sulfur centers. It will also use NADPH as a substrate, however, with a lower V_{max}.

Puget & Michelson (1972) demonstrated the presence of soluble NADH:FMN oxidoreductases from several different bacteria; however, they have not been purified or well characterized. The role of these enzymes is unknown at present. Studies are under way to further characterize these interesting enzymes.

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Glycoproteins of the CHO Cell Membrane: Partial Fractionation of the Receptors for Concanavalin A and Wheat Germ Agglutinin Using a Lectin Immunoprecipitation Technique†

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ABSTRACT: The lectin-binding glycoproteins of the CHO cell plasma membrane have been analyzed using an immunoprecipitation technique. Membranes from cells labeled via metabolic incorporation of [^{3}H]glucosamine were solubilized in deoxycholate, the solubilized material was treated with concanavalin A, *Ricinus communis* agglutinins, or wheat germ agglutinin, and the lectin–glycoprotein complexes were precipitated with specific antisera directed against the lectins. The immunoprecipitates were analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate and fluorescence-enhanced autoradiography. The CHO cell membrane contained two major glucosamine-labeled glycoprotein classes, A and B, which migrated as diffuse bands on polyacrylamide gels, and which had apparent molecular weights of 100 000 and 130 000, respectively. Several minor labeled components were also apparent. Lectin immunoprecipitation of solubilized CHO cell membranes with wheat germ agglutinin resulted in the precipitation of material primarily of class A, while immunoprecipitation with concanavalin A produced material of class B. Thus a degree of subfractionation of the membrane glycoproteins according to lectin-binding specificity has been obtained. The utility of the lectin immunoprecipitation technique is discussed in terms of analyzing the molecular associations between subclasses of membrane glycoproteins and nonglycosylated membrane macromolecules.

The biochemical nature, surface distribution, and lateral mobility of cell surface glycoproteins seem to be directly related to important biological processes such as the expression of malignant transformation, cell to cell recognition, and control of cell growth (Nicolson, 1974). Since glycoproteins have an affinity for lectins, the carbohydrate binding proteins derived from plant sources, surface glycoproteins frequently have been termed lectin "receptors". In the past few years a large number of investigations have dealt with the biological effects of lectin binding (Burger, 1973), and with the relationship between the mobility and distribution of lectin receptors and the actions of the cytoskeleton (Nicolson, 1976). Most of the studies im-

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plicating the cytoskeleton in the control of lectin receptor distribution on the cell surface have relied on drugs such as colchicine, cytocholasin B, or procaine which apparently interdict the action of microtubules and microfilaments (Poste et al., 1975).

Unfortunately, except in the case of glycophorin, the wheat germ agglutinin and phytohemagglutinin receptor from human erythrocytes (Tomita & Marchesi, 1975), very little is known biochemically about lectin receptors or about their putative linkage to the cytoskeleton. It has often been assumed that the various "lectin receptors" detected on cells might represent different carbohydrate moieties attached to the same polypeptides. In some cases this seems to be true; thus glycophorin has receptor activity for the lectins from kidney bean, wheat germ, and *Agaricus bisporous* (Fukuda & Osawa, 1973). However, in other cases it seems clear that carbohydrate moieties with affinities for different lectins reside on different macromolecules. For example, in the erythrocyte, glycophorin is distinct from the receptor for concanavalin A which is a subcomponent of band 3 (Findlay, 1974). Both biochemical (Adair & Kornfeld, 1974) and electron microscopic (Triche et al., 1975) evidence suggests that the receptors for the *Ricinus communis* agglutinins are also distinct from glycophorin. In other cell types not much is known about the molecular identity of lectin receptors. In thymocytes, indirect evidence (from capping experiments) (Lustig & Pluznik, 1976) suggests that concanavalin A receptors and wheat germ receptors reside on different populations of molecules. Recently, Nachbar et al. (1976) have shown that the macromolecular receptors for concanavalin A, *Ricinus communis,* and wheat germ agglutinins in Ehrlich ascites cells comprise partially overlapping but distinct populations of glycoproteins.

