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THE CONCANAVALIN A RECEPTOR FROM HUMAN ERYTHROCYTES IN LIPID BILAYER MEMBRANES

INTERACTION WITH CONCANAVALIN A AND SUCCINYL-CONCANAVALIN A

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The concanavalin A receptor from human erythrocyte membranes has been isolated by affinity chromatography using the mild, readily-dialyzable detergent dodecyltrimethylammonium bromide. The purified protein has been reincorporated into large unilamellar phospholipid vesicles using a detergent dialysis technique. The mean diameter of these vesicles increases as the lipid:protein ratio decreases. Binding of succinyl-concanavalin A to these vesicles was quantitated using ^{125}I-labelled lectin in a filtration assay. The concanavalin A receptor in lipid bilayer vesicles provides specific high affinity binding sites for succinyl-concanavalin A with an association constant of $2.13 \cdot 10^6$ M^{-1}. Scatchard plots indicate positive cooperativity of binding at very low lectin concentrations, a characteristic also seen in concanavalin A binding to intact human erythrocytes. The presence of bovine serum albumin has little effect on lectin binding and is not required for expression of cooperativity. Concanavalin A effectively competes with succinyl-concanavalin A for binding to the vesicles with an association constant of $4.83 \cdot 10^6$ M^{-1}. Receptor-bearing vesicles are readily agglutinated by concanavalin A but not by its succinylated derivative. The kinetics of vesicle agglutination are biphasic, with an initial rapid phase followed by a pseudo-first order process. We suggest that studies on reassembled receptor proteins in lipid bilayers can provide valuable insight into receptor involvement in transmembrane signalling events and the factors involved in cell membrane behaviour and cell agglutination.

Introduction

Glycoproteins, particularly transmembrane glycoproteins, are important components of the cell membrane, acting as receptors for numerous external agents such as plant lectins [1], viruses [2] and hormones [3]. Glycoproteins are also involved in many important transmembrane signalling events such as lymphocyte activation [4]. Little is known about glycoprotein behaviour at the molecular level although the characteristics of receptor binding, particularly positive cooperativity, seem to be important in determining cellular response [5]. Despite enormous numbers of studies characterizing lectin binding to high affinity receptors on cell surfaces (see, for example, Refs. 6–8) it is still not clearly understood how these binding characteristics are related to glycoprotein behaviour at the

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Abbreviations: PC, phosphatidylcholine; PS, phosphatidylserine; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate; RCA 120, *Ricinus communis* agglutinin; RCA 60, *Ricinus communis* lectin.

molecular level. The role played by receptor rearrangement and the level of control exerted by interactions with other membrane components is also not clear. Recently there has been some interest in studying the ability of glycoproteins to act as high-affinity lectin receptors in lipid bilayers [9,10]. Lipid bilayers are an attractive alternative to cell surfaces for receptor binding studies in that they offer the researcher a much greater degree of control over the entire system. Lipid composition, fluidity and domain structure, lipid : protein ratios, the presence or absence of other important membrane components may all be manipulated in model systems.

The concanavalin A receptor from human erythrocytes provides an excellent model for studying the factors controlling glycoprotein behaviour in membranes in that it is fairly typical of the type of receptor glycoprotein found at the mammalian cell surface. (To date, only glycophorin, a somewhat atypical integral glycoprotein, has been studied in lipid bilayer systems [9,10].) The concanavalin A receptor is a large (molecular weight 95 000) transmembrane glycoprotein which spans the membrane several times [11]. It is part of the Band 3 group of proteins, and as such is the carrier involved in anion transport across the red blood cell membrane. It is also known to interact with several other proteins in the membrane, both integral (glycophorin [12]) and peripheral (ankyrin [13] and several glycolytic enzymes [14]).

We have incorporated the concanavalin A receptor glycoprotein into large unilamellar phospholipid vesicles. Concanavalin A was found to have a relatively high nonspecific binding component when tested with these vesicles (Chicken, C.A. and Sharom, F.J., unpublished data). We therefore chose to use its succinylated derivative in our binding studies. Succinyl-concanavalin A is a divalent derivative of concanavalin A with a much higher surface negative charge [15]. It retains the biological activity of concanavalin A, but seems to lack many of the nonspecific binding and toxic effects associated with the parent lectin [16]. We have shown that the human erythrocyte concanavalin A receptor provides fully functional high affinity binding sites for succinyl-concanavalin A at the vesicle surface, and have characterized binding over a 10^5-fold range of lectin concentrations. Positive cooperativity is seen at low lectin concentrations, suggesting that characteristics normally associated with intact cells can be duplicated in model systems using purified receptors.

