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Lectin affinity chromatography of glycopeptides and oligosaccharides from normal and lectin-resistant Chinese-hamster ovary cells

Lawrence A. HUNT
Department of Microbiology and Immunology, University of Louisville School of Medicine, Louisville, KY 40292, U.S.A.

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The [^{3}H]mannose-labelled glycopeptides from two lectin-resistant lines of Chinese-hamster ovary cells were fractionated by chromatography on lentil lectin–Sepharose and concanavalin A–agarose columns and subsequently analysed by gel filtration in comparison with the glycopeptides of the parental cell line. Essentially all of the [^{3}H]mannose-labelled asparaginyl-oligosaccharides from the 'single-mutant' cells selected for resistance to phytohaemagglutinin and the 'double-mutant' cells selected for additional resistance to concanavalin A were not bound to lentil lectin, whereas approximately one-sixth of the parental-cell glycopeptides were bound and specifically eluted with α-methyl mannoside. These bound and eluted glycopeptides represented a specific subset of the complex acidic-type asparaginyl-oligosaccharides. The percentage of radiolabelled glycopeptides and oligosaccharides from each cell line that were specifically bound to concanavalin A was consistent with the relative sensitivities of the three cell lines to this lectin. The major radiolabelled species in the endoglycosidase digest of the 'double-mutant'-cell glycopeptides ($Man_4GlcNAc_1$-size neutral oligosaccharides) were not bound to concanavalin A, whereas essentially all of the other neutral-type oligosaccharides were bound. In addition, the larger neutral-type oligosaccharides ($Man_{8-9}GlcNAc_1$) were more strongly bound to concanavalin A than were either the smaller neutral-type or the di-antennary acidic-type structures.

Chinese-hamster ovary (CHO) cells selected in a single step for resistance to phytohaemagglutinin (Pha) and in a second step to concanavalin A (ConA) were previously shown to be 200-fold less sensitive to the cytotoxicity of lentil lectin from *Lens culinaris* than were the parental CHO cells (CHO-Parent) and bound decreased amounts of this lectin at their cell surface (Stanley *et al.*, 1975*a*; Stanley & Carver, 1977). In addition, the 'single-mutant' cells (CHO-Pha^R) were 4–5 times more sensitive than the CHO-Parent cells to the cytotoxicity of concanavalin A and bound 2–4 times more concanavalin A at their surface, whereas the 'double-mutant' cells (CHO-Pha^{R}ConA^R) were less sensitive and bound less concanavalin A than did either the CHO-Pha^R or the CHO-Parent cells (Stanley *et al.*, 1975*a*; Stanley & Carver, 1977).

The collection of asparagine-linked oligosaccharides for viral and cellular membrane glycoproteins synthesized in these two lectin-resistant cell lines was shown to be drastically altered in structure (Robertson *et al.*, 1978; Hunt, 1980*a*,*b*, 1981) as a result of: (i) the absence from both the CHO-Pha^R and the CHO-Pha^{R}ConA^R cells of a specific *N*-acetylglucosaminyltransferase (Stanley *et al.*, 1975*b*) that is necessary for the synthesis of complex acidic-type oligosaccharides [$(NeuAc \pm Gal\text{-}GlcNAc)_{2-4}Man_3\text{-}GlcNAc_2$-(±fucose)-Asn] and (ii) the synthesis of a truncated precursor oligosaccharide (seven instead of nine mannose residues; Fig. 1*c*) in the CHO-Pha^{R}ConA^R cells (Hunt, 1980*b*). The structures of the major mature oligosaccharide species from the CHO-Pha^R and CHO-Pha^{R}ConA^R cells are shown in Fig. 1 along with the precursor oligomannosyl core structure.

Because of the major alterations in lectin-sensitivity and lectin binding of the intact cells, it was decided to determine whether the asparagine-linked

Abbreviation used: CHO cells, Chinese-hamster ovary cells.

