A with *p*-nitrophenyl α-D-glucoside is optimal at pH 3–8 [17] and with dextrans and α-mannans at pH 6.1–7.2 [18] and 5.7–6.7 [19], respectively. The optimal pH for the interaction of concanavalin A with human erythrocytes was in a relatively narrow range around pH 7 (Fig. 3). At higher pH values above pH 8, nonspecific binding of ^{125}I-labelled concanavalin A increased sharply and specific binding decreased. This sudden change of the binding property may be due to the irreversible denaturation of the lectin in the higher pH range [20].

Concanavalin A binding to human erythrocytes was time and temperature dependent. Though equilibrium was reported to be attained in 60 min [6], a longer incubation time was needed in the present study in agreement with more recent reports [4,21]. Although the reasons for these discrepancies are unknown, it seems that the lower the concentration of concanavalin A in the reaction mixture, the longer the incubation time which is needed to reach equilibrium. Other factors such as sugar component on the cell membrane may also participate, since native and mildly oxidized bovine erythrocytes required different incubation times to reach the equilibrium with concanavalin A [4].

Inhibition studies showed that methyl α-D-glucoside was as potent as methyl α-D-mannoside in inhibiting binding between concanavalin A and human erythrocytes (Fig. 4). However, about 100-times more methyl β-D-glucoside than methyl α-D-glucoside was required to achieve 50% inhibition. Methyl α- and β-D-galactosides had no inhibitory activity.

Positive cooperative binding of concanavalin A to erythrocytes at lower concanavalin A concentrations (less than 1 μg/ml) was demonstrated in both buffer 1 and PBS-CM (Fig. 6), as previously reported [11, 12]. Interestingly, a positive cooperative effect at higher lectin concentrations (more than 100 μg/ml) was observed only in buffer 1 and not in PBS-CM. The latter finding may be similar to the reported positive cooperative binding of concanavalin A to fat cells [13]. We do not know the actual mechanism responsible for such a cooperativity, but the effect observed at the lower lectin concentrations may be explained by a concentration-dependent association of concanavalin A subunits with a change of binding affinity [21] or a lectin-induced reorientation of binding sites as proposed for wheat germ agglutinin [24]. A dimer-tetramer interconversion dependent on concanavalin A concentration [11,22] and the formation of the precipitable aggregates at the higher lectin concentrations [16] may be responsible for the positive cooperativity observed in buffer 1 at concanavalin A concentrations above 100 μg/ml. The nature of the incubation medium employed appears to be impor-

tant both for self-aggregation (Table I) as well as for cell-lectin interaction. Schnebli et al. [11] and Gordon and Young [21] used phosphate-buffered saline and PBS-CM, respectively, and showed positive cooperativity at lower lectin concentrations but not at higher lectin concentrations. Cuatrecasas [13] employed Krebs-Ringer bicarbonate buffer and observed a positive cooperative effect at higher lectin concentrations. We showed a positive cooperative effect at lower concanavalin A concentrations in the two buffer systems used but that at higher lectin concentrations in one buffer system only.

Since the molecular species of concanavalin A which bind to erythrocytes is unknown [21], the number of binding sites calculated from the amount of concanavalin A bound must be accepted with reservations. If it is assumed that the bound concanavalin A is in the tetrameric form, the number of binding sites per erythrocytes calculated in the present study is about 150 000. This is in agreement with some previous values [3,11,21], but is substantially different from the approx. $2 \cdot 10^6$ binding sites/cell found by Phillips et al. [10]. The reason for this discrepancy may be that the latter authors calculated the number of binding sites from data obtained at concanavalin A concentrations of more than 150 μg/ml, where bound concanavalin A might be in self-aggregated forms. However, the steeply descending part of the Scatchard plots, on which calculation of the binding sites in the present study was based, was derived from the binding data at a concanavalin A concentration around 10 μg/ml.

Comparison of the concanavalin A bound and the type of hemagglutination observed (Table II) suggests that the amount and nature of bound lectin on the cell surface may determine the mode of hemagglutination. In the range of less than 1 μg/ml concanavalin A concentration, on the ascending limb of the Scatchard plots (Fig. 6B) where probably dimer and protomer forms are present [21], no hemagglutination was observed. Hemagglutination was seen at concanavalin A concentrations higher than 5 μg/ml in buffer 1 and 10 μg/ml in PBS-CM, where the Scatchard plots show a descending limb and the major portion of concanavalin A may be in the tetrameric form. At much higher concanavalin A concentrations in buffer 1 where the amount of bound concanavalin A increased abruptly (Figs. 5 and 6) and probably self-aggregated concanavalin A is predominant, hemagglutination with big clusters of erythrocytes were observed (Table II).

It was reported that bound ^{125}I-labelled concanavalin A could be dissociated by methyl α-D-mannoside [8,13] but not by native concanavalin A [13]. In the present study, we showed that 1 mg/ml native concanavalin A dissociated a major portion of bound ^{125}I-labelled concanavalin A (Fig. 7). The dissociation rate was dependent on the buffers used as was the binding rate. The ^{125}I-labelled concanavalin A which remained bound after 5 h incubation may correspond to the labelled lectin which became bound in the presence of excess amounts of native concanavalin A (Fig. 5). A larger amount of radioactively labelled concanavalin A was bound at equilibrium in buffer 1 than in PBS-CM in both the association and dissociation phases. Methyl α-D-mannoside effected a complete dissociation of lectin in a relatively short time (Fig. 7), as reported previously [13]. The dissociation rate was also temperature dependent (Fig. 8). At 0°C, bound concanavalin A was stable and was not dissociated even in the presence of 2 mg/ml native concanavalin A. Binding of concanavalin A to erythrocytes at 0°C was demonstrated, although the total bound was small (Fig. 1). Conformational changes in a concanavalin A [20] and/or its receptor sites after lectin-cell interaction [24] may render binding more stable at lower temperature.

In the present study, we showed that concanavalin A at lower concentrations inhibited the dissociation of bound ^{125}I-labelled concanavalin A (Fig. 8). The reason for this inhibition may be the same as for the positive cooperative binding at lower concanavalin A concentration found in the association phase. In the medium not containing native concanavalin A, bound tetrameric concanavalin A may dissociate into dimer and protomer [21,22] at the higher temperatures, which may then dissociated from erythrocytes. In the presence of a small amount of native concanavalin A in the medium, concentration-dependent dissociation of concanavalin A into subunits is inhibited and the fraction of the bound ^{125}I-labelled concanavalin A which remained attached may be more than that in the absence of native concanavalin A. At higher native concanavalin A concentration, ^{125}I-labelled concanavalin A on cell surface may be replaced by native concanavalin A.

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