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CHROMATOGRAPHIC BEHAVIOR OF PEA LECTIN RECEPTORS FROM THE 6C3HED MURINE ASCITES TUMOR

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Summary

We have previously shown that pea lectin and concanavalin A bind to different receptors as well as to common receptors on the cell surface of 6C3HED murine ascites tumor cells (Allen, H.J. and Johnson, E.A.Z. (1976) Biochim. Biophys. Acta 436, 557—566). We have characterized the metabolically labeled pea lectin receptor from trypsinates of viable tumor cells by gel filtration and affinity chromatography. The pea lectin receptor eluted as an apparent high molecular weight glycopeptide (M_r 200 000) from Sephadex G-200 columns. Other glycopeptides were present in the trypsinates but they did not bind to either concanavalin A or to pea lectin in significant amounts. The high molecular weight glycopeptide was resistant to digestion by a high concentration of trypsin but it was absent in non-specific protease digests of viable cells. Sequential lectin affinity chromatography of Nonidet P-40 extracts of cells permitted the probable identification of the native glycoprotein corresponding to the high molecular weight glycopeptide which binds to pea lectin.

Introduction

We have selected the 6C3HED murine ascites tumor as a model system from which to isolate and characterize the cell surface glycoproteins which may play a role in cellular recognition events. That such components are involved in cellular recognition phenomena is strongly supported by numerous studies which have compared cell surface glycoproteins and glycopeptides of transformed cells with those of their normal counterparts [1—5], which have compared cell surface glycoproteins and glycopeptides among variant strains of a given tumor [6—8] and which have examined the effects of carbohydrates [9—11] and

lectins [12] on cell behavior. One approach to understanding how cells recognize self from non-self and how recognition phenomena influence cell replication is to isolate those cell surface macromolecules which are likely to be involved in those phenomena and to carry out their physico-chemical characterization.

Toward this end, we have previously carried out studies in the 6C3HED murine ascites tumor cell receptors for D-mannosyl-binding lectins [13,14]. By reciprocal-inhibition experiments, we have shown that there are cell surface receptors present which bind both concanavalin A and pea lectin as well as receptors which bind only concanavalin A or pea lectin [13,14]. Receptors site heterogeneity was also demonstrated by Scatchard analysis of lectin binding data.

Trypsin and non-specific protease digestion of viable cells resulted in a decrease in the pea lectin binding capacity of the cells whereas the binding of concanavalin A was unaffected [14]. We report here the identification of the pea lectin receptor present in trypsinates and its correlation with an intact glycoprotein extracted from whole cells.

Materials and Methods

The 6C3HED ascites tumor was harvested as previously described [14]. Tumor cell glycoproteins were metabolically labeled by injecting 10 μCi of D-[6-^{3}H]glucosamine or L-[6-^{3}H]fucose interperitoneally per mouse 18 h before harvesting the tumor. For double-label experiments reported, separate tumor-bearing mice were injected with 5 μCi of D-[U-^{14}C]-glucosamine.

Tumor extracts were prepared as follows: about 2 ml of packed cells were suspended in 8 ml of cold affinity-columm buffer (0.1 M Tris/0.1 M glycine/1.0 M NaCl/0.05% NaN_3, pH 7.2) containing 1 mM L-1-tosylamide-2-phenylethylchloromethyl ketone, 1% Nonidet P-40 and 50 μg/ml DNAase I. The suspension was stirred in the cold for 24—48 h. The suspension was then centrifuged at 49 000 × g for 60 min and the supernatant was dialyzed in the cold against affinity-column buffer/0.1% Nonidet P-40. The retentate was then diluted 4-fold with the preceding solution prior to use. This extraction procedure solubilized all of the D-[6-^{3}H]-glucosamine-labeled components present in the tumor cells.

Proteolytic digestion of viable cells was carried out as previously reported [14] for 2 h unless otherwise stated. These conditions gave maximal release of D-[6-^{3}H]glucosamine-labeled components (20—25% of the total cell label) while maintaining essentially constant cell count and viability. Control cell suspensions lacking exogenous proteolytic enzyme released 3—5% of cell label. L-[6-^{3}H]Fucose-labeled cells released about one-half of the above percentages. On occasion, the cell-free trypsinate was heated at 100°C for 15 min to stop trypsin activity rather than adding soybean trypsin inhibitor. After enzyme inactivation, the digests were clarified by centrifugation and then concentrated 20—40-fold by vacuum dialysis to give retentate and filtrate fractions. The retentates were frozen until further use. The filtrates were lyophilized, dissolved in 0.1 N acetic acid and then desalted on a 2.5 × 95 cm column of Bio Gel P-2 equilibrated with 0.1 N acetic acid at 18°C. The glycopeptides, which

eluted in the void volume, were lyophilized and strored dessicated at −20°C until further use.