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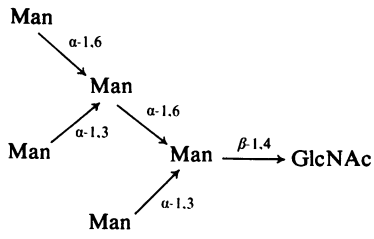
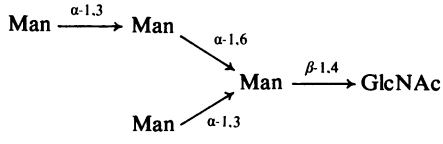
(*a*) ($Man_5GlcNAc_1$)



(*b*) ($Man_4GlcNAc_1$)



(*c*) ($Man_9GlcNAc_1$)

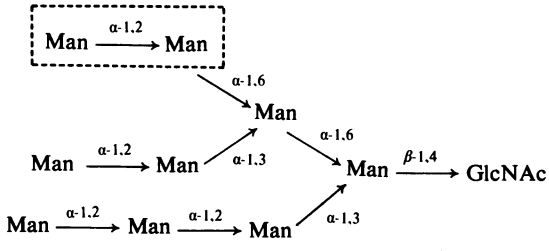


Fig. 1. *Structures of mature and precursor oligomannosyl cores*

$Man_5GlcNAc_1$ (*a*) and $Man_4GlcNAc_1$ (*b*) structures are those reported for the products of endo-β-*N*-acetylglucosaminidase D digestion of the glycopeptides from vesicular stomatitis virus replicated in the CHO-Pha^R and CHO-Pha^RConA^R cells respectively (Robertson *et al.*, 1978; Etchison & Summers, 1979). The $Man_9GlcNAc_1$ structure (*c*) is that reported for the endoglycosidase digestion product of the lipid-linked precursor oligosaccharide from vesicular-stomatitis-virus-infected CHO cells (Li & Kornfeld, 1978), shown here without the terminal glucose residues. The specific α-1,6-linked mannose residue and the additional terminal α-1,2-linked mannose residue that are presumably absent from the truncated (seven-mannose-residue) precursor oligosaccharides of the CHO-Pha^RConA^R cells (Hunt, 1980*b*) are indicated by the 'box' in (*c*).

oligosaccharides isolated from the lectin-resistant CHO cells, compared with the parental cells, would also exhibit altered affinity for concanavalin A and lentil lectin. In the present studies, [^{3}H]mannose-labelled glycopeptides and endo-β-acetylglucosaminidase-digested oligomannosyl cores were analysed by gel filtration after fractionation by lectin affinity chromatography on concanavalin A–agarose and lentil lectin–Sepharose columns.

Materials and methods

Cells and radiolabelling

The CHO parental and mutant cell lines were obtained from Dr. Pamela Stanley, Albert Einstein College of Medicine. The cell lines were Gat^-2 (CHO-Parent; a glycine-, adenosine- and thymidine-requiring auxotroph), $Gat^-2Pha^{R}1$ (CHO-Pha^R; selected from Gat^-2 for resistance to phytohaemagglutinin) and $Gat^-2Pha^{R}1ConA^{R}3B$ (CHO-Pha^RConA^R; selected from $Gat^-2Pha^{R}1$ for resistance to concanavalin A). Their nomenclature and genetic characterization have been previously described in more detail (Stanley *et al.*, 1975*a*; Stanley & Siminovitch, 1977). Cells were grown in monolayer cultures as previously described (Hunt, 1980*b*). Confluent cultures (one 75 cm^2 flask for the CHO-Parent cells and two 75 cm^2 flasks for each of the two mutant cell lines, containing approx. 1×10^7–2×10^7 cells per flask) were labelled for 48 h at 37°C in medium containing 2% (v/v) foetal bovine serum, one-third the normal amount of glucose (0.33 mg/ml) and 100 μCi of [2-^{3}H]mannose (14 Ci/mmol; New England Nuclear)/ml.