Materials and Methods

Egg PC and PS (from bovine brain) were obtained from Sigma. Egg PC was further purified by column chromatography on Bio-Sil A (BioRad). Both phospholipids were pure as judged by thin-layer chromatography on Silica gel G.

SDS-polyacrylamide gel electrophoresis was carried out in 1.5 mm slabs by the procedure of Laemmli [17]. Protein was assayed by the method of Peterson [18]. Human erythrocyte ghosts were prepared from outdated bank blood by the procedure of Dodge et al. [19].

Concanavalin A receptor isolation

The concanavalin A receptor was isolated by affinity chromatography using a modification of the method previously described [20]. The affinity column buffer was 0.1 M NaCl, 10 mM Tris, 0.7 mM in each of Ca^{2+}, Mg^{2+} and Mn^{2+}, 0.2 mM dithiothreitol, 0.02% sodium azide pH 7.4. A 30–50 ml volume of packed ghosts (90–150 mg protein) was mixed on ice with an equal volume of column buffer containing 200 mM dodecyltrimethylammonium bromide (Sigma). After stirring for 30 min at 0°C the solution was centrifuged at 29 000 $\times g$ for 20 min at 4°C and the small pellet discarded. The supernatant was run onto a concanavalin A-Sepharose 4B column (30 ml bed volume) at 4°C and washed with 4–5 bed volumes of 25 mM dodecyltrimethylammonium bromide in column buffer. Extensive washing was found to be necessary to reduce contamination of the receptor protein with band 4.2 and band 6, which tend to copurify with it. Bound protein was eluted with column buffer containing 25 mM dodecyltrimethylammonium bromide and 0.1 M α-methylmannoside (Sigma). Protein-containing fractions were pooled and concentrated five times by pressure ultrafiltration in a stirred cell at 0°C, using an Amicon PM 50 membrane. The concentrated receptor protein was immediately frozen in small aliquots and stored at −20°C. Aliquots used for

further reincorporation studies were thawed only once.

Reincorporation into large unilamellar vesicles

A mixture of egg PC and PS in a weight ratio of 4 : 1 was used for all reincorporation experiments. Di[1-^{14}C]palmitoyl-L-α-phosphatidylcholine (Amersham, 80–120 mCi/mmol) was included as a tracer. The lipid : protein ratio could be varied by using appropriate amounts of the phospholipid mixtures and concanavalin A receptor. Phospholipids were dried down under a stream of nitrogen and pumped under vacuum for 30 min to ensure complete removal of organic solvents. Lipids were dissolved in 200 mM dodecyltrimethylammonium bromide/5 mM Hepes-buffered saline pH 7.4, at a concentration of 1.5 mg/ml, receptor glycoprotein was added and the mixture incubated for 1–2 h at 4°C. The mixture was dialyzed against four changes of 5 mM Hepes-buffered saline, pH 7.4, for 48 h at 4°C using Spectrapor 2 membrane tubing (Spectrum Medical Industries). Vesicles were harvested at 78 500 $\times g$ for 20 min at 4°C and resuspended in Hepes-buffered saline at a lipid concentration of 5–10 mg/ml using small diameter glass beads. Vesicles were frozen in small aliquots and those used for further studies were thawed only once.

Electron microscopy

Negative staining with ammonium molybdate was carried out as described by Munn [21]. Grids were examined in a Philips 300 electron microscope. Phase-contrast microscopy was used to monitor the presence of amorphous material in the vesicle preparations.

Dynamic light scattering studies

Dynamic light scattering measurements were carried out using a thermally jacketed scattering chamber at 20°C, a helium-neon laser (wavelength 632.8 nm), a quantum photometer and a 64 channel autocorrelator (Langley-Ford Model 1096). A small aliquot of vesicles (containing 15–20 μg protein) was diluted to 2 ml with phosphate-buffered saline, pH 7.4, and scattering was recorded at an angle of 90°. Measurements were made at several sample ('bin') times. Analysis of the resulting autocorrelation functions was carried out using the method of cumulants. The average hydrodynamic diameter (Stoke's radius) of the vesicles was computed assuming they were spherical.