We have attempted to analyze different categories of lectin receptors in cultured wild type Chinese hamster ovary (CHO) cells in the following manner. We have solubilized CHO plasma membranes with mild detergents, allowed the solubilized membrane glycoproteins to react with lectins, and then precipitated the lectin–glycoprotein complexes with antibodies directed against the lectin. The antibody in this system serves merely to ensure the efficient precipitation of the complexes, which are then analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. We believe that this approach offers some advantages over affinity chromatography using lectin conjugated absorbents, in that a variety of experimental conditions involving comparison of different lectins, different ratios of lectin to glycoprotein, or different cell lines can be analyzed rapidly. These advantages also apply to the recently described technique of "staining" glycoproteins in polyacrylamide gels with [^{125}I]lectins (Tanner & Anstee, 1974; Gurd & Evans, 1976; Burridge, 1976). However, we believe that "lectin immunoprecipitation" as described here offers an advantage over "lectin staining" in that the latter allows only the identification of isolated, denatured glycoproteins, while "lectin immunoprecipitation" can be used, in conjunction with chemical cross-linking (see Ji, 1976, for example), to examine possible associations between surface glycoproteins and other nonglycosylated membrane components. Other workers have recently applied similar "lectin immunoprecipitation" techniques to lymphocyte membrane glycoproteins (Sela et al., 1975; Henkart & Fisher, 1975).

Materials and Methods

Cells. Chinese hamster ovary (CHO)[1] cells were maintained in exponential growth in suspension culture as previously described (Juliano & Behar-Bannelier, 1975a, b).

Labeling Conditions, Membrane Isolation, and Detergent Solubilization. Cells were labeled in the oligosaccharide portions of their membrane glycoproteins by the metabolic incorporation of [^{3}H]glucosamine (0.2 μCi/cm^3) in complete culture medium for a period of at least one doubling time, followed by incubation with 1 μCi/cm^3 of isotope in glucose free medium for 3 h. Incubating Chinese hamster cells with [^{3}H]glucosamine gives rise to oligosaccharides labeled primarily in their *N*-acetylglucosamine residues (75%) and to a lesser degree in galactosamine and sialic acid residues (Gottlieb et al., 1975). The level of labeling obtained in this manner was on the order of 1 μCi/mg of membrane protein. Glucosamine would seem to be an appropriate label since *N*-acetylglucosamine is almost universally present in membrane glycoproteins (Sturgess et al., 1978).

Plasma membranes were prepared by a modification of the aqueous polymer two phase technique of Brunette & Till (1971) as described in detail elsewhere (Juliano & Behar-Bannelier, 1975a, b; Juliano & Gagalang, 1975). Glycoproteins were solubilized from partially purified membrane preparations by incubation in solutions of mild detergents such as deoxycholate (DOC), Triton X-100, and Noniodet P-40 in 10 mM Tris (pH 8); phenylmethanesulfonyl fluoride (1 mM) was included so as to inhibit auto digestion by membrane proteases. Eventually solubilization in 1% DOC proved to be the method of choice and DOC-solubilized material was used in most of the studies described. Usually a few milligrams of membranes was suspended in 1% DOC for 1 h at 4 °C. Undissolved material was pelleted by centrifugation at 30 000 rpm for 1 h in an IEC-B-60 machine and the supernatant was recovered for further use. Protein concentrations in membranes and in DOC extracts were measured according to the method of Lowry et al. (1951) using bovine albumin as a standard.