Preparation of glycopeptides and oligosaccharides

Clarified cell homogenates were prepared from the radiolabelled cells as described previously for vesicular-stomatitis-virus-infected cells (Hunt & Summers, 1976). Protein was extracted from the homogenates with butan-1-ol, washed with ethanol and digested extensively with Pronase (Calbiochem) (Hunt & Summers, 1976). These radiolabelled glycopeptides were derived from total cell protein, rather than just cell-surface glycoproteins. Glycopeptides were desalted on a column of Sephadex G-15/G-50 (Pharmacia) before glycosidase digestions or analytical gel-filtration analysis (Hunt & Summers, 1976).

Glycosidase digestions

Glycopeptides were digested with endoglycosidases as previously described (Hunt *et al.*, 1978). Purified endo-β-*N*-acetylglucosaminidase D from *Diplococcus pneumoniae* and endo-β-*N*-acetylglucosaminidase H from *Streptomyces griseus* were purchased from Miles Laboratories. This endo-β-*N*-acetylglucosaminidase D preparation lacks the exoglycosidase activities (neuraminidase, galactosidase, hexosaminidase) present in crude glycosidase mixtures from *Diplococcus pneumoniae*, and is therefore unable to digest complex acidic-type oligosaccharides to a $Man_3GlcNAc_1$ core (Hunt, 1981). The [^{3}H]mannose-labelled products of digestion with endo-β-*N*-acetylglucosaminidases D and H were

shown to be neutral oligomannosyl cores ($Man_n^{\alpha}Man^{\beta}GlcNAc$) by chromatography on Dowex AG1-X2 (formate form) (Robertson *et al.*, 1978) and digestion with jack-bean α-mannosidase (Hunt, 1980*b*).

Lectin affinity chromatography

Columns (4cm) of concanavalin A–agarose (Miles Laboratories or Sigma Chemical Co.) or lentil lectin–Sepharose (Pharmacia) were poured in 12.7cm (5in) Pasteur pipettes stuffed at the bottom with glass-wool, and washed with at least 10 column volumes of 10mM-Tris/HCl buffer, pH7.4. Samples were adjusted to pH7–8, and added to the columns in a volume of 0.5–1.0ml at room temperature. Non-bound glycopeptides/oligosaccharides were eluted with 10mM-Tris buffer, pH7.4, and bound glycopeptides/oligosaccharides were eluted with 10mM-Tris buffer, pH7.4, containing 100mM-α-methyl mannoside. Fractions of 2ml volume were collected, and 0.1ml portions were assayed for radioactivity. The total radiolabel recovered in the non-bound and the α-methyl mannoside-eluted fractions (the total radioactivity in the 0.1ml portions multiplied by 20) was approx. 90% or more of the radiolabel originally added to the lectin affinity columns. Peak fractions of non-bound or bound and eluted radiolabel were concentrated by freeze-drying before further analysis by gel filtration. Lectin affinity column resins could be re-used after very extensive washing with 10mM-Tris/HCl buffer, pH7.4, containing 1mM-$CaCl_2$ and 1mM-$MnCl_2$. Resins were stored at 4°C in the same buffer.

Gel filtration

[^{3}H]Mannose-labelled glycopeptides and oligosaccharides released by digestion with endo-β-*N*-acetylglucosaminidases D and H were analysed by gel filtration through columns (120cm × 1.5cm) of Bio-Gel P-4 (−400 mesh; Bio-Rad Laboratories) along with various unlabelled and ^{14}C-labelled gel-filtration markers (Hunt & Summers, 1976; Hunt *et al.*, 1978). These internal standards were not utilized as molecular-weight standards, but were useful in comparing the elution profiles of the same sample on different columns, or different samples run separately on the same column. Before the unknown ^{3}H-labelled samples were run, each column was calibrated for endoglycosidase digestion products by determining the elution positions of [^{3}H]mannose- or glucosamine-labelled neutral oligosaccharides of known composition ($Man_nGlcNAc_1$, $n = 3$–9) relative to the same internal standards. The oligomannosyl cores included $Man_3GlcNAc_1$, $Man_4GlcNAc_1$ and $Man_5GlcNAc_1$ from mature glycoprotein of vesicular stomatitis virus released from wild-type and lectin-resistant CHO cells (Hunt, 1980*a*), $Man_9GlcNAc_1$ and $Man_7GlcNAc_1$ from pulse-labelled 'precursor' glycopeptides derived from wild-type and lectin-resistant CHO cells (Hunt, 1980*b*), and mixtures of $Man_{5-9}GlcNAc_1$ from neutral-type glycopeptides of Rous sarcoma virus (Hunt *et al.*, 1981) or Sindbis virus (Hunt, 1981).