Gel filtration chromatography

A 200 μl aliquot of vesicles (phospholipid concentration 8 mg/ml, lipid : protein ratio 10 : 1) was applied to a small column of Sepharose 2B equilibrated in 5 mM Hepes-buffered saline, pH 7.4. The sample was eluted with the same buffer and 300 μl fractions collected. Each fraction was assayed for protein and [^{14}C]PC was quantitated by liquid scintillation counting.

Binding of succinyl-concanavalin A to lipid vesicles

Succinyl-concanavalin A (Vector Laboratories) was radioiodinated using Na^{125}I (carrier free, Amersham) and 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril ('Iodogen', Pierce) by the basic method of Markwell [22]. Lectin (5 mg) and radioiodine (0.5 mCi) were incubated at room temperature for 10 min. Unreacted ^{125}I was removed by two successive gel filtration steps on Bio-Gel P-10 (BioRad). Protein recovery was 80–85% and > 95% of the radioactivity in the final product was precipitable by trichloroacetic acid. Specific activity of ^{125}I-labelled succinyl-concanavalin was usually close to $5 \cdot 10^4$ cpm/μg. Iodinated lectin was stored in aliquots at −70°C with 0.02% (w/v) sodium azide as a preservative.

Binding assays were carried out in 500-μl microtest-tubes in a total incubation volume of 100 μl. ^{125}I-labelled succinyl-concanavalin A, the appropriate amount of carrier succinyl-concanavalin A, and the required amount of vesicle suspension were incubated in Dulbecco's phosphate-buffered saline (+Ca^{2+}, Mg^{2+}), pH 7.4 [23]. After a 1 h incubation at 22°C, an equal volume of cold 25% (w/v) poly(ethylene glycol) 6000 (Sigma) in phosphate-buffered saline was added, and the samples were incubated on ice for 15 min. Vesicles and bound ^{125}I-labelled succinyl-concanavalin A were collected on 13 mm fiberglass filters (Gelman) using a vacuum filtration manifold. Filters were washed several times with 10% (w/v) poly(ethylene glycol) in phosphate-buffered saline, dried and counted. All binding assays were carried out in duplicate; controls for nonspecific binding con-

tained 0.1 M α-methylmannoside.

For inhibition studies, concanavalin A (150 μg/ml) was added immediately after the ^{125}I-labelled succinyl-concanavalin A. Succinyl-concanavalin A concentrations were chosen to be in the linear region of the Scatchard plot.

Binding of concanavalin A to human erythrocytes

Concanavalin A (Sigma) was radioiodinated by the same method as previously described for succinyl-concanavalin A. Unreacted ^{125}I was removed by the method of Phillips et al. [24]. Binding of ^{125}I-labelled concanavalin A to human erythrocytes was measured using two different techniques [24,25]. Very similar binding curves were obtained in each case.

Agglutination of vesicles

Vesicle agglutination was studied by monitoring the increase in absorbance of the vesicle suspension at 500 nm and 22°C in a Unicam SP-1800 double-beam spectrophotometer. Semi-micro cuvettes were used containing 400 μl of a vesicle suspension (2 mg/ml phospholipid, lipid : protein ratio 10 : 1) in phosphate-buffered saline, pH 7.4, and the appropriate concentration of lectin. In the absence of lectin, no significant change in the absorbance of the vesicle suspension was seen over a period of a few hours. Vesicles made up of phospholipid alone were not agglutinated by concanavalin A.

Results and Discussion

Reassembly of the concanavalin A receptor into large unilamellar vesicles

The concanavalin A receptor glycoprotein was isolated from erythrocyte membranes using the mild, readily dialyzable detergent dodecyltrimethylammonium bromide. Detergent concentrations were minimized and dithiothreitol was included to reduce the possibility of protein denaturation. 6–8% of the total membrane protein was eluted by α-methylmannoside and the product showed a single broad band on SDS-polyacrylamide gel electrophoresis, corresponding to Band 3 of the erythrocyte membrane [20].