Concanavalin A and pea lectin were isolated as described by Agrawal and Goldstein [15] and Entlicher et al. [16], respectively. Affinity adsorbents were prepared by coupling concanavalin A and pea lectin to Sepharose 2B. The gel was activated using the buffer system of Porath et al. [17] and with the CNBr being pre-dissolved in acetonitrile (1.44 g CNBr/80 ml gel). The lectins were dissolved in 10% sucrose/0.1 M $NaHCO_3$ prior to mixing with the activated gel. After stirring the lectin/gel suspension for 15 min at room temperature, the pH was asjusted to 7.4 with 2 N HCl. The suspension was then stirred in the cold for 72 h. After washing the gel with deionized water, it was suspended in cold 0.1 M ethanolamine, pH 8.0, for 20 h. The gel was then washed and stored in 0.15 M NaCl/0.015 M phosphate/0.05% NaN_3, pH 7.0 (phosphate-buffered saline) at 4°C. This procedure gave a coupling efficiency of 95% and 5.4 mg lectin/ml gel.

Affinity chromatography of glycopeptides was carried out at 4°C in affinity-column buffer. For affinity chromatography of cell extracts, the buffer was made 0.1% in Nonidet P-40. After washing off unbound materials, bound components were eluted with 0.1 M methyl-α-D-mannopyranoside in affinity-column buffer. Gel filtration was carried out as described in the figure legends.

Diphenylcarbamyl chloride-treated type XI trypsin, type VI protease, type-1-S soybean trypsin inhibitor, beef pancreatic DNAase I, L-1-tosylamide-2-phenylethylchloromethyl ketone and molecular weight standards were obtained from Sigma Chemical Co., St. Louis, MO. Mini-Vials, Aquasol, D-[6-^{3}H]glucosamine (specific activity 10—20 Ci/mM), D-[U-^{14}C]glucosamine (specific activity 228 mCi/mM) and L-[6-^{3}H]fucose (specific activity 10—20 Ci/mM) were obtained from New England Nuclear Corp., Boston, MA. Sephadex and Sepharose were products of Pharmacia Fine Chemicals, Piscataway, NJ. Bio Gel P-2 was obtained from Bio-Rad Laboratories, Inc, Rockville Center, NY. Nonidet P-40 was obtained from Particle Data Laboratories, Elmhurst, IL. ^{3}H and ^{14}C were quantitated by liquid scintillation counting. Molecular weight standards were Blue Dextran 2000, catalase (M_r 250 000), aldolase (M_r 158 000), bovine serum albumin (M_r 68 000), chymotrypsinogen A (M_r 25 000), lysozyme (M_r 14 000) and RNAase (M_r 13 700).

Results

The vacuum dialysis retentate of trypsinates, prepared as described in Materials and Methods, contained 80—90% of the radioactivity, derived from D-[^{3}H]glucosamine incorporation, present in the cell-free trypsinates. The retentate was chromatographed on a calibrated Sephadex G-200 column. The results of a representative experiment are shown in Fig. 1. A minor peak of radioactivity was present in the void volume with two major retarded peaks also present. Peak 2 consistently eluted at a position corresponding to an apparent molecular weight of M_r about 200 000. The distribution of peak 3 was somewhat variable in different experiments and it was usually spread over an apparent molecular weight range of about M_r 25 000—50 000.