Results and discussion

Lentil lectin affinity chromatography of normal and mutant-cell glycopeptides

When the products of the digestion of glycopeptides from [^{3}H]mannose-labelled CHO-Parent cells by endo-β-*N*-acetylglucosaminidases D and H were chromatographed on lentil lectin–Sepharose, approx. 16% of the radiolabel was bound to the column and specifically eluted with α-methyl mannoside (Fig. 2*a*, fractions 7–9). With the equivalent chromatography of the corresponding digested glycopeptides from [^{3}H]mannose-labelled CHO-Pha^R and CHO-Pha^R-ConA^R cells, essentially all (greater than 99%) of the radiolabel was not bound and apparently eluted unretarded from the columns in the first three fractions (results not shown). This difference between the parental and lectin-resistant cell samples was expected from the previously demonstrated absence of [^{3}H]mannose-labelled acidic-type oligosaccharides from the glycoproteins of the two mutant cells (Hunt, 1980*b*) and the apparent requirement for both inner-core mannose residues and outer-branch *N*-acetylglucosamine for the tight binding of glycopeptides to lentil lectin (Kornfeld *et al.*, 1971).

The gel filtration of the lentil-lectin-non-bound and -bound glycopeptides and oligosaccharides from the CHO-Parent cells is shown in Fig. 3(*a*). Essentially all of the neutral oligomannosyl cores released by digestion with endo-β-*N*-acetylglucosaminidases D and H (fractions 70–90; $Man_{5-9}GlcNAc_1$) were in the non-bound fraction along with larger-size glycopeptides, whereas the bound and eluted fraction contained the medium-size glycopeptides (fractions 45–60). These glycopeptides resistant to digestion with endo-β-*N*-acetylglucosaminidases D and H have been shown previously to contain complex acidic-type oligosaccharides with three mannose cores by digestion with a mixture of exoglycosidases and endo-β-*N*-acetylglucosaminidase D (Hunt, 1980*b*). The endoglycosidase-released oligosaccharides were previously shown to have a common neutral oligomannosyl structure ($Man_n^{\alpha}Man^{\beta}GlcNAc$) (Hunt, 1980*b*).

Concanavalin A affinity chromatography of normal and mutant-CHO-cell glycopeptides

Compared with the chromatography on lentil lectin–Sepharose columns, a significantly higher fraction (66%) of the [^{3}H]mannose-labelled CHO-Parent-cell glycopeptides and the oligosaccharides