Lectins. Ricinus communis agglutinins (RCA I plus RCA II) were prepared according to Nicolson et al. (1974). Concanavalin A (Con A) was purchased from Pharmacia and wheat germ agglutinin was obtained from Sigma; in some cases, these lectins were repurified by affinity chromatography on Sephadex (Con A) or by affinity elution from fixed erythrocytes according to the method of Reitherman et al. (1974). Radiolabeled lectins were prepared by reaction with ^{125}I and lactoperoxidase. The lectins (10 mg) were dissolved in 2 cm^3 of 10 mM Tris (pH 8) containing 100 mM haptene[2] and 1 μmol of NaI. Lactoperoxidase (20 μg) and ^{125}I (0.1 mCi) were added and the reaction was initiated by the addition of 50 μL of 0.003% H_2O_2. The reaction proceeded at room temperature for 45 min during which two further aliquots of H_2O_2 were added. The reaction was terminated by the addition of 1 mmol of NaI and the samples were dialyzed vs. cold phosphate-buffered saline. The iodinated lectins were repurified by affinity chromatography on Sephadex or Sepharose respectively for concanavalin A and *Ricinus communis* and by affinity elution from fixed erythrocytes for wheat germ agglutinin. The lectins were labeled to a level of 1 μCi/mg by this procedure.

Lectin Binding to CHO Membranes. The binding of [^{125}I]lectins to fixed CHO cell membranes was measured as follows. The membranes were fixed by incubation with 5% gluteraldehyde in phosphate buffered saline for 2 h at room temperature. The fixation was stopped by washing the mem-

[1] Abbreviations used: CHO, Chinese hamster ovary cells; $NaDodSO_4$, sodium dodecyl sulfate; DOC, deoxycholate; Con A, concanavalin A.

[2] The haptenes used were mannose, galactose, and *N*-acetylglucosamine for concanavalin A, *Ricinus* communis agglutinin, and wheat germ agglutinin, respectively.

TABLE I: Extraction of Membrane Proteins and Glycoproteins by Detergents.[a]

	% radioact. remaining in pellet	
	[^{3}H]Amino acid	[^{14}C]Glucosamine
Buffer	100	100
Noniodet P 40	33	54
Emulphogene	44	69
Deoxycholate	8	9
Triton X-100 (in 56 mM borate)	76	37

[a] The solubilization of CHO cell membrane proteins (labeled with ^{3}H-labeled amino acid mixture) and carbohydrate ([^{14}C]glucosamine labeled) was examined. The radiolabeled membranes were extracted with 2 cm^3 of a 1% detergent solution in 10 mM Tris (pH 8) for a period of 1 h at 4 °C. The residual insoluble material was pelleted by centrifugation at 10 000 rpm for 1 h, dissolved in 1.0% sodium dodecyl sulfide, and counted in Aquasol (New England Nuclear). The results represent the means of duplicate determinations.

branes in 1 M glycine, followed by further washes in phosphate-buffered saline. In order to measure lectin binding, 25 μg of fixed membranes was placed in tubes containing 2 cm^3 of phosphate-buffered saline plus 0.1% albumin and in some cases, 100 mM haptene sugar. Radioiodinated lectins were then added and incubated for 1 h at room temperature. The membranes were pelleted by centrifugation at 10 000 rpm for 20 min and washed twice with phosphate-buffered saline. The membrane associated radioactivity was then determined and the amount of bound and residual free lectin calculated from the known specific activity of the lectin. The data were corrected for nonspecific binding in the presence of haptene and were analyzed according to the method of Scatchard (see Tanford, 1968) using lectin molecular weights taken from Brown & Hunt (1977).

Preparation and Characterization of Anti-Lectin Sera. Rabbit antisera to lectins were raised by weekly subcutaneous injections of graded doses of lectins in Freund's complete adjutant over a period of 1–2 months. It was necessary in some cases (e.g., *Ricinus* agglutinins) to begin with very low doses (1 μg) of lectin and to work up to the final dose level (5 mg), because of the toxic properties of the molecules involved (Olsnes et al., 1974). The plasma obtained by ear vein puncture was allowed to clot, and the serum was recovered. Prebleeds from the antibody producing animals were kept as control sera. The specificity of the antisera was tested by immunodiffusion vs. lectins using plates made up of 1.2% agarose plus a 100 mM concentration of the appropriate haptene sugars in Tris buffer. The equivalence zone for each lectin–antilectin serum pair was established by assay of the immunoprecipitation of 10 μg of ^{125}I-labeled lectin by the serum, in the presence of 100 mM haptene sugar.