We chose to reassemble the concanavalin A receptor into large unilamellar vesicles rather than multilamellar liposomes since they are much easier to control and characterize. They form a much more homogeneous population of structures and display the maximum number of receptor sites at their surface, which is obviously important for any studies involving ligand binding. In recent years, techniques have evolved for the production of large unilamellar vesicles of lipid alone, using dialyzable detergents such as octyl glucoside [24], and we have developed a similar method for reconstitution of membrane proteins. Previous methods for reassembly of the concanavalin A receptor gave rise to a large proportion of jumbled multilamellar structures [1]. In the present technique, several factors are crucial to the production of a uniform population of unilamellar vesicles. The type of dialysis membrane used has a marked effect on the phospholipid structures formed; use of ordinary dialysis tubing rather than 'fast' dialysis tubing gave rise to a much more heterogeneous assortment of lipid structures, some very large and amorphous. The inclusion of a negatively charged phospholipid (in this case PS) was found to greatly improve the homogeneity of the resulting vesicles. Use of egg PC alone resulted in preparations containing more vesicle aggregates as well as some large amorphous structures. Presumably PS exerts its effects via surface charge, leading to repulsion between vesicles and prevention of aggregation.

Phospholipid and protein recoveries were monitored over a wide range of lipid : protein ratios (from 15 : 1 to 2 : 1). In all cases, lipid : protein ratios measured for the harvested vesicles were within 3% of the initial ratio in detergent solution. Actual recoveries were always over 85% and usually more than 95% for vesicles with a higher protein content. The concanavalin A receptor remains tightly associated with the phospholipids during gel filtration on Sepharose 2B implying that effective reassembly has occurred. The vesicles elute as a single sharp peak at the void volume of the column, suggesting homogeneity in size. Phase-contrast microscopy showed only very small amounts of amorphous material in the vesicle preparations. Negative staining electron microscopy showed a reasonably homogeneous population of large vesicles (Figs. 1A and 1B) and thin-sectioning revealed that they were unilamellar (not shown). Dynamic laser light scattering was used to