Non-specific protease (from *Streptomyces griseus*) digestion of [^{3}H]gluco-

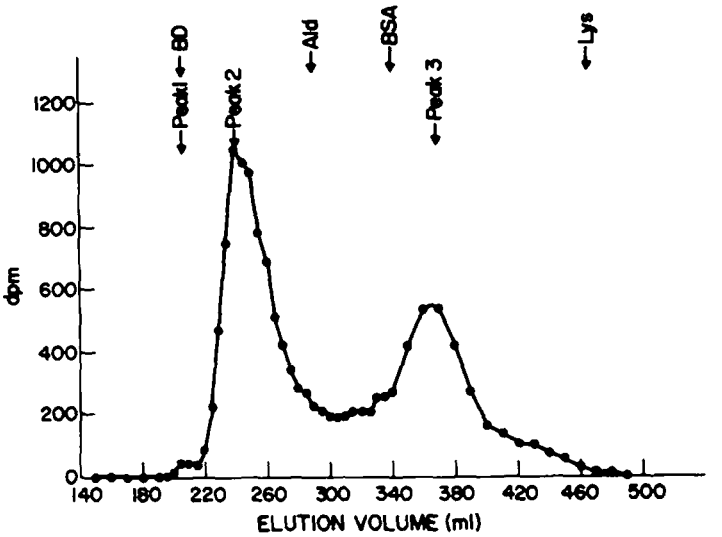

Fig. 1. Sephadex G-200 chromatography of trypsinate retentate. The vacuum dialysis retentate was applied via ascending flow to a 93.1 X 2.6 cm Sephadex G-200 column equilibrated with phosphate-buffered saline at 18°C. Aliquots of fractions were assayed for ^{3}H. The elution positions of Blue Dextran 2000 (BD), aldolase (Ald), bovine serum albumin (BSA), and lysozyme (Lys) are indicated. Flow rate 20.1 ml/h. Load volume 5.8 ml. Fraction volume 5.0 ml.

samine-labeled cells released a similar percentage of cellular radioactivity as did trypsin. However, considerably more radioactivity (40—50%) was recovered in the filtrate after vacuum dialysis. The retentate was chromatographed on Sephadex G-200. The results are shown in Fig. 2. The most striking difference, compared to the trypsinate, was the complete absence of the peak 2 glycopeptide shown in Fig. 1.

Digestion of L-[^{3}H]fucose-labeled cells with trypsin and non-specific protease released a smaller percentage of radioactivity and gave rise to a more complex mixture of glycopeptides when analyzed by gel filtration than was the case with [^{3}H]glucosamine-labeled cells. These glycopeptides were not studied further.

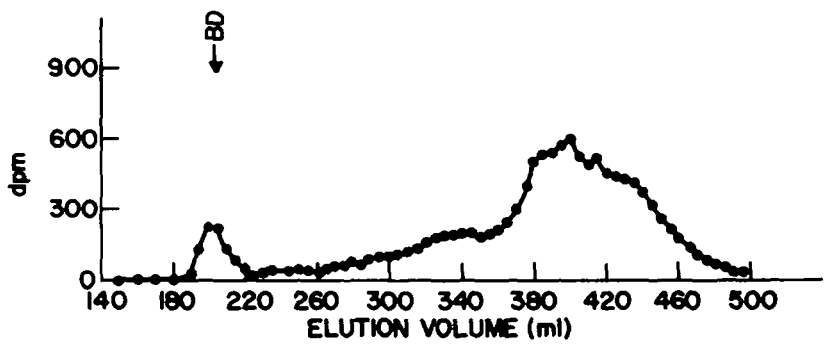

Fig. 2. Sephadex G-200 chromatography of vacuum dialysis retentate from non-specific protease digest. The retentate was applied via ascending flow to the column described in Fig. 1. Aliquots of fractions were assayed for ^{3}H. Flow rate 19.7 ml/h. Load volume 4.8 ml. Fraction volume 5.0 ml.

In contrast to the results from enzyme digests, the vacuum dialysis retentate of control incubates contained radioactivity which eluted only in the void volume of Sephadex G-200 corresponding to peak 1 of Fig. 1 (results not shown).

To determine if the variability in the elution profile for peak 3 was due to variable and incomplete digestion of these glycopeptides by trypsin after their cleavage from the cell surface, the following experiment was carried out to permit efficient digestion of glycopeptides appearing in solution: a cell trypsinization was conducted in which 100 μg trypsin/ml was added to the cell suspension at 0, 30, 60 and 90 min to give a final concentration of 400 μg/ml. The digestion was stopped at 120 min and the cell-free trypsinate was desalted on a column of Bio Gel P-2. The glycopeptides, which appeared in the void volume, were then chromatographed on Sephadex G-200 as described in Fig. 3. Two major peaks of radioactivity were present: peak 2 glycopeptides which overlapped with the void volume; and a peak which eluted in the salt volume representing degraded peak 3 glycopeptides.