Lectin Immunoprecipitations. Detergent solubilized membrane extracts were incubated with lectins in 10 mM Tris pH 8 plus 0.2% DOC for 1–3 h at 4 °C. Thereafter an appropriate amount of antiserum was added and the sample was incubated for an additional h at 4 °C. In some cases haptene sugars were added prior to the lectins, thus inhibiting the formation of lectin–glycoprotein complexes. Usually 5 or 10 μg of lectin plus sufficient antisera for maximal precipitation was used, and the amount of detergent solubilized membrane extract was varied so as to vary the ratio of lectin to membrane glycoproteins. The data were usually expressed as % of [^{3}H]glucosamine label precipitated vs. the amount of membrane extract in terms of μg of protein.

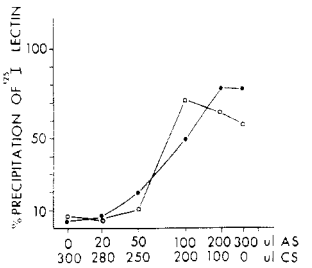

FIGURE 1: Equivalence zones of lectins plus antilectin sera. The equivalence zones of lectin–antilectin sera combinations were measured by incubating 10 μg of [^{125}I]lectin in 10 mM Tris plus 0.2% DOC with 300 μL of serum for 1 h at 4 °C. The proportion of antiserum (AS) to control serum (CS) in the sample was varied from 0 to 100%. The equivalence zones for two typical batches are illustrated. (O—O) Anti-concanavalin A; (●—●) anti-wheat germ agglutinin.

Polyacrylamide Gel Electrophoresis. Membranes and immunoprecipitates were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate ($NaDodSO_4$) and reducing reagent. A continuous buffer system (Fairbanks et al., 1971) and slab gels were used, and the pattern was analyzed by fluorescence enhanced autoradiography and densitometry as described elsewhere (Juliano & Ling, 1976): this system yields a linear relationship between the densitometric peak area and the amount of radioactivity. Molecular weight standards included chymotrypsinogen, ovalbumin, albumin, and myosin which were labeled by reductive alkylation with [^{14}C]formaldehyde (Rice & Means, 1971).

Results

Extraction of Membrane Glycoproteins. As seen in Table I deoxycholate seems to be more efficient than other detergents in extracting membrane proteins and glycoproteins. This detergent has been shown to be compatible with ligand binding to lectins and with antibody antigen reactions (Letarte-Muirhead et al., 1975). Although high concentrations of deoxycholate are reported to denature lectins, the concentration employed for lectin immunoprecipitation in this study (usually 0.2%) does not affect the properties of concanavalin A, wheat germ agglutinin, or *Ricinus communis* agglutinins (Lotan et al., 1977). Deoxycholate is also conveniently removed by dialysis, whereas the detergents NP40 and Triton X-100 are less easily dialyzed away. Deoxycholate also seems to extract a random sample of the membrane proteins as visualized by gel electrophoresis and Coomassie blue staining (data not shown).

Specificity of Antisera to Lectins. The specificity of antilectin sera was tested by immunodiffusion. Immunization with relatively pure lectins gave rise to potent highly specific antisera as evidenced by sharp immunoprecipitin lines and the lack of cross reactivity. Haptene sugars were included in the immunodiffusion tests so as to prevent binding of the lectin to the agarose matrix of the immunodiffusion plates and to interdict possible interactions between the lectin and the carbohydrate side chains of the immunoglobulins. None of the sera were directly reactive with CHO cell membrane extracts.

The equivalence zones of the lectin and anti-lectin combinations varied among the different batches of serum used. However, generally about 50–100 μL of antiserum was sufficient to precipitate 10 μg of lectin (see Figure 1). In further experiments sufficient antiserum was used so as to effect maximal precipitation of the amount of lectin employed.