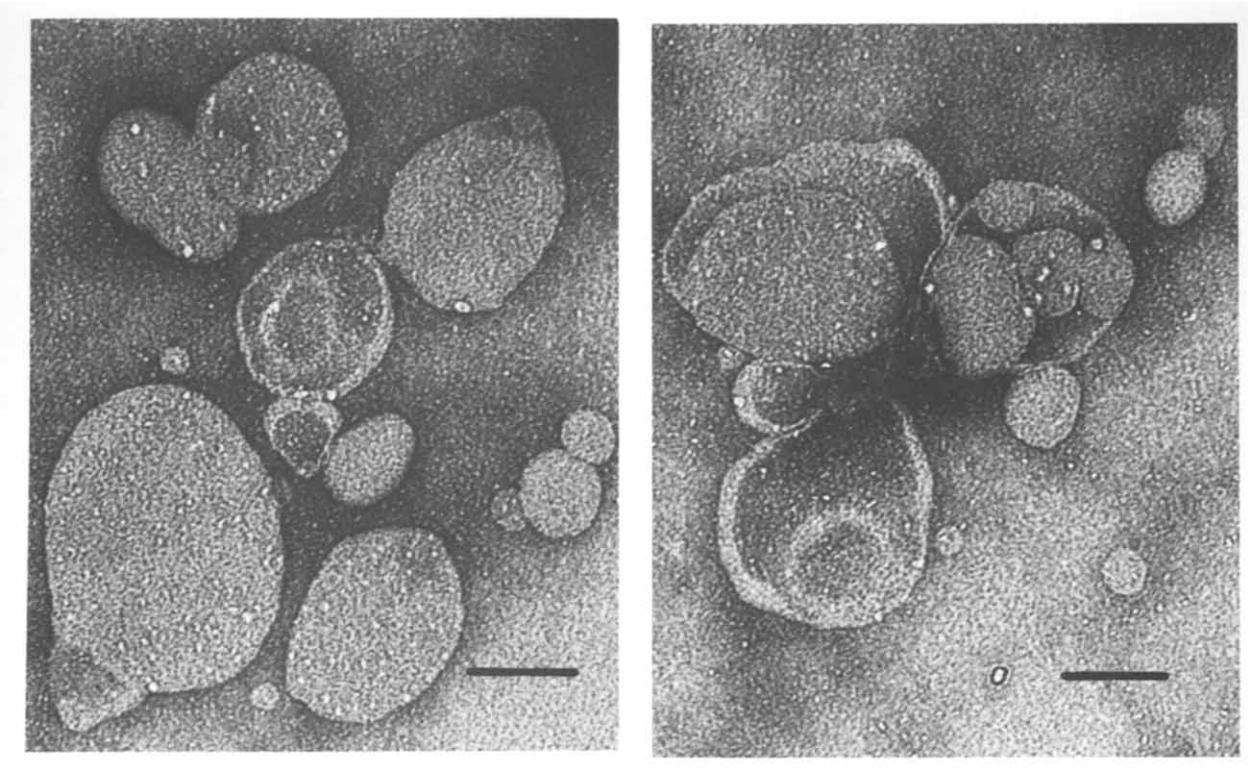

Fig. 1. Large unilamellar vesicles containing the concanavalin A receptor negatively stained with ammonium molybdate. Bar indicates 0.1 μm.

measure the mean hydrodynamic radius (Stoke's radius) of the vesicles at a variety of lipid : protein ratios. It was found that the mean vesicle diameter increased linearly from 0.27 μm at a lipid : protein ratio of 15 : 1 to 0.40 μm at a ratio of 4 : 1. Clearly, as the receptor content of the vesicles is increased, the vesicle population shifts to one of larger diameter. We have previously noted that the concanavalin A receptor has a dramatic effect on lipid bilayers [1], perhaps because several segments of the protein penetrate the hydrophobic region of the membrane. It thus seems likely that the observed dependence of vesicle diameter on receptor content is related to a very strong lipid-protein interaction.

Lectin binding studies

Lectin binding to unilamellar vesicles was quantitated by a rapid filtration assay using fiberglass filters. In the absence of poly(ethylene glycol), less than 50% of the vesicles (4 : 1 lipid : protein) were retained on the filters. Addition of poly(ethylene glycol) caused rapid aggregation of the vesicles such that more than 85% of the phospholipids were recovered on the filters after repeated washing. Poly(ethylene glycol) is a well-known membrane fusogen and has been used by others in similar receptor binding assays. It had no effect on the nonspecific binding of succinyl-concanavalin A to either filters or phospholipid vesicles. The amount of succinyl-concanavalin A bound to the reassembled vesicles increased linearly with the volume of vesicle suspension used in the assay. Nonspecific binding, measured in the presence of 0.1 M α-methylmannoside, represented less than 10% of the total succinyl-concanavalin A bound. Specific binding was calculated as the difference in binding in the absence and presence of the sugar inhibitor. Succinyl-concanavalin A showed a very small amount of 'specific' binding to phospholipid alone (less than 0.18 μg per 100 μg of phospholipid). To demon-

strate that binding of succinyl-concanavalin A was indeed protein-related, we measured the amount of lectin bound to vesicles of varying lipid : protein ratio, in the range 15 : 1 to 2 : 1. Fig. 2 shows that lectin binding is linearly proportional to the concanavalin A receptor content of the vesicles. The fact that this relationship is maintained over a very wide range of lipid : protein ratios implies that the vesicle preparations are indeed unilamellar; any multilamellar character would result in the internalization of receptors and a reduction in the amount of lectin bound.

The binding curve for interaction of succinyl-concanavalin A with the receptor appears Michaelis-Menten in character at moderate to high lectin concentrations (Fig. 3). However, Carver and coworkers [6] have demonstrated the importance of making binding measurements over a wide range of concentrations. We have thus measured binding of succinyl-concanavalin A to vesicles over a 10^5-fold range of lectin concentrations. At very low lectin concentrations (< 2 μg/ml) the binding curve (Fig. 4) shows evidence of positive cooperativity. A Scatchard plot of the binding data (Fig. 5) is concave downwards and clearly shows that binding of succinyl-concanavalin A is positively cooperative at low lectin concentrations. The slope

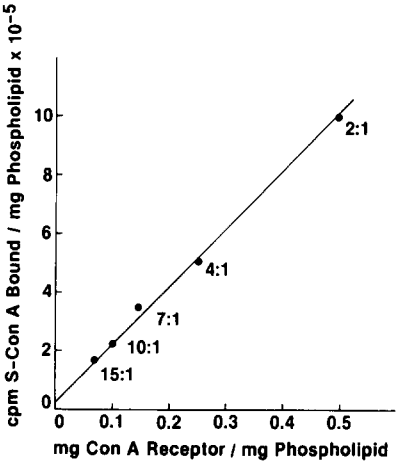

Fig. 2. Effect of concanavalin A receptor content of the vesicles on binding of ^{125}I-labelled succinyl-concanavalin A. Vesicles containing 15 μg of receptor protein and varying amounts of phospholipid were incubated with 50 μg/ml ^{125}I-labelled succinyl-concanavalin A at 22°C. Lipid : protein ratios are indicated for each vesicle preparation.