Since the vacuum dialysis filtrates of trypsinates contained only 10—20% of the radioactivity present in cell-free trypsinates, it was possible that this was due to non-specific leakage.

To determine if this was so, the filtrates were analyzed by gel filtration on Sephadex G-50. The results are shown in Fig. 4. The major peak of radioactivity eluted at a position corresponding to an apparent molecular weight of M_r 13 700. Hence, very little non-specific leakage occurred during vacuum dialysis of trypsinates and the glycopeptides present in the filtrate may represent 'limit' glycopeptides observed in Fig. 3.

To determine whether peak 2 glycopeptide(s) shown in Fig. 1 were cleaved directly from the cell surface or were derived from cleavage of peak 1 glycoprotein(s) after being released into the incubation medium, a double-label exper-

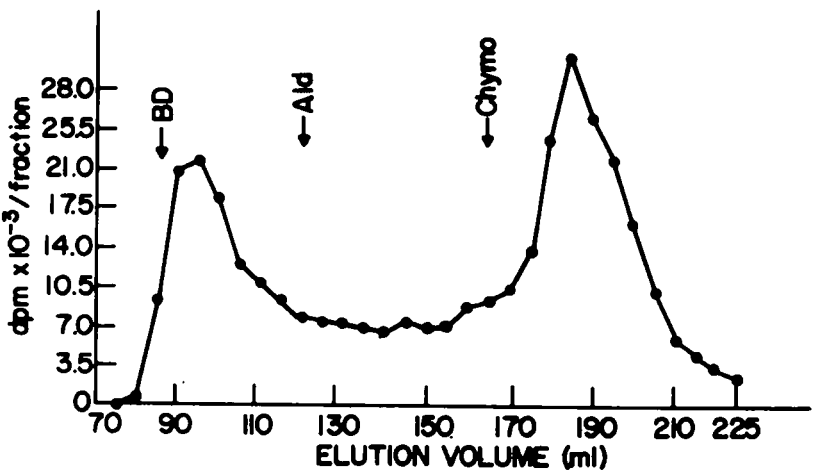

Fig. 3. Sephadex G-200 chromatography of 'limit' tryptic glycopeptides present in Bio Gel P-2 void volume. The trypsinate was desalted on Bio Gel P-2 and the void volume peak was freeze-dried. The sample was dissolved in deionized water and applied via ascending flow to a 95.0 X 1.6 cm Sephadex G-200 column equilibrated with deionized water at 18°C. Aliquots of fractions were assayed for ^3H. The elution positions of BD, Ald and chymotrypsinogen A (chymo) are indicated. Flow rate 11.6 ml/h. Load volume 2.6 ml. Fraction volume 5.0 ml.

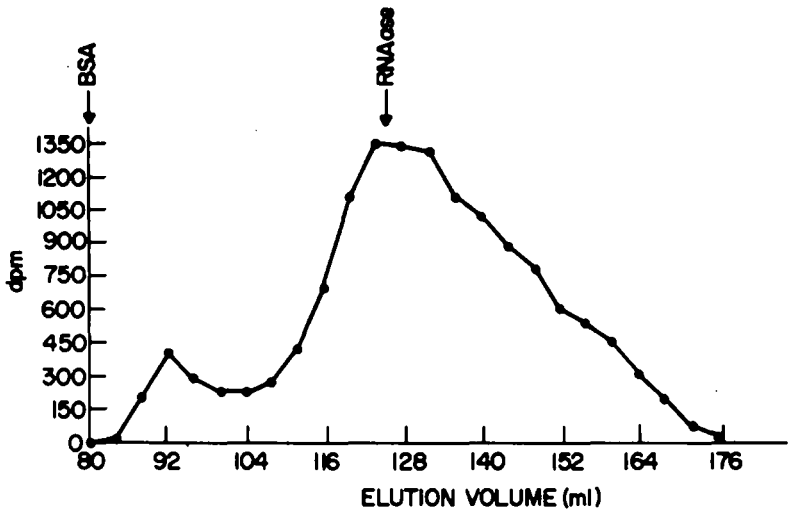

Fig. 4. Sephadex G-50 chromatography of trypsinate filtrate. The vacuum dialysis filtrate was desalted on Bio Gel P-2 and freeze-dried. The glycopeptide fraction (void volume) was dissolved in 0.1 N acetic acid and applied via ascending flow to a 95.0 X 1.6 cm Sephadex G-50 column equilibrated with 0.1 N acetic acid at 18°C. Aliquots of fractions were assayed for ^{3}H. The elution positions of BSA and RNase are indicated. Flow rate 6.1 ml/h. Load volume 2.0 ml. Fraction volume 4.0 ml.