Immunoprecipitation of Lectin Binding Glycoproteins. Figure 2 displays the results of immunoprecipitation using

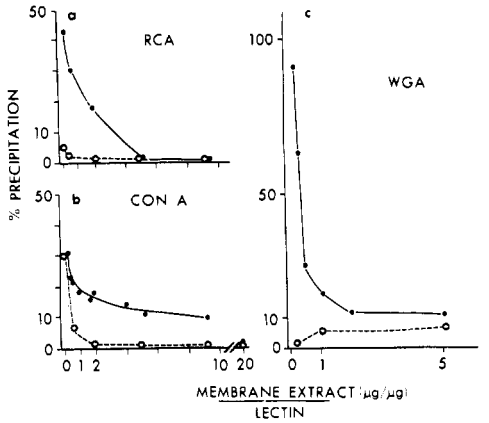

FIGURE 2: Lectin immunoprecipitation of [^{3}H]glucosamine-labeled membrane extract. Labeled membranes were solubilized in 1% DOC as described in Materials and Methods and aliquots of membrane extract were added to tubes containing 5 cm^3 of 0.2% DOC in 10 mM Tris (pH 8). Lectins (5 or 10 μg) were added and the tubes were incubated for 1 h at 4 °C. Thereafter sufficient antiserum was added to maximally precipitate the lectins and the incubation was continued a further hour. The immunoprecipitates were pelleted by centrifugation at 2000 rpm for 15 min and were washed two times with Tris buffer prior to assay for radioactivity. The results are the means of triplicate determinations. (a) *Ricinus* agglutinin (●—●); *Ricinus* + 100 mM galactose (○---○). (b) Concanavalin A (●—●); concanavalin A + 100 mM mannose (○---○). (c) Wheat germ agglutinin (●—●); wheat germ agglutinin + 100 mM *N*-acetylglucosamine (○---○).

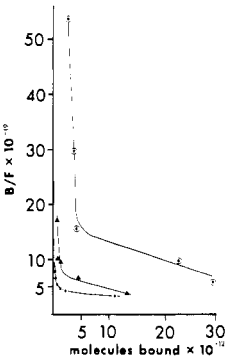

FIGURE 3: Binding of [^{125}I]lectins to gluteraldehyde fixed CHO cell membranes. Binding assays using ^{125}I-labeled concanavalin A, wheat germ agglutinin, and *Ricinus* agglutinins were carried out as described in Materials and Methods. The data show binding to 25 μg of membrane protein which represents the membrane derived from roughly 2.5 × 10^6 cells. Lectin binding was measured over the range of 0.25 to 25 μg/cm^3. The amount of lectin specifically bound was calculated by difference from the amounts bound in the presence and absence of 100 mM haptenes. (⊙—⊙) Wheat germ agglutinin; (▲—▲) *Ricinus* agglutinin; (●—●) concanavalin A.

different ratios of solubilized membrane glycoproteins and glycolipids (i.e., DOC extract) to lectin. In all three cases shown (concanavalin A, *Ricinus* agglutinins, wheat germ agglutinin), when low ratios (<1) of extract to lectin were used, a large fraction of the [^{3}H]glucosamine-labeled material was precipitated, ranging from about 30% in the case of concanavalin A to about 90% in the case of wheat germ agglutinin. The percentage of the material precipitated declined rapidly with increasing amounts of extract and was negligible at very high ratios (>20) of membrane extract to lectin. In the region where the amount of extract was moderately in excess of the amount of lectin, a plateau was evident in the case of concanavalin A. In all cases the percentage of material which was precipitated in the presence of haptenes was far less than that precipitated in the absence of these sugars over a wide range of DOC extract to lectin ratios, indicating that the immunoprecipitation reactions involved specific binding between lectins and membrane oligosaccharides. Antisera alone, in the absence of lectins, did not precipitate appreciable amounts of material from DOC extracts of membranes. It should be noted that the "high affinity" regions of the Scatchard plots shown in Figure 3 correspond to ratios of membrane protein to lectin of ~50/1 to ~5/1. Thus the lectin immunoprecipitates formed at the higher ratios of DOC extract to lectin (~10/1) may reflect lectin binding to "high affinity" receptors. At very high ratios of concanavalin A to membrate extract (i.e., low amounts of extract), some nonspecific precipitation which was not inhibited by mannose was evident. This may result from the ability of concanavalin A to enter into nonspecific hydrophobic interactions with glycoproteins (Davey et al., 1976).