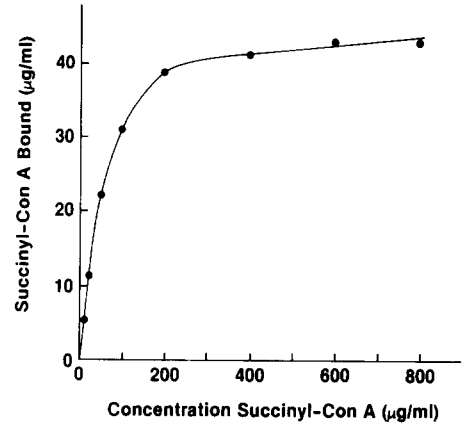

Fig. 3. Binding of ^{125}I-labelled succinyl-concanavalin A to lipid vesicles containing the concanavalin A receptor at moderate to high lectin concentrations. Vesicles containing 15 μg of receptor (4 : 1 lipid : protein ratio) were incubated with varying concentrations of ^{125}I-labelled succinyl-concanavalin A at 22°C.

of the linear portion of the Scatchard plot gives a value for the association constant of $2.13 \cdot 10^6$ M^{-1} demonstrating that the reassembled receptors form high-affinity binding sites for succinyl-concanavalin A. The maximal amount of succinyl-concanavalin A bound per 100 μl of assay mixture, from the Scatchard plot is 4.42 μg, or 80.4 pmol assuming a molecular weight of 55 000 for succinyl-concanavalin A. The amount of receptor protein in each 100 μl assay tube is 15 μg, corresponding to 158 pmol. We suggest that the con-

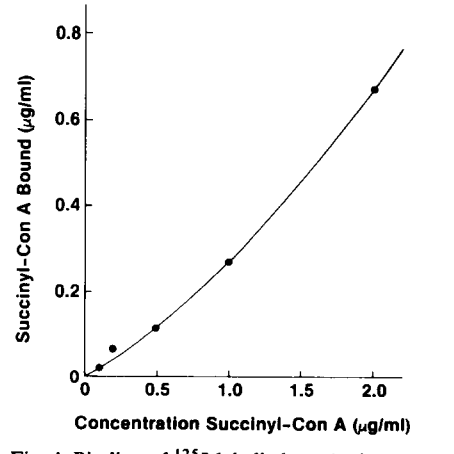

Fig. 4. Binding of ^{125}I-labelled succinyl-concanavalin A to lipid vesicles containing the concanavalin A receptor at very low lectin concentrations. Conditions as for Fig. 3.

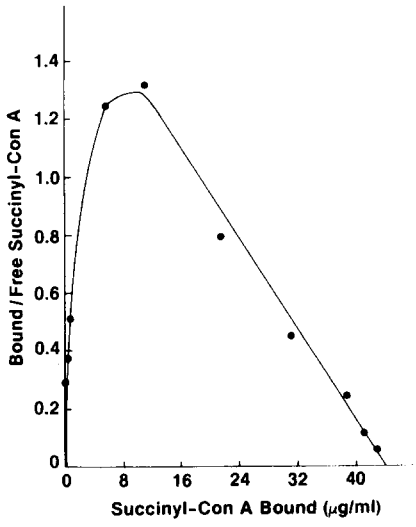

Fig. 5. Scatchard plot of the ^{125}I-labelled succinyl-concanavalin A binding data.

canavalin A receptor is reassembled symmetrically in the bilayer membrane, that is 50% faces inwards and is unavailable for lectin binding. We have recently used a detergent-dialysis method to reconstitute lentil lectin receptor glycoproteins from lymphocyte plasma membrane and have shown that the enzyme 5'-nucleotidase is also reassembled in a symmetric fashion (Campbell, C.D. and Sharom, F.J., unpublished results). Asymmetric orientation of reconstituted glycoproteins has so far been observed in very small sonicated vesicles [26] where steric hindrance of bulky sugar headgroups would be expected to be a dominant factor. There should be minimal steric effects associated with vesicles of the size used in this study. If symmetrical reassembly is assumed, then this implies that essentially all the concanavalin A receptor molecules are functional and bind lectin at the vesicle surface.