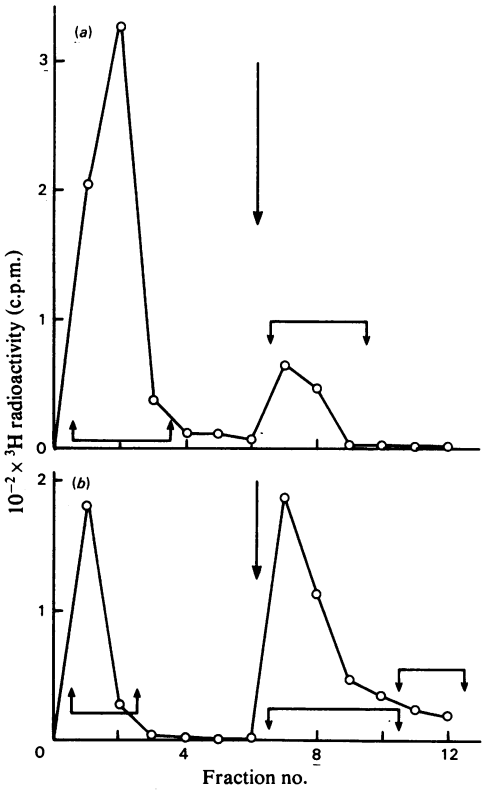


Fig. 2. *Lectin affinity chromatography of [^3H]mannose-labelled glycopeptides and oligosaccharides from CHO-Parent cells*

The products of the digestion of glycopeptides by endo-β-*N*-acetylglucosaminidases D and H were fractionated on columns of lentil lectin–Sepharose (*a*) or concanavalin A–agarose (*b*). Bound radiolabel was eluted with α-methyl mannoside starting with fraction 7 (indicated by vertical arrow), and peak tubes of non-bound or bound and eluted material were pooled for further analysis, as indicated by the bracketed arrows. Approx. 15000 c.p.m. was added to each column, and the total amount of radiolabel in the 0.1 ml portions of fractions 1–12 that were assayed for radioactivity was 717 c.p.m. for the lentil lectin column (*a*) and 650 c.p.m. for the concanavalin A column (*b*).

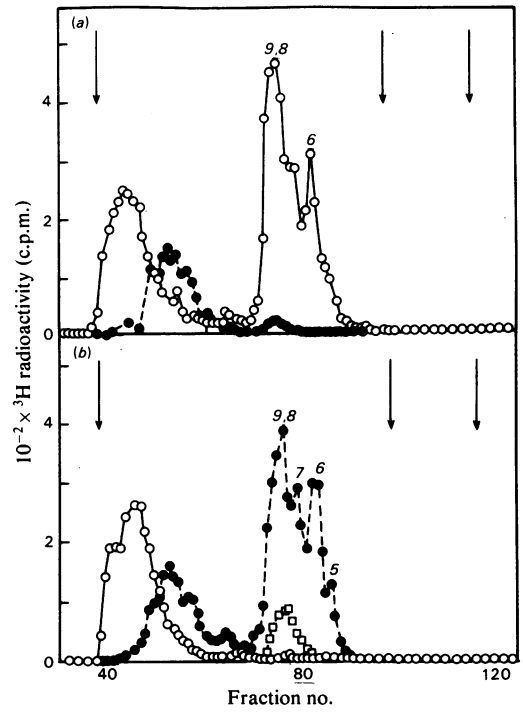


Fig. 3. *Gel-filtration analysis of lectin-bound compared with lectin-non-bound CHO-Parent-cell glycopeptides and oligosaccharides*

The fractions from the lentil lectin–Sepharose column are shown in (*a*), and those from the concanavalin A–agarose column in (*b*). Profiles of non-bound (○) or bound and eluted (●) glycopeptides and oligosaccharides analysed separately on the same column were superimposed by aligning the peak elution positions of the three internal standards (vertical arrows, from left to right: Blue Dextran, stachyose, mannose). In this and subsequent Figures the numerals (*3* to *9*) indicate the peak elution positions of oligomannosyl core structures ($Man_nGlcNAc_1$) with $n = 3$ to 9. (*b*) also shows the profile (□, fractions 73–82) of the oligosaccharides in the later-eluted fractions from the concanavalin A–agarose column (fractions 11 and 12 in Fig. 2*b*).