Thus, while the reasons for the precise shapes of the precipitation curves are obscure, it does seem evident that over a wide range of lectin to glycoprotein ratios, specific immunoprecipitates can be formed which reflect the carbohydrate binding capacity of the lectin. Although the carbohydrate side chains on serum proteins could potentially act as competitive binding sites for the lectins, this does not seem to be a problem in practice. In the presence of immune sera, precipitates are visible very quickly (~5 min) and are abundant, while in the presence of nonimmune sera, little precipitate formation occurs. It is likely that antibody population in the immune serum "traps" the lectin–glycoprotein complexes too quickly to allow reequilibration of the lectins.

NaDodSO$_4$–Polyacrylamide Gel Patterns of CHO Membranes and lectin Immunoprecipitates. As seen in Figure 4c, the [^{3}H]glucosamine-labeled glycoproteins of the CHO cell membrane gave rise to two broad major peaks and several minor ones when analyzed by NaDodSO$_4$–polyacrylamide gel electrophoresis and autoradiography. The major peaks, which have been designated A and B, contained greater than 90% of the total radioactivity and migrated with apparent molecular weights of 100 000 and 130 000, respectively, while minor peaks were evident between 45 000 and 80 000 and at about 230 000. The reason for the diffuse appearance of peaks A and B is unclear at this time: it may be due to polydispersity within each peak, or to the highly glycosylated nature of these components (the major red cell glycoproteins, band 3 and glycophorin, also appear diffuse in NaDodSO$_4$ gels) (Juliano, 1973). The overall pattern of membrane glycoproteins visualized by [^{3}H]glucosamine labeling was very similar to that reported previously using the galactose-oxidase–[^{3}H]borohydride surface labeling system (Juliano & Behar-Bannelier, 1975a, b; Juliano & Ling, 1976).

The NaDodSO$_4$–polyacrylamide gel patterns of lectin immunoprecipitates formed at high ratios of DOC extract to lectin are seen in Figures 4a, b. It is clear that the concanavalin A immunoprecipitate contained mainly material in the region corresponding to peak B, while the wheat germ agglutinin immunoprecipitate contained material migrating in the region corresponding to peak A. In addition, the wheat germ agglutinin sample contained a small amount of material migrating at about 45 000 and at 230 000. Experiments with *Ricinus communis* agglutinins (data not shown) gave immunoprecipitates containing material migrating in both the A and B regions. It should be noted that the optical density scale (Figure 4a) in the wheat germ agglutinin sample is reduced relative to that in Figures 4b, c: this is because the wheat germ agglutinin samples tended to give fainter gel patterns even though equal amounts of radioactivity were applied in all cases (see Discussion).