iment was carried out. D-[^{14}C]Glucosamine-labeled cells were incubated in the cold as control cells. The cell-free incubation medium was collected and trypsin was added to give 100 μg/ml. After 120 min, soybean trypsin inhibitor was added. D-[^{3}H]Glucosamine-labeled cells were also incubated as control cells. The ^{3}H-labeled cell-free incubate (no trypsinization) was mixed with the trypsinized ^{14}C-labeled incubate, concentrated by vacuum dialysis and then chromatographed on Sephadex G-200. The elution profiles are shown in Fig. 5. The

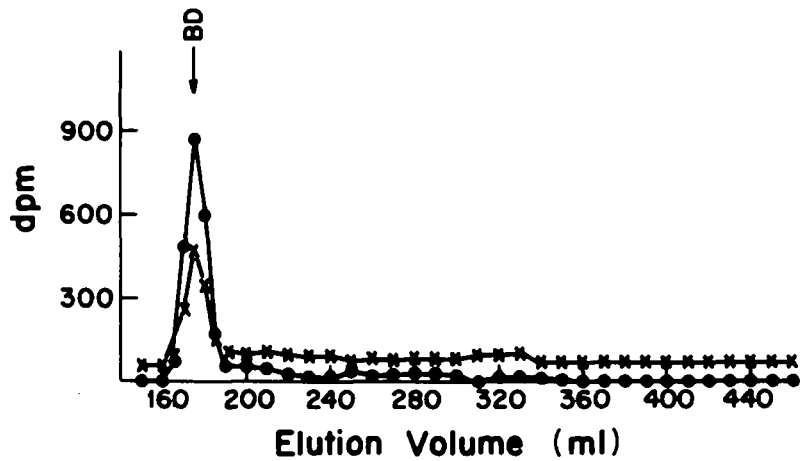

Fig. 5. Sephadex G-200 chromatography of control media retentate. The ^{14}C-labeled control medium was trypsinized and then mixed with ^{3}H-labeled control medium which was not trypsinized. After concentration by vacuum dialysis, the retentate was applied via ascending flow to a 93.1 X 2.6 cm Sephadex G-200 column equilibrated with phosphate-buffered saline at 18°C. Aliquots of fractions were assayed for ^{14}C and ^{3}H and cross-over corrections were applied to the data. The elution position of BD is indicated. Flow rate 17.8 ml/h. Load volume 5.3 ml. Fraction volume 5.0 ml. X———X, ^{14}C; ●———●, ^{3}H.

TABLE I

CONCANAVALIN A AND PEA LECTIN AFFINITY CHROMATOGRAPHY OF TRYPTIC GLYCOPEPTIDES

A concanavalin A-Sepharose column (0.9 × 15 cm) was coupled to a pea lectin-Sepharose column (0.9 × 29 cm) equilibrated with affinity-column buffer at 4°C. The samples were dissolved in affinity-column buffer and applied to the concanavalin A column via descending flow (2.3 ml/h). After passing through 2 bed vols. of buffer, the columns were disconnected and then eluted separately with 0.1 M methyl-α-D-mannopyranoside in affinity-column buffer.

Sample	Load (dpm)	% of total		
		Wash-through	Pea-bound	Concanavalin A-bound
G-200 peak 2	1 146 770	31.7	63.8	4.5
G-200 peak 3	474 000	74.4	22.8	2.8
G-50	216 160	76.0	19.3	4.8

^{3}H- and ^{14}C-elution profiles were identical demonstrating that peak 2 glycopeptide(s) were not derived from shed peak 1 glycoprotein(s).