released by digestion with endo-β-*N*-acetylglucosaminidases D and H were bound to the concanavalin A–agarose column and specifically eluted with α-methyl mannoside (Fig. 2*b*). The results of gel filtration of the concanavalin-A-non-bound and -bound fractions (Fig. 3*b*) were similar to those with the lentil-lectin–Sepharose fractions (Fig. 3*a*) for the acidic-type glycopeptides: the non-bound fraction contained the larger-size acidic-type glycopeptides, and the bound and eluted fraction contained the medium-size glycopeptides. The previously described binding specificity of concanavalin A for various acidic-type asparaginyl-oligosaccharides (Ogata *et al.*, 1975; Baenziger & Fiete, 1979) suggested that the medium-size glycopeptides contained the di-antennary acidic-type structures [$(NeuAc \pm Gal\text{-}GlcNAc)_2Man_3GlcNAc_2$-(±fucose)-Asn], whereas the larger glycopeptides contained tri- and/or tetra-antennary acidic-type structures [$(NeuAc \pm Gal\text{-}GlcNAc)_{3-4}Man_3GlcNAc_2(\pm fucose)$-Asn]. In addition, the gel-filtration

analysis indicated that the neutral-type oligomannosyl structures ($Man_{5-9}GlcNAc_1$) were all bound to the concanavalin A–agarose column, with the later-eluted fractions from the concanavalin A–agarose column (fractions 11 and 12 in Fig. 2*b*) enriched for the largest-size neutral oligosaccharides ($Man_{7-9}GlcNAc_1$; fractions 72–82 in Fig. 3*b*). It was obvious from this comparison of concanavalin A and lentil lectin affinity chromatography that only a specific subset of the total concanavalin-A-bound asparaginyl-oligosaccharides were specifically bound by lentil lectin, and recent studies with the acidic-type glycopeptides from viral glycoprotein (Hunt, 1982) suggested that only fucose-containing diantennary acidic-type structures were tightly bound to lentil lectin. This proposed specificity is similar to that reported by Kornfeld *et al.* (1981) for both lentil lectin and pea lectin from *Pisum sativum*. Their studies also indicated that a specific subset of triantennary acidic glycopeptides (containing an additional β-1,6-linked *N*-acetylglucosamine and not bound to concanavalin A) could be specifically bound to lentil lectin and pea lectin affinity columns.

The radiolabelled profiles for the corresponding concanavalin A–agarose affinity chromatography of the products of the digestion of glycopeptides from the [^{3}H]mannose-labelled CHO-Pha^R and CHO-Pha^{R}ConA^R cells by endo-β-*N*-acetylglucosaminidases D and H are shown in Fig. 4. Approx. 89% of the total radiolabel in the CHO-Pha^R-cell oligosaccharides was bound and specifically eluted (fractions 7–11), whereas only 36% of the radiolabel in the oligosaccharides from the concanavalin-A-resistant CHO-Pha^{R}ConA^R cells was bound. Thus the order of increasing concanavalin-A-sensitivity of the CHO-cell lines (Stanley *et al.*, 1975*a*) was consistent with the relative amounts of [^{3}H]mannose-labelled glycopeptides and oligosaccharides specifically bound to concanavalin A–agarose columns: CHO-Pha^{R}ConA^R (36%), CHO-Parent (66%) and CHO-Pha^R (89%).

The gel-filtration profiles of the unfractionated oligosaccharides and peak fractions of concanavalin-A-bound and -non-bound oligosaccharides from the two mutant cell samples are shown in Fig. 5 for the CHO-Pha^R sample and in Fig. 6 for the CHO-Pha^{R}ConA^R sample. The non-bound fraction of the CHO-Pha^R oligosaccharides (Fig. 5*b*) contained a peak of $Man_4GlcNAc_1$-size oligosaccharides (fractions 97–100) plus a small fraction of the major $Man_5GlcNAc_1$-size species (fractions 92–95), whereas the peak fractions of bound and eluted oligosaccharides recovered from the concanavalin A–agarose column (fractions 7–9 in Fig. 4*a*) included a major $Man_5GlcNAc_1$ species (proposed structure shown in Fig. 1*a*), smaller amounts of $Man_6GlcNAc_1$- and $Man_3GlcNAc_1$-size oligosaccharides, and minor amounts of $Man_{7-9}GlcNAc_1$-size oligosaccharides. The apparent tighter binding and inefficient recovery of larger oligomannosyl structures ($Man_{7-9}GlcNAc_1$) was similar to that seen with the neutral-type oligosaccharides for the CHO-Parent-cell glycopeptides in Fig. 3(*b*), and suggested that the 89% value for the concanavalin-A-bound oligosaccharides may have been an underestimate of the actual value. A similar result was