The immunoprecipitation experiments of Figure 4 were

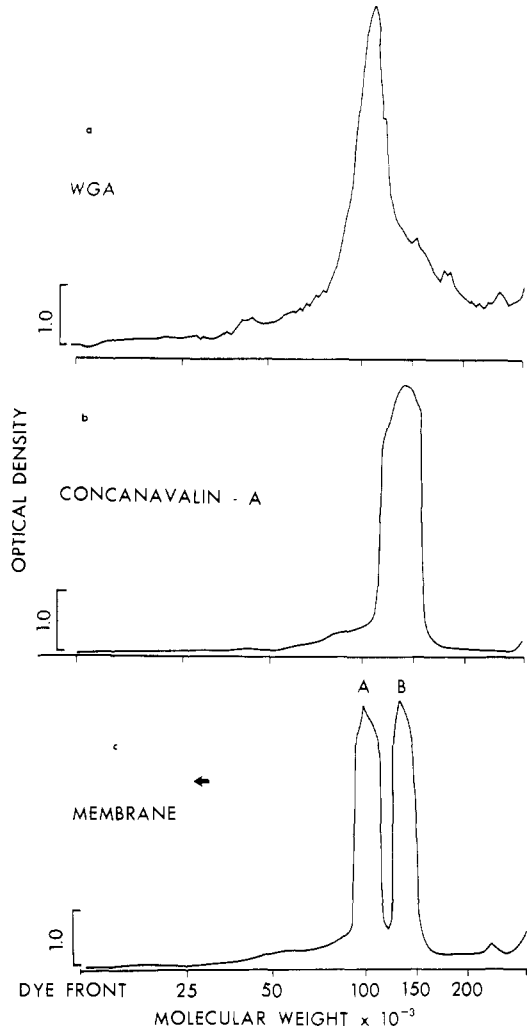

FIGURE 4: $NaDodSO_4$-polyacrylamide gel patterns of CHO cell membranes and lectin immunoprecipitates. CHO cell plasma membranes labeled with [^{3}H]glucosamine were solubilized in 1% DOC and then subject to lectin immunoprecipitation as described in Materials and Methods. The ratio of lectin to solubilized membrane protein was 1/10 (μg/μg) in each case. The specific activity of the original plasma membrane was 1768 dpm/μg of protein. The immunoprecipitates and the plasma membranes were analyzed by electrophoresis on 7.5% polyacrylamide slab gels containing $NaDodSO_4$ and under reducing conditions; molecular weight markers were run in parallel. Approximately 15 000 dpm of each sample was applied to the gel. The gel patterns were analyzed by autoradiography and densitometry as described in Materials and Methods. (a) Wheat germ agglutinin immunoprecipitate; (b) concanavalin A immunoprecipitate; (c) membranes.

performed at lectin/solubilized glycoprotein ratios which corresponded to the "high affinity" portion of the plots in Figure 3. Thus it seems likely that the material precipitated in the presence of concanavalin A or of wheat germ agglutinin represents a sampling of the high affinity macromolecular membrane receptors for these lectins.

The process of lectin immunoprecipitation represents a specific tripartite interaction between membrane glycoprotein, lectin, and antilectin serum. When all these elements were present, an abundant precipitate formed which was amenable to gel analysis. If the lectin–glycoprotein interaction was interrupted (as in the presence of haptene), or if the interaction between lectin and serum was ineffective (as with control serum), then only meager precipitates were produced which yielded extremely diffuse patterns upon gel electrophoresis. For example, treatment of a sample of DOC extract (100 μg, 3.6×10^5 dpm) with 10 μg of concanavalin A plus 200 μL of anti-concanavalin A serum yielded an immunoprecipitate containing 5×10^4 dpm (19%), while treatment of similar samples with control serum, or in the presence of 100 mM mannose, yielded immunoprecipitates containing 2.6×10^3 dpm (1%) and 6×10^3 dpm (2%), respectively.

Discussion

In this report, we have described a technique for the analysis of membrane glycoproteins based on their interaction with lectins followed by precipitation of the lectin–glycoprotein complex with antilectin serum. This technique is shown to be specific in that the immunoprecipitate fails to form in the presence of a haptene sugar, or if a nonimmune serum is used. The technique of lectin immunoprecipitation offers some advantages over that of lectin affinity chromatography in terms of the ease of manipulation of experimental conditions; it is not, however, useful as a preparative technique. As indicated in the introductory section, the most interesting application of lectin immunoprecipitation techniques may be in analyzing the molecular associations between subpopulations of the membrane glycoproteins defined by lectin affinity and other, carbohydrate-free, membrane components; studies of this type are now under way in our laboratory.

In terms of the biochemistry of the CHO cell membrane, the major finding of the present report is that the bulk of the [^{3}H]glucosamine-labeled glycoproteins migrate in two broad bands, A and B, and that wheat germ agglutinin precipitates primarily components of band A, while concanavalin A precipitates primarily components of band B. Thus we have affected a partial resolution of two different categories of lectin binding glycoproteins in the CHO cell membrane.