In order to identify which glycopeptides present in the cell trypsinates were pea lectin receptors, the major glycopeptide fractions pooled from Sephadex G-200 and G-50 chromatography (Figs. 1 and 4) were chromatographed on insolubilized concanavalin A and pea lectin, sequentially. The results are summarized in Table I with the experimental details being given in the legend. Due to negligable amounts present, peak 1 material shown in Fig. 1 was not studied. The results presented in Table I show that very little of the tryptic glycopeptides released from the 6C3HED cells bound to concanavalin A. This is in agreement with previous observations [14] which showed that trypsinization of viable cells did not result in decreased tritiated concanavalin A-binding. As predicted, however, glycopeptides capable of binding to pea lectin were present.

The major pea lectin receptor was located in the Sephadex G-200 peak 2 glycopeptides for which about 65% of the radioactivity present was adsorbed by the pea lectin affinity column and was subsequently eluted with 0.1 M methyl-α-D-mannopyranoside. None of the pea-bound glycopeptide was adsorbed by concanavalin A upon re-chromatography.

The pea lectin-binding glycopeptides present in Sephadex G-200 peak 3 could have been due to cross-over contamination from peak 2 arising from pooling of fractions for analysis. To determine if this was the case and to compare apparent molecular weight distributions, the pea lectin-binding glycopeptides and the affinity chromatography wash-through glycopeptides from peak 3 were re-chromatographed on Sephadex G-200. The results are shown in Fig. 6. The two sets of glycopeptides had very similar elution profiles and they were not contaminated with peak 2 glycopeptides.

We attempted to identify the intact cell surface glycoprotein(s) which is the parent molecule giving rise to the high molecular weight tryptic glycopeptide(s) which binds to pea lectin. Pea lectin receptors, devoid of concanavalin A receptors, were isolated from cell extracts by sequential affinity chromatography on columns of insolubilized concanavalin A and pea lectin. The results for two

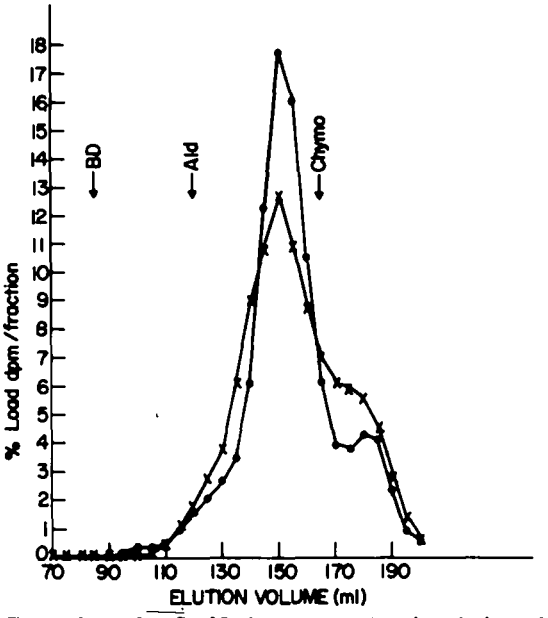

Fig. 6. Sephadex G-200 chromatography of wash-through and pea-bound glycopeptides. The wash-through and pea-bound fractions, from G-200 peak 3, prepared in Table I were dialyzed and lyophilized. The samples were dissolved in phosphate-buffered saline and applied separately via ascending flow to a 95 × 1.6 cm Sephadex G-200 column equilibrated with phosphate-buffered saline at 18°C. Aliquots of fractions were assayed for ^{3}H. The elution position of BD, Ald and chymo are indicated. Flow rate 13.2 ml/h. Load volume 3.5 ml. Fraction volume 4.9 ml. X———X, wash through; load 261 760 dpm. ●———●, pea-bound; load 81 450 dpm.

such affinity chromatography experiments are summarized in Table II with experimental details being given in the legend. About 10% of the total extracted D-[^{3}H]glucosamine-labeled macromolecules were eluted from the pea lectin column by methyl-α-D-mannopyranoside. These pea lectin-binding glycoproteins were then dialyzed, lyophilized and redissoved in phosphate-buffered saline 2.0% Nonidet P-40. They were then chromatographed on Sepharose 6B. The results are shown in Fig. 7. Three peaks of radioactivity were resolved. Peaks A and B were the components which eluted at an apparent molecular

TABLE II

CONCANAVALIN A AND PEA LECTIN AFFINITY CHROMATOGRAPHY OF CELL EXTRACTS

The affinity columns were as described for Table I. After loading the samples on the affinity column, they were washed with 4 bed vols. of affinity column buffer/0.1% Nonidet P-40. The columns were then disconnected and eluted separately with 0.1 M methyl-α-D-mannopyranoside in the above buffer.