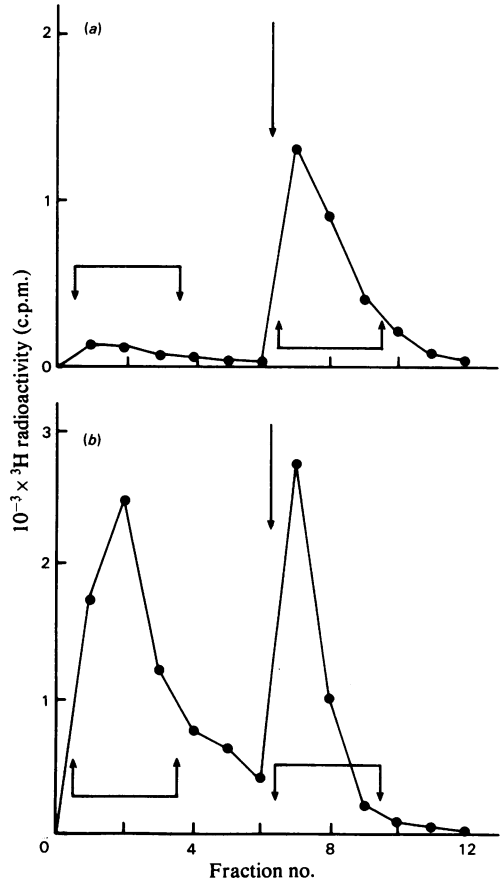


Fig. 4. *Concanavalin A affinity chromatography of [^{3}H]mannose-labelled oligosaccharides from mutant CHO cells*

The conditions were identical with those described for Fig. 2(*b*) for the products of the digestion of CHO-Parent glycopeptides by endo-β-*N*-acetylglucosaminidases D and H. (*a*, CHO-Pha^R cells; *b*, CHO-Pha^{R}ConA^R cells.) The CHO-Pha^R-cell oligosaccharides added to the column contained approx. 70000c.p.m., and the total radiolabel in the 0.1 ml portions of fractions 1–12 (*a*) was 3305 c.p.m. The CHO-Pha^{R}ConA^R-cell oligosaccharides added to the column contained approx. 240000c.p.m., and the total radiolabel in the 0.1 ml portions of fractions 1–12 (*b*) was 11310c.p.m.

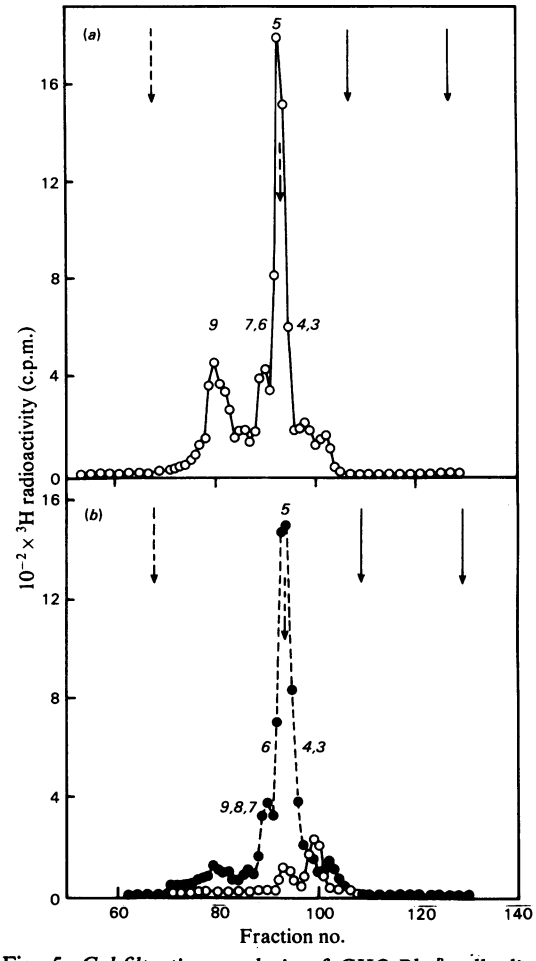


Fig. 5. *Gel-filtration analysis of CHO-Pha^R-cell oligosaccharides before and after concanavalin A–agarose fractionation*