We have measured binding parameters for interaction of concanavalin A with intact human erythrocytes using two different techniques and find approximately $2.7 \cdot 10^5$ binding sites per cell, with an association constant of $6 \cdot 10^6$ M^{-1}. This agrees well with previous values [25,29]. Succinyl-concanavalin A has a very low association constant for binding to intact erythrocytes ($5 \cdot 10^4$ M^{-1}, see Ref. 29) presumably because both the lectin and the cell have a high surface negative charge. Positive cooperativity (as shown by Scatchard plots which are concave downwards) was observed at concanavalin A concentrations below 2 μg/ml. This has also been observed by others and has been proposed to be due to association of concanavalin A subunits with a subsequent change in binding affinity, or to a lectin-induced reorientation of binding sites [25,29]. Interestingly, the incubation medium used for binding studies seems to affect the characteristics of the cooperativity quite dramatically [25]. Since positive cooperativity is seen in our system, where dimer-tetramer equilibrium is presumably not a complication, we suggest that the cooperativity is due to reorientation of the receptor sites induced by the lectin.

Other studies on lectin binding to lipid-protein bilayers, using glycophorin as a receptor, have found that the presence of bovine serum albumin or dextran as a surface coat was obligatory for high-affinity binding to be observed [9,10]. In our studies, this surface coat was not found to be necessary to obtain high-affinity binding. It is possible that these differences stem from the two distinct approaches used for measuring binding. The rapid filtration assay we have used allows very little dissociation of bound lectin during the course of vesicle harvesting. Repetitive centrifugations on the other hand, allow more opportunity for dissociation of lectin from the vesicle surface. It seems likely that the effect of these 'surface coats' may be to dramatically reduce the rate of dissociation of the lectin from its receptor.

Direct measurement of concanavalin A binding to our reassembled vesicles is complicated by nonspecific binding of the lectin to both phospholipids and receptor. Lectin binding can, however, be measured less directly by inhibition studies. Concanavalin A inhibits binding of ^{125}I-labelled succinyl-concanavalin A to vesicles containing the receptor in a competitive fashion as shown in Fig. 6. The inhibition constant for concanavalin A binding to the receptor was estimated at $4.8 \cdot 10^6$ M^{-1}, which is almost equal to the association constant measured for the intact cell.

These experiments suggest that properties such as cooperativity, normally associated with intact cells, can be duplicated in lipid bilayers using

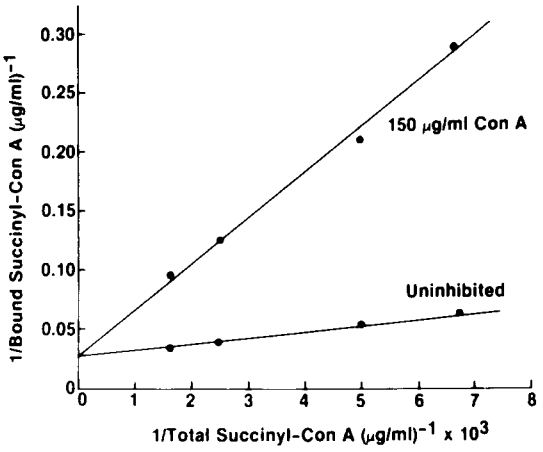

Fig. 6. Lineweaver-Burk plot of binding data showing competitive inhibition of ^{125}I-labelled succinyl-concanavalin A binding to vesicles by concanavalin A. Lectin binding to vesicles containing 15 μg of receptor was carried out in the absence and presence of 150 μg/ml concanavalin A. Succinyl-concanavalin A concentrations were chosen from the linear portion of the Scatchard plot.