Sample	Load (dpm)	% of load		
		Wash-through	Pea-bound	Concanavalin A-bound
Experiment 1	1 225 440	42.3	9.1	33.7
Experiment 2	1 291 200	42.8	10.4	33.0
Recovery of load dpm approx. 85%				

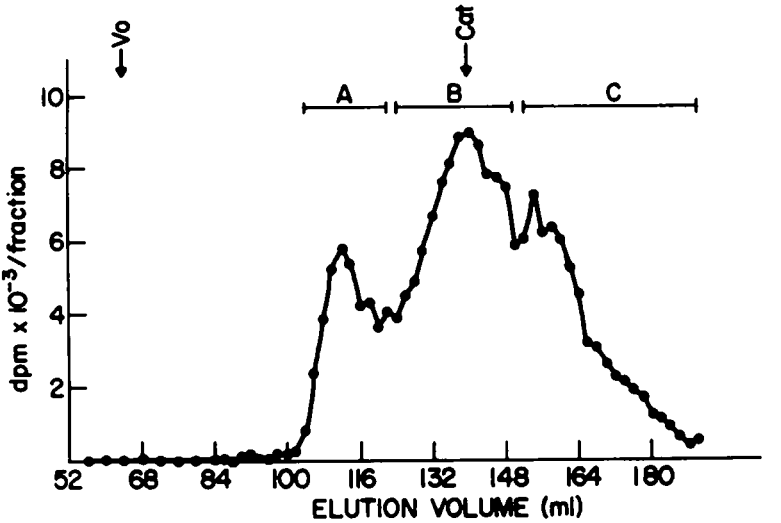

Fig. 7. Sepharose 6B chromatography of pea lectin receptors present in cell extracts. The pea-bound fractions prepared in Table II were dialyzed, lyophilized and then re-dissolved in phosphate-buffered saline/2% Nonidet P-40. The sample was applied via ascending flow to a 92.5 X 1.6 cm Sepharose 6B column equilibrated with phosphate-buffered saline/0.1% Nonidet P-40 at 18°C. Aliquots of fractions were assayed for ^{3}H. The void volume and elution position of catalase (cat) are indicated. Flow rate 10.4 ml/h. Load volume 1.5 ml. Fraction volume 2.0 ml.

weight greater than that of the Sephadex G-200 peak 2 glycopeptide(s) in Fig. 1.

Peak A was pooled, dialyzed and lyophilized. It was then digested with trypsin followed by chromatography on Sephadex G-200. Two peaks of radioactivity were resolved; one was near the void volume and one was near the salt volume (results not shown).

When peaks B and C were digested with trypsin and subsiquently chromatographed on Sephadex G-200, no radioactivity near the void volume was detected but rather it eluted near the salt volume. Hence, it appeared that Sepharose 6B peak A contained the intact cell surface pea lectin receptor which gave rise to the pea lectin-binding tryptic glycopeptide(s) present in peak 2 of Fig. 1.

Discussion

In order to understand how cell surface glycoproteins may function in cellular recognition phenomena and influence cell replication, it is necessary to identify those components which can interact with environmental solutes and surfaces. Once such components are identified, it is then possible to pursue their isolation and characterization.

While a vast number of studies on cell surface glycoproteins have been carried out over the years by numerous skilled investigators, very few such studies have led to the isolation and characterization of cell surface glycoproteins which are known to be capable of interacting with the cell environment. The most notable examples are the HLA glycoproteins [18], the H-2 glycoproteins [19], the MN glycoproteins [20], epiglycanin [21] and fibronectin [22].

The continued study of structure-function relationships for cell surface glycoproteins [23] is of paramount importance for an understanding of how metabolic modification, deletion or addition of such components may be significant in the neoplastic state.

We have, therefore, described here the identification of a pea lectin receptor present in cell trypsinates. It is reasonable to assume that the high molecular weight glycopeptide described here, which when linked to its parents glycoprotein in native form, provides a site for pea lectin binding at the cell surface. For reasons of methodology it is fortunate, however fortuitous, that this glycopeptide does not bind to concanavalin A. As can be seen from the data presented, very little of the trypic glycopeptides binds to concanavalin A.

This is in agreement with earlier observations [14] that protease treatment of the tumor cells reduced pea lectin binding but not concanavalin A binding to the cell surface.

The major cell surface pea lectin receptor described here was present in trypsinates as a glycopeptide of apparent molecular weight 200 000. This molecular weight is no doubt an overestimate; however, its large size is still quite extraordinary with respect to the sizes of tryptic glycopeptides which are usually observed with other cell systems. The most notable exeption is the tryptic glycopeptide derived from epiglycanin present on the cell surface of the TA3-Ha murine ascites tumor [24]. Because of the unexpected large size of the pea lectin receptor, we wanted to be certain that it was cleaved directly from a cell surface glycoprotein and not cleaved from glycoprotein(s) as it was shed (or secreted) into the incubation medium. That the pea lectin receptor was cleaved from a cell surface glycoprotein was demonstrated by a double-label experiment whereby it was evident that trypsinization of control incubation medium did not generate the pea lectin receptor.

The large size of the pea lectin receptor was due to cleavage at a unique trypsin-sensitive peptide bond since high levels of trypsin did not alter the gel filtration profile of the pea lectin receptor although other glycopeptides present were further degraded. We can conclude that the pea lectin-binding glycopeptide does contain other protease-sensitive bonds, however, and is not a glycosaminoglycan since the glycopeptide was absent in non-specific protease digests of cells even though up to 40% of the pea lectin receptors are cleaved from the cell surface [14]. The pea lectin-binding glycopeptide was not derived from adsorbed mouse glycoproteins accumulated during in vivo metabolic labeling because tumor cells labeled in vitro also gave rise to the glycopeptide when trypsinized (results not shown).

To begin isolation of the parent molecule which gave rise to the pea lectin-binding glycopeptide, we took advantage of the fact that concanavalin A will bind many types of D-mannosylated glycoproteins whereas the glycopeptide will not bind to concanavalin A but will bind to pea lectin. Hence, most of the contaminating glycoproteins could be removed by affinity chromatography of cell extracts on a column of concanavalin A-Sepharose which was coupled to a column of pea lectin-Sepharose.

Elution of the pea lectin column with methyl-α-D-mannopyranoside yielded a fraction of D-mannosylated (based upon pea lectin monosaccharide specificity) glycoproteins incapable of binding to concanavalin A. This fraction

accounted for 10% of the starting radioactivity derived from D-[6-^{3}H]glucosamine incorporation.

Although binding specificity studies have been carried out on pea lectin and concanavalin A [25—29] and subtle differences exist, we cannot state what oligosaccharide structure is responsible for the observed lectin-binding behavior of the pea lectin receptor parent molecule. It could be due to sialic acid content and/or to linkage position of substituents on D-mannosyl residues.

As a first attempt to determine which of the cell glycoproteins adsorbed by and eluted from pea lectin columns was the parent molecule of the pea lectin-binding glycopeptide, the fraction was chromatographed on Sepharose 6B in the presence of Nonidet P-40. Three major fractions, obviously heterogeneous with respect to molecular weight, were resolved. From apparent molecular weight considerations and from the results of Sephadex G-200 chromatography of trypsinates (results not shown), it appeared as though the parent molecule of the pea lectin-binding glycopeptide was present in peak A (Fig. 7) material. We are presently engaged in preparative isolation of this component for further characterization.

Other pea lectin-binding glycopeptides, in addition to the high molecular weight glycopeptide, were present in tryptic digests of viable cells labeled either in vivo or in vitro with D-[6-^{3}H]glucosamine. These were represented by limit tryptic glycopeptides of apparent molecular weight 13 700 and they accounted for a minor proportion of the total pea lectin-binding glycopeptides present in trypsinates. These glycopeptides have not been studied further; it is possible that they are derived from the same parent molecule as is the high molecular weight glycopeptide. It is worth noting that about 50% (by radioactivity measurements) of the glycopeptides released by trypsin bind neither to concanavalin A nor to pea lectin. Part of this material elutes from Sephadex G-200 with an apparent molecular weight of 200 000. It will be of interest to compare this component(s) with the pea lectin-binding glycopeptide.

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