The conditions of gel filtration were identical with those in Fig. 3, except that two additional [^{14}C]-glucosamine-labelled internal standards (indicated by broken vertical arrows) were included: a large neutral-type glycopeptide and $Man_5GlcNAc_1$ oligosaccharide from CHO-Pha^R cells (Hunt, 1980*b*). The elution position of Blue Dextran (void volume, approximately fraction 40) is not shown in these profiles, and the radioactivity profiles for fractions 31–60 are also not shown because only background levels of radioactivity were detected in these fractions. (*a*) Products of the digestion of glycopeptides by endo-β-*N*-acetylglucosaminidases D and H without concanavalin A–agarose fractionation. (*b*) Profiles of the [^{3}H]mannose-labelled oligosaccharides from the concanavalin-A-non-bound (○) and concanavalin-A-bound and eluted fractions (●) from Fig. 4(*a*) were superimposed.

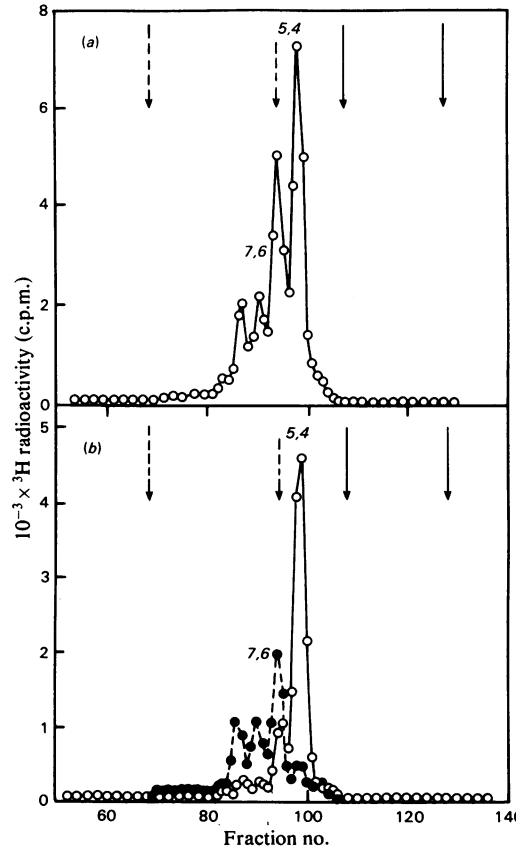


Fig. 6. *Gel-filtration analysis of CHO-Pha^{R}ConA^R-cell oligosaccharides before and after concanavalin-A–agarose fractionation*

The conditions of gel filtration were identical with those in Fig. 5, and the superimposed profiles in (*b*) represent the concanavalin-A-non-bound compared with concanavalin-A-bound and eluted fractions shown in Fig. 4(*b*).

previously reported in comparative studies with $Man_{5-6}GlcNAc_2$–peptides from ovalbumin and $Man_9GlcNAc_2$–peptides from calf thyroglobulin (Ogata *et al.*, 1975). Baenziger & Fiete (1979) reported that the presence of additional α-linked mannose residues did not significantly increase the association constants of glycopeptides binding to concanavalin A, but they compared only structures with three, five and six mannose residues.

With the corresponding gel-filtration profiles of the CHO-Pha^{R}ConA^R-cell-derived oligosaccharides, almost all of the major peak of $Man_4GlcNAc_1$-size (proposed structure shown in Fig. 1*b*) and a minor fraction of the $