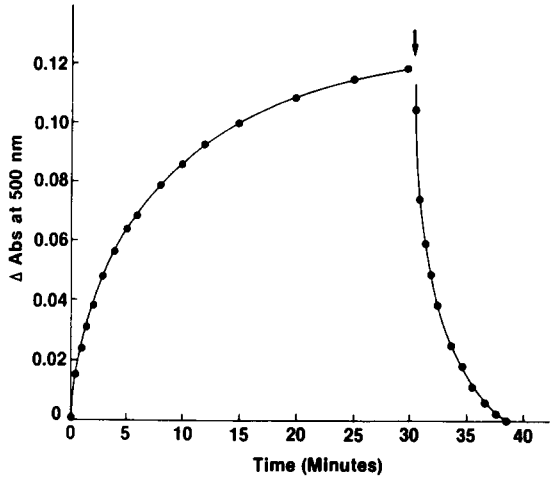

Fig. 7. Time course of the agglutination of receptor-bearing vesicles by concanavalin A. Vesicles of 4:1 lipid:protein at 2 mg/ml phospholipid were agglutinated by 250 μg/ml lectin in phosphate-buffered saline. Turbidity changes were monitored by measuring the absorbance at 500 nm. The suspension was made 0.1 M in α-methylmannoside as indicated by the arrow.

purified receptors. It also provides a system whereby factors such as lipid composition, fluidity and domain structure, and the presence of other surface molecules may be tightly controlled, enabling researchers to investigate if and how they modulate these binding phenomena.

Vesicle agglutination

Previous work on lipid bilayers containing glycoprotein receptors showed the value of macroscopic agglutination as a tool for probing receptor behaviour [1]. The earlier studies used fairly large multilamellar structures (which were visible to the naked eye after agglutination) and were rather qualitative in nature. We have attempted to quantitate the agglutination process to the point where the influence of several important variables can be tested. When concanavalin A was added to large unilamellar vesicles containing the receptor, the turbidity of the suspension increased with time as shown in Fig. 7. This increase in turbidity is presumably due to the formation of vesicle clusters which resemble cell agglutination. That this process is due to agglutination and not fusion is shown by the fact that the addition of α-methylmannoside causes a very rapid and complete reversal of the process (see arrow, Fig. 7). A first-order plot of the kinetic data (Fig. 8) shows that the agglutination is biphasic. There is an initial rapid phase, followed by a pseudo-first-order process giving rise to a linear region. This bimodal type of behaviour has been noted by other researchers using glycolipid receptors in lipid bilayers [30] and its origin is unknown. Rate constants for the two phases may be measured directly from the kinetic plot.

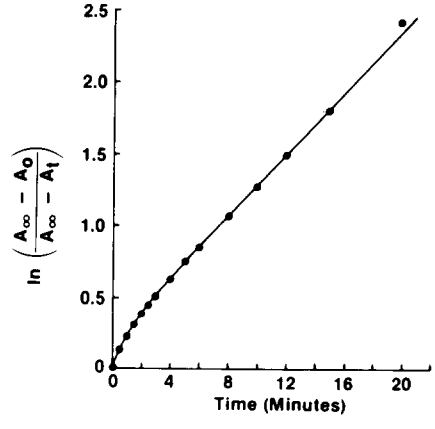

Fig. 8. First order kinetic plot of concanavalin A-induced vesicle agglutination.

Vesicles containing the concanavalin A receptor are non-agglutinable by succinyl-concanavalin A, despite the fact that it binds with high affinity, suggesting that multivalent character is needed to form stable vesicle-vesicle crosslinks. Lymphocytes are similarly agglutinable by concanavalin A but not by succinyl-concanavalin A, although the affinity of the two lectins for cell surface receptors is very similar [31]. Receptor-bearing vesicles are also agglutinable by lentil lectin and pea lectin. This is not unexpected since these lectins have the same sugar specificity as concanavalin A. RCA 120 also caused dramatic agglutination, while monovalent RCA 60 did not. These results suggest that the concanavalin A receptor is also the main erythrocyte receptor for RCA 120. Previous work in this area, although not clear-cut, showed that RCA 120 affinity columns resulted in a Band 3-enriched protein fraction [32]. Carbohydrate sequencing of the sugar headgroup of Band 3 has revealed that it has several terminal galactose residues which would be available for binding RCA 120 [33].

Cell agglutination by lectins has been used as an important probe of the architecture of normal and tumour cell surfaces, although very little is known about the factors controlling cell agglutinability. The concanavalin A receptor is agglutinable by several lectins with different specificities and the kinetics of agglutination can be easily quantitated. This system is thus ideal as a model for agglutination studies where membrane fluidity, receptor density and topology may be controlled.

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