The binding of iodinated lectins to CHO cell membranes manifests nonlinear Scatchard plots indicating several binding sites with different affinities; similar observations have been made in other cell types (Sandvig et al., 1976; Schmidt-Ullrich et al., 1975). Presumably most lectins combine to some degree with a variety of carbohydrate-containing molecules so long as one or more appropriate sugar residues are present (Brown & Hunt, 1978). However, it seems clear (Young & Leon, 1974) that the high affinity binding of lectins requires more than the presence of a single sugar and is a function of the sequence, branching and linkage configuration of an oligosaccharide region containing several residues. Presumably the steeper portions of the Scatchard plots reflect the binding of lectins to one or more types of high affinity sites on membrane glycoproteins, while the remainder of the plot may reflect the binding to more numerous low affinity sites on glycoproteins and/or glycolipids. Lectins have been reported to bind to glycolipids but the affinities are lower than those for whole membranes or cells (Redwood et al., 1975; Surolia et al., 1975). It is important to note that lectin binding is, at least in some cases, a reversible equilibrium phenomenon, and that this provides the basis for discrimination of high and low affinity receptors (Sandvig et al., 1976).

The lectin immunoprecipitation curves of Figure 2 reflect some of the characteristics of the lectin binding data. Thus when the amount of lectin is large relative to the amount of solubilized membrane material (DOC extract), a large portion of the [^{3}H]glucosamine label is precipitated. This may reflect the binding of the lectin to a variety of high and low affinity receptors when it is present in excess. It should be noted that a greater fraction of the total labeled material could be precipitated by *Ricinus communis* and wheat germ agglutinins than by concanavalin A; this may result from the reported ability of these first two lectins to bind to glycolipids as mentioned above. In all cases, there are regions of the immunoprecipitation curves where only a modest fraction (about

10%) of the total labeled material is precipitated in the absence of haptene, but here the amount of precipitate in the presence of haptene is still lower. This may represent regions where the higher affinity receptors for the lectin in question are preferentially precipitated.

Polyacrylamide gel patterns observed at moderately high ratios of membrane extract to lectin seem to support the notion that one can specifically precipitate higher affinity lectin-binding glycoproteins.[3] Thus as mentioned above, concanavalin A and wheat germ agglutinin each tend to precipitate components from one of the two major glycoprotein classes in the CHO cell membrane. By contrast, experiments using lectin affinity chromatography have, with only a few exceptions (Hunt et al., 1975; Schmidt-Ullrich et al., 1975), been rather unsuccessful in subfractionating membrane glycoproteins. A possible explanation for this is that our immunoprecipitation experiments are performed under conditions where the membrane glycoproteins are in excess and the lectin tends to bind to high affinity components. On the other hand, most affinity chromatography runs are probably performed under conditions where the lectin is present in excess and is likely to bind both higher and lower affinity receptors. The importance of column load in lectin affinity chromatography has been emphasized previously (Adair & Kornfeld, 1974).

Our biochemical analysis of the CHO cell membrane tends to support earlier studies using indirect techniques (Greene et al., 1976; Jacobsen et al., 1976; Lustig & Pluznik, 1976) which suggest that the receptors for wheat germ agglutinin and concanavalin A might reside on different populations of membrane macromolecules in a variety of cell types. Differences in lateral mobility and ease of capping of wheat germ agglutinin vs. concanavalin A receptors may reflect varying levels of linkage between subpopulations of surface glycoproteins and elements of the cytoskeletal system. It would be of interest to test this possibility through use of lectin immunoprecipitation techniques and chemical cross-linking in order to search for associations between cell surface components and cytoskeletal components.

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[3] The fainter gel patterns of the wheat germ immunoprecipitates may be due to the presence of radiolabeled glycolipid in these samples; because of the dimethyl sulfoxide fixation step used in the autoradiography procedure, radioactivity due to glycolipids is lost in our gel analysis system.

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