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FUNCTIONAL REASSEMBLY OF LYMPHOCYTE LENTIL LECTIN RECEPTOR GLYCOPROTEINS INTO LIPID BILAYER VESICLES

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Plasma membrane vesicles purified from pig mesenteric lymph nodes were solubilized using the mild, readily-dialyzable detergent dodecyltrimethylammonium bromide, and lentil lectin receptor glycoproteins were isolated by affinity chromatography. The receptor fraction showed 12 major bands on SDS-polyacrylamide gel electrophoresis representing 8–9% of the membrane protein. 5'-Nucleotidase (EC 3.1.3.5) was very effectively solubilized by the detergent and was recovered in high yield in the receptor fraction. Receptor glycoproteins were reassembled into large unilamellar vesicles of phosphatidylcholine/phosphatidylserine (mean diameter 0.235 μm) using a detergent dialysis technique. Sixty to seventy percent of the glycoprotein and most of the 5'-nucleotidase activity is associated with the phospholipid vesicles. 5'-Nucleotidase is reassembled in a symmetrical fashion and is inhibited by binding of concanavalin A, lentil lectin and pea lectin but not by succinyl-concanavalin A. Measured values for K_i and maximal inhibition are similar to those observed with intact plasma membrane vesicles. Hemagglutination inhibition studies showed that the reassembled receptors effectively bind lentil lectin. Thus lymphocyte membrane glycoproteins reassembled into phospholipid vesicles seem to retain at least part of their function in that enzyme activities such as 5'-nucleotidase remain intact and the receptors effectively bind lentil lectin.

Introduction

The activation of B and T lymphocytes by polyclonal mitogens such as plant lectins and lipopolysaccharide is a classic example of a transmembrane signalling event, and is used by immunologists as a model for lymphocyte activation by antigens in vivo. The primary event in the activation process is the binding of the mitogen to its specific receptor on the cell surface. This binding is followed by a large number of complex biochemical events both at the membrane level and inside the cell [1] eventually leading to cell proliferation. Binding of mitogenic lectins to receptor glycoproteins at the lymphocyte cell surface has been the focus of much study (see, for example, Refs. 2–4) and it seems likely that the characteristics of lectin binding, particularly positive cooperativity, are tightly linked to the activation process [5]. However, given the complexity of the lymphocyte membrane, only limited information on glycoprotein organization and dynamics can be obtained from binding studies on the intact cell or plasma membrane.

In the case of the human erythrocyte membrane, studying lectin binding to receptor proteins

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Abbreviations: DTAB, dodecyltrimethylammonium bromide; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; PC, phosphatidylcholine; PS, phosphatidylserine; SDS, sodium dodecyl sulfate.

reassembled into lipid bilayers has proved to be a very fruitful approach in obtaining information on glycoprotein behaviour [6–9]. Recently, methods have been developed for the isolation of relatively large quantities of lymphocyte membrane glycoproteins [10–12] thus making possible the reassembly of these receptors into lipid bilayers. This study describes the isolation of milligram quantitites of lentil lectin receptor glycoproteins from pig lymphocytes using the detergent DTAB and their reassembly into large unilamellar phospholipid vesicles by a detergent dialysis technique. The reassembled glycoproteins retain certain enzymatic activities and their capacity to bind lectins, thus providing a useful tool for further studies on the biochemical and immunological properties of the membrane proteins involved in lymphocyte activation.

Materials and Methods

Egg PC was obtained from Sigma and was further purified by column chromatography on Bio-Sil A (Bio-Rad). Bovine brain PS was purchased from Sigma and used without further purification. Both phospholipids were pure as judged by thin-layer chromatography on silica gel plates. Di[1-^{14}C]palmitoyl-L-α-phosphatidylcholine (80–120 mCi/mmol) and [2-^{3}H]adenosine 5′-monophosphate (10–20 mCi/mmol) were obtained from Amersham. Concanavalin A and lentil lectin were obtained from Sigma, succinyl-concanavalin A from Vector Laboratories, and pea lectin from Sigma and Calbiochem.

Negative staining of lipid vesicles using 1% ammonium molybdate was carried out by the method of Munn [13]. Grids were examined in a Philips 300 electron microscope.

Dynamic laser light scattering measurements were carried out using 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 10–20 μg protein) was diluted to 2 ml with phosphate-buffered saline pH 7.4 and scattering was recorded at an angle of 90° in a thermally jacketed chamber at 20°C. Analysis of the resulting autocorrelation function was carried out using the method of cumulants.

Protein was assayed by the method of Peterson [14] using bovine serum albumin (Sigma, crystallized and lyophilized) as a standard. SDS-polyacrylamide gel electrophoresis was carried out in slabs according to Laemmli [15]. Gels were fixed and stained with Coomassie brilliant blue.

Isolation of lentil lectin receptor glycoproteins

Plasma membrane vesicles were prepared from pig mesenteric lymph node tissue using the tissue-press method of Snary et al. [16]. Approximately 100 g of tissue was processed during one preparation and the resulting plasma membrane vesicles were stored frozen in aliquots at −70°C. Plasma membrane samples used for receptor isolation were thawed only once. Plasma membrane vesicles (75–85 mg protein) were solublized in affinity column buffer (10 mM Hepes in isotonic saline pH 7.4 with 0.7 mM Ca^{2+}, Mg^{2+}, Mn^{2+}, 0.2 mM dithiothreitol and 0.02% (w/v) sodium azide) containing 100 mM DTAB (Sigma) at a protein concentration of about 2 mg/ml. The mixture was stirred on ice for 1 h, centrifuged at 100000 × *g* for 30 min at 4°C and the supernatant used for affinity chromatography. In general, 35–45 ml of supernatant was run onto a lentil lectin-Sepharose 4B column (Pharmacia, 25 ml bed volume) at 4°C and washed through with column buffer containing 25 mM DTAB. Bound protein was subsequently eluted with column buffer containing 25 mM DTAB and 0.2 M α-methyl-D-mannoside (Sigma). Fractions were read at 280 nm and those containing eluted receptors were pooled and concentrated at 0°C using a pressure ultrafiltration cell with an Amicon PM 10 membrane. Receptor glycoprotein was stored frozen in small aliquots at −20°C.

Incorporation of receptors into lipid vesicles

Five mg of 4:1 egg PC:PS (w/v) in chloroform-ethanol was evaporated to dryness under a stream of nitrogen and then pumped under vacuum for 30 min. [^{14}C]PC (usually 0.125 μCi) was often included as a tracer to enable the determination of lipid recovery. The phospholipids were then dissolved in 8 ml of 100 mM DTAB in 10 mM Hepes-buffered saline, pH 7.4. Lentil lectin receptors (usually 1 mg protein) were added and the mixture incubated at 4°C for 30 min to allow

thorough mixing. The lipid/protein mixture was then dialyzed against four changes of 10 mM Hepes-buffered saline pH 7.4 at 4°C over 48 h. Following dialysis, lipid vesicles were harvested by centrifugation at 120000 × *g* for 2 h at 4°C and resuspended in 10 mM Hepes-buffered saline, pH 7.4, at a lipid concentration of approx. 4–5 mg/ml. Reassembled lipid vesicles were stored frozen in small aliquots at −20°C.

Assay for 5'-nucleotidase

Assays for 5'-nucleotidase at various stages of the lymphocyte plasma membrane isolation and the lentil lectin receptor purification were carried out by the method of Michell et al. [17]. Released inorganic phosphate was quantitated as described by Kates [18]. 5'-Nucleotidase activity in lipid vesicles containing reassembled receptors was quantitated using the much more sensitive radiometric assay of Sharom and Mellors [19]. In studies on inhibition of 5'-nucleotidase by lectins, the lipid vesicles were preincubated with the appropriate lectin concentration for 30 min before the enzyme assay was carried out. Enzyme activity was expressed as percent control and the concentration of lectin which produced half-maximal inhibition was defined as the K_i for that lectin.

Gel filtration chromatography

Lipid vesicles (100 μl) containing lentil lectin receptors (8.83 mg/ml lipid, 1.03 mg/ml protein) was applied to a small column of Sepharose 2B (Pharmacia) equilibrated in 10 mM Hepes-buffered saline, pH 7.4. The column was eluted with the same buffer and 325-μl fractions were collected. Samples from each fraction were assayed for protein and [^{14}C]PC was quantitated by liquid scintillation counting.

Hemagglutination inhibition

The effectiveness of lipid vesicles containing purified receptor glycoproteins at binding lentil lectin was tested by their ability to inhibit lentil lectin-mediated hemagglutination of human erythrocytes. Freshly outdated red cells obtained from a blood bank were washed four times with phosphate-buffered saline, pH 7.4, at 1000 × *g* for 10 min at 4°C. Packed washed cells were resuspended at a concentration of 1% (v/v) in phosphate-buffered saline containing 3.5 mg/ml bovine serum albumin. Several sets of serial 2-fold dilutions of lentil lectin were made into 100 μl of cold phosphate-buffered saline (containing bovine serum albumin) in a disposable microtitre plate. The first row served as a control, while to following rows was added 10 μl per well of an appropriate lipid vesicle suspension (either with or without lymphocyte receptor glycoproteins) in 10 mM Hepes-buffered saline, pH 7.4. A 100-μl aliquot of the red blood cell suspension was added to each well and the plate incubated for several hours at 4°C. Endpoints were read as the lowest concentration of lectin showing agglutination of red blood cells.

Results and discussion

Isolation of lentil lectin receptors

The detergent DTAB was chosen as the solublizing agent for lymphocyte membrane glycoproteins because it is mild, a very effective solublizer and easily removed by dialysis. These properties make it a valuable detergent for use in reconstitution studies where recovery of protein function is desirable [7,20,21]. Receptor glycoproteins in general suffer from the drawback that functionality is difficult to assess. The lymphocyte membrane ectoenzyme 5'-nucleotidase is known to be a lentil lectin receptor glycoprotein [22] and we have chosen to monitor the activity of this enzyme during both purification and reconstitution. Preliminary experiments showed that DTAB concentrations of up to 100 mM had no effect on the 5'-nucleotidase activity of the intact plasma membrane and enzyme activity was reduced by only 20% at a detergent concentration of 200 mM. It thus seems likely that DTAB itself will have a minimal effect on the native structure and function of lymphocyte membrane components. That DTAB is also an excellent solublizing agent for these membrane components is shown in Fig. 1. 100 mM DTAB solublized about 92% of the 5'-nucleotidase activity of the plasma membrane after treatment for 1 h on ice. 100 mM DTAB was thus chosen as the optimum detergent concentration compatible with both maximal solublization and minimal disruption of protein structure and function. Treatment of plasma membrane vesicles with 100 mM DTAB

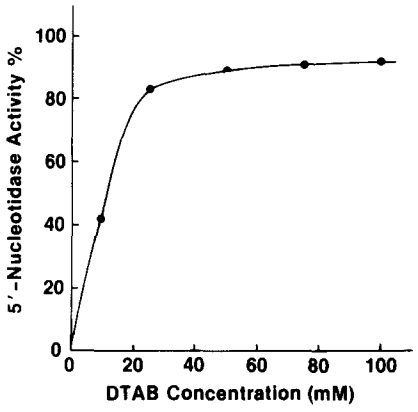


Fig. 1. Solublization of 5'-nucleotidase from lymphocyte plasma membrane vesicles by increasing concentrations of DTAB. Plasma membrane was incubated for 1 h on ice with various DTAB concentrations at a protein concentration of 2 mg/ml. After centrifugation at 100000 × *g* for 30 min at 4°C the supernatant containing solubilized membrane components was assayed for 5'-nucleotidase activity.

resulted in solublization of 60–70% of the total membrane protein. This is similar to that observed using deoxycholate as the solublizing detergent [11] and probably represents more than 85% of the membrane glycoprotein judging from the final recovery of receptors from the affinity column. The detergent-insoluble fraction probably represents a membrane-associated cytoskeletal network [23].

Fig. 2 shows a typical elution profile for affinity chromatography of the solublized membrane protein on lentil lectin-Sepharose 4B. Approximately 8% of the solublized protein loaded onto the column was eluted with 0.2 M α-methylmannoside (range 6–10%). This represents virtually all the bound glycoprotein fraction since α-methylmannoside concentrations up to 1.0 M did not elute any further protein. The 5'-nucleotidase specific activity of the eluted receptors (about 48 μmol 5'-AMP hydrolysed/h per mg protein) was enriched more than 7-times over that of the plasma membrane and about 77-times with respect to lymphocyte homogenates. This data agrees favourably with values previously reported using sodium deoxycholate as a solublizing detergent [22].

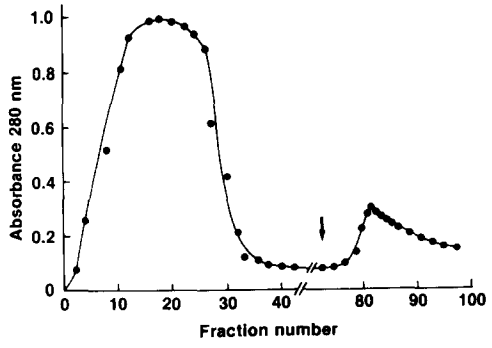


Fig. 2. Elution profile for lentil lectin affinity chromatography of lymphocyte plasma membrane solublized in 100 mM DTAB. Arrow indicates the point at which elution with 0.2 M α-methylmannoside was started.

SDS-polyacrylamide gel electrophoresis of the lentil lectin receptor fraction followed by Coomassie blue staining revealed a complex mixture of proteins with 12 major bands and several minor ones (see Fig. 3). The overall pattern seems similar to that reported using deoxycholate-solublized plasma membranes [11]. The very high molecular weight glycoproteins appear somewhat under-represented but this probably reflects differing sensitivity to the detection methods used (Coomassie blue staining vs. ^{125}I-labelling and autoradiography), since these proteins also do not stain well with Coomassie blue in the intact plasma membrane. Several of these proteins have recently been identified [11] including the major histocompatibility (SLA) antigen, the α and β subunits of the Ia-like antigens, β_2-microglobulin and membrane-bound immunoglobulins. It should be noted that treatment with *N*-ethylmaleimide or iodoacetamide before and during detergent solublization has frequently been used in studies on lymphocyte membrane proteins to prevent disulfide bond formation [11,12]. Since the objective of this research was to maintain the glycoproteins in their functional native state, the use of alkylating agents has been deliberately avoided. The presence of a small amount of lentil lectin (heavy chain 18 kDa, light chain 8 kDa) was noted in the eluted receptors. This phenomenon was recently observed when lentil lectin-Sepharose was used in conjunction with ionic detergents [24]. From the intensities of the stained bands on SDS gels, we estimate that lentil lectin accounts for less than 7% of the total protein in the eluted receptor fraction.

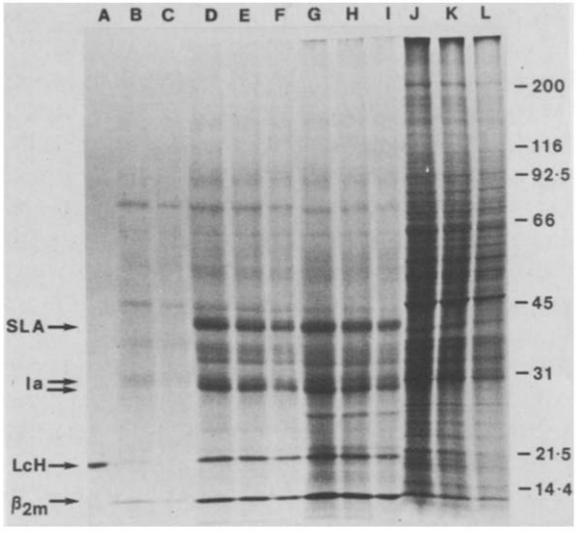
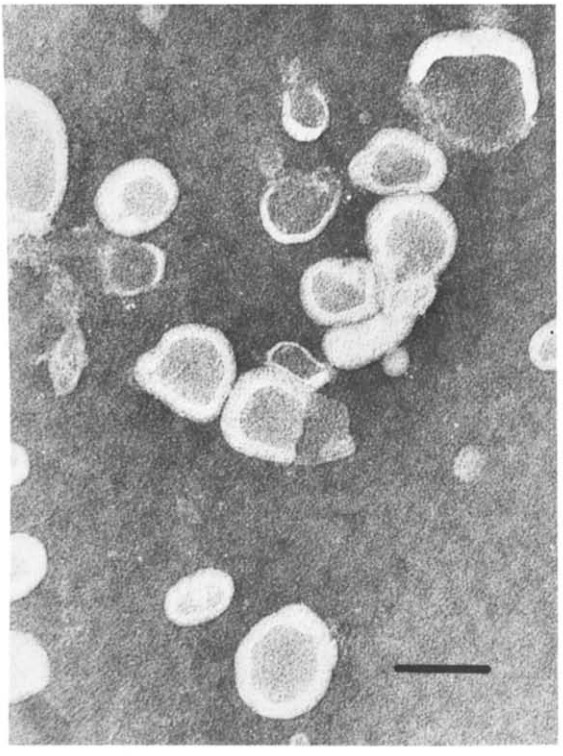


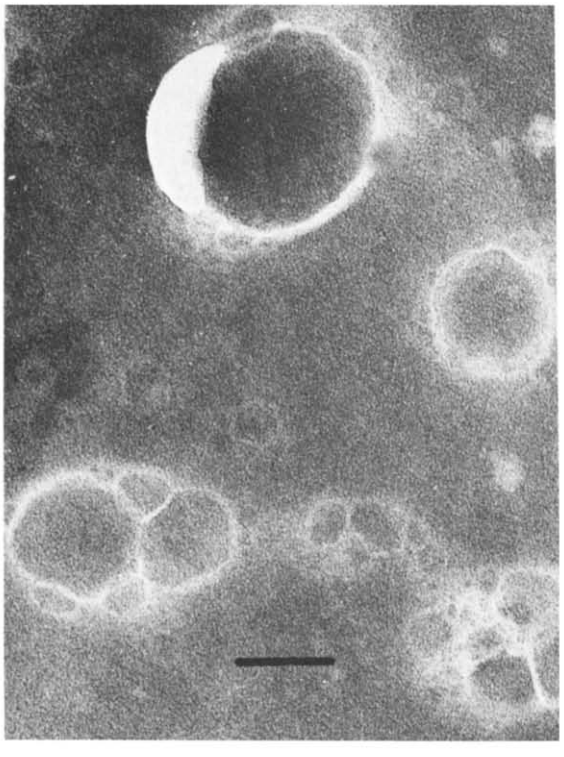
Fig. 3. SDS-polyacrylamide gel electrophoresis of lentil lectin (track A), lentil lectin receptors from vesicle supernatant (tracks B, C), lentil lectin receptors prepared from plasma membrane treated with *N*-ethylmaleimide (see Ref. 11, tracks D–F), lentil lectin receptors from untreated plasma membrane (tracks G–I) and plasma membrane (tracks J–L). Samples were run on a 7–13% polyacrylamide gradient gel under reducing conditions. The positions of molecular weight markers are indicated on the right. Bands corresponding to the 18 kDa subunit of lentil lectin (LcH), β_2-microglobulin (β_{2m}), the Ia α (upper arrow) and β (lower arrow) polypeptides and the SLA α chain have been labelled. Bands were assigned by comparison with SDS-polyacrylamide gel patterns for receptors isolated using sodium deoxycholate as the solublizing detergent (see Ref. 11).

Reassembly of receptor glycoproteins into large unilamellar vesicles

Techniques have been developed recently in our laboratory for reassembly of integral membrane proteins into large unilamellar phospholipid vesicles by detergent dialysis. Initial studies using the human erythrocyte concanavalin A receptor (a 95 kDa transmembrane glycoprotein) showed that large unilamellar vesicles with diameters in the range 0.27–0.40 μm could be produced over a wide range of lipid : protein ratios from 15 : 1 to 2 : 1 [25]. These vesicles are ideal for use in studies on lectin binding since they display the maximum number of receptor sites at their surface. Applying the same approach to lymphocyte glycoproteins should provide us with a means for studying lectin binding to these receptors in model systems. These have the advantage that certain characteristics of the system (for example lipid composition, fluidity and domain structure, lipid : protein ratio, presence of other membrane components) may be controlled to a much greater degree than in the intact membrane.

Lymphocyte lentil lectin receptors have been incorporated into vesicles composed of 4 : 1 (w/w) egg PC : PS with an initial lipid : protein ratio of 5 : 1. About 60–70% of the protein was found to be associated with the vesicles on harvesting, together with 90–95% of the phospholipid. Final lipid : protein ratios for the harvested vesicles were in the range 6.4–7.9. The fraction of protein incorporated was increased only slightly by providing a larger amount of matrix phospholipid. Reconstitutions at lipid : protein ratios of 10 : 1 and 15 : 1 both produced 70% incorporation of protein, at the high end of the range, but not a dramatic increase. It is possible that the use of a lipid mixture more closely resembling that of the intact plasma membrane might increase the fraction of protein which is reassembled. The incorporated proteins remaining in the supernatant probably exist as fairly small hydrophobic aggregates. Concentration and electrophoresis of the supernatant proteins showed that certain bands, particularly those at 12 kDa (β_2-microglobulin), 19 kDa (lentil lectin), 46 and 73 kDa, were more abundantly represented than in the original protein mixture. This differential reincorporation probably reflects the molecular properties of the individual glycoproteins. That the reassembly process is somewhat selective is further illustrated by measurement of the 5′-nucleotidase activity of the vesicles. Vesicles showed enzyme specific activities of around 37 μmol 5′-AMP hydrolysed/h per mg protein, representing 50% of the total initial 5′-nucleotidase activity. Since these vesicles are sealed and normally impermeable to the substrate, only enzyme molecules oriented with the active site facing outwards will be detected in the assay. Addition of Triton X-100 to the vesicles to allow penetration of the substrate to any inward-facing enzyme should result in an increase in measured activity. The magnitude of this increase will be dependent on the symmetry of reconstitution i.e. what fraction of 5′-nucleotidase molecules face inwards. When 1% Triton X-100 was added to several ves-





icle preparations reassembled independently using different batches of lentil lectin receptors, increases in measured 5′-nucleotidase activity in the range 195–219% were observed. This reproducible doubling of enzyme activity indicates that the glycoprotein is reassembled symmetrically, that is, 50% faces inwards and 50% faces outwards, and the total activity (inside and out) measured in the vesicles represents essentially quantitative recovery of the enzyme. These data also confirm the unilamellar nature of the vesicles; if they were multilamellar, considerably more than 50% of the enzyme activity would be cryptic in the intact vesicles. 5′-Nucleotidase thus seems to be reincorporated preferentially into phospholipid vesicles and the same may also be true of other lentil lectin receptors although to a lesser extent.

Negative-staining electron microscopy showed a reasonably homogeneous population of vesicles (Figs. 4A and 4B). Because of the problems associated with size analysis of phospholipid vesicles by electron microscopy, a laser light scattering technique was used to obtain a mean vesicle diameter of 0.235 μm. Gel filtration chromatography of the reassembled vesicles on Sepharose 2B (Fig. 5) showed that the lentil lectin receptors remain tightly associated with the phospholipids, 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 preparation. The amount of [^{3}H]5′-AMP hydrolysed in the radiometric assay for 5′-nucleotidase was linear with the volume of vesicles added in the range 1–10 μg of protein (Fig. 6). Vesicles composed of phospholipid alone were not able to hydrolyse 5′-AMP.

Interaction of lectins with reassembled receptors

Preparations of lipid vesicles containing purified lentil lectin receptors were capable of effectively inhibiting lentil lectin-mediated agglutination of human red blood cells (see Table I). Hu-

Fig. 4. (A and B) Large unilamellar vesicles containing lentil lectin receptors (lipid:protein ratio about 7:1) negatively stained with ammonium molybdate. Bar indicates 0.1 μm.

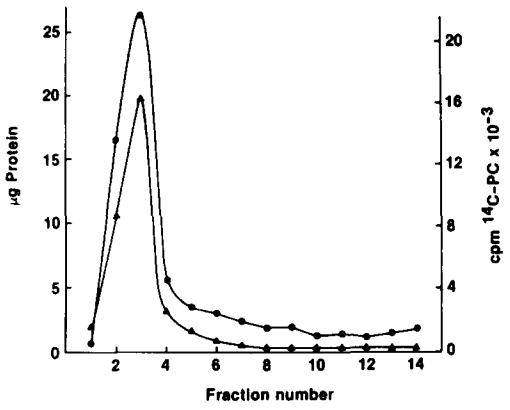


Fig. 5. Sepharose 2B gel filtration chromatography of large unilamellar vesicles containing lentil lectin receptors. 325-μl fractions were collected and assayed for protein (●——●) and [^{14}C]PC (▲——▲).

man erythrocytes are normally agglutinable by lentil lectin at a concentration of 6.25 μg/ml, whereas in the presence of vesicles containing receptors 25 μg/ml of the lectin was required to cause agglutination. From these numbers it can be estimated that 4 μg of reassembled receptors is capable of binding 1.25–3.75 μg of lentil lectin. Since half of the glycoproteins face inwards and are unavailable for lectin binding, it appears that a large fraction of the receptors at the vesicle surface can bind lentil lectin. Lymphocyte lentil lectin receptors thus retain their lectin binding activity when reassembled into phospholipid vesicles.

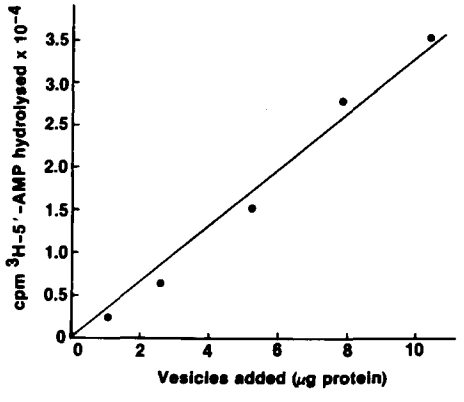


Fig. 6. Effect of vesicle concentration on the hydrolysis of [^{3}H]5'-AMP. Vesicles containing between 1 and 10 μg of lentil lectin receptor protein (lipid:protein ratio 7.7:1) were incubated for 30 min in the 5'-nucleotidase assay mixture as described in Materials and Methods.

TABLE I

INHIBITION OF LENTIL LECTIN-MEDIATED HEMAGGLUTINATION BY LIPID VESICLES CONTAINING LENTIL LECTIN RECEPTORS

Serial 2-fold dilutions of lentil lectin were made in microtitre plates. To selected rows was added 10 μl per well of either (a) vesicles of phospholipid alone, prepared by vortexing dried lipids in buffer, or (b) vesicles containing reassembled lentil lectin receptors (protein concentration = 0.4 mg/ml). 100 μl of a 1% red blood cell suspension was then added to each well. Endpoints were read as the lowest concentration of lectin showing agglutination.

Sample added	Inhibition	Endpoint (μg/ml)
None	–	6.25
Phospholipid [a] alone	–	6.25
Phospholipid [a]-receptors	+	25

[a] Phospholipid is 4:1 (w/w) egg PC:PS.

The use of lectins as a tool for studying the cell surface is complicated by the fact that multiple receptors of differing properties are often involved, making interpretation of results difficult. 5'-Nucleotidase is known to be inhibited by binding of concanavalin A and other lectins [26,27] and thus provides a means of studying lectin interactions with a single receptor species. 5'-Nucleotidase in lipid vesicles is effectively inhibited by binding of the glucose/mannose-specific lectins concanavalin A, lentil lectin and pea lectin. Fig. 7 shows a typical inhibition curve measured for concanavalin A. The K_i values for 50% enzyme inhibition by these lectins are slightly higher than those observed for plasma membrane vesicles under the same conditions (see Table II). The maximum inhibition obtainable by lectin binding to vesicles was generally slightly lower than that seen in plasma membrane (see Table II) but the data confirms that only outside-facing enzyme is being measured and this has the potential to be almost completely inhibited by lectin binding. The behaviour of 5'-nucleotidase in lipid vesicles is very similar to that seen in plasma membrane, suggesting that the enzyme is maintained very close to its native state.

Divalent succinyl-concanavalin A is not an ef-

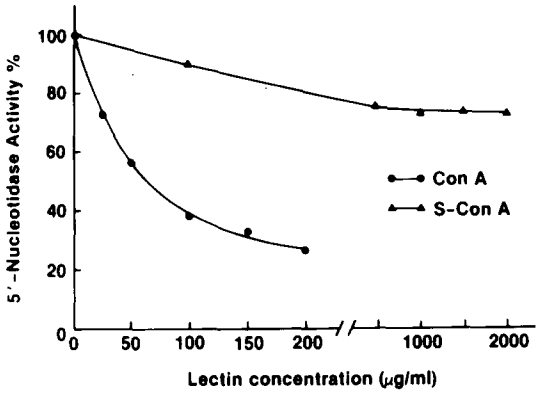


Fig. 7. Inhibition of 5'-nucleotidase activity in vesicles containing reassembled lentil lectin receptors by the lectins concanavalin A (con A) and succinyl-concanavalin A (S-con A). Enzyme activity is expressed as percent control.

fective inhibitor of 5'-nucleotidase in either the plasma membrane or vesicles. This may be due to its divalent character; multivalent crosslinking may be necessary for enzyme inhibition. This seems unlikely, however, since treatment of both plasma membrane and vesicles with anti-concanavalin A antibody after succinyl-concanavalin A binding did not restore inhibition. Similar observations have been made by Dobson and Mellors [28] in a study of lymphocyte acyltransferase (EC 2.3.1.23) which is also inhibited by concanavalin A binding, and they have suggested that the hydrophobic binding tendencies shown by concanavalin A (but not by succinyl-concanavalin A) may play a role in the inhibition. Alternatively, the divalent derivative may have a much lower affinity constant for binding to 5'-nucleotidase than the parent lectin. The two lectins seem to bind to the lymphocyte surface with similar overall affinities, but this may not necessarily be true of individual receptor species.

TABLE II

EFFECT OF LECTINS ON 5'-NUCLEOTIDASE IN LYMPHOCYTE PLASMA MEMBRANE AND IN LIPID VESICLES CONTAINING LENTIL LECTIN RECEPTORS

Plasma membrane vesicles (PM) or large unilamellar vesicles containing receptors (LUV) were preincubated with lectin for 30 min before being assayed for 5'-nucleotidase at 37°C by the radiometric assay described in Materials and Methods. The concentration of lectin producing 50% inhibition was defined as the K_i for that lectin.

Lectin	Sample	K_i (μg/ml)	Maximal inhibition (%)
Concanavalin A	PM	65	93
	LUV	63	77
Succinyl-concanavalin A	PM	> 2000	34
	LUV	> 2000	28
Lentil	PM	25	90
	LUV	41	70
Pea	PM	56	77
	LUV	100	60

Lymphocyte 5'-nucleotidase is somewhat unique in that it is an integral membrane glycoprotein with both enzymatic and lectin binding activities. Carraway and co-workers have already pointed out [27] that studies on purified or partially purified 5'-nucleotidase in model membrane systems would provide valuable information on lectin-glycoprotein interactions. The reconstitution procedure we have described produces functional enzyme in high yield and provides a useful starting point for detailed study of 5'-nucleotidase in lipid bilayers. This would allow investigation of the role of phospholipids in both enzyme activity and lectin binding and may clarify the exact mechanism of enzyme inhibition by lectins and the possible involvement of other receptor species [26].

Cytolytic T lymphocytes specific for allogeneic cells recognize certain cell surface glycoproteins on these cells. These antigens are present in the lentil lectin receptor fraction and it has recently been demonstrated that reconstituted membranes containing these antigens are very useful for studying antigen recognition by cytotoxic T lymphocytes at the molecular level [29,30]. The model systems used in our study have the advantages of being both well-characterized and unilamellar and should also prove useful in investigations into lymphocyte recognition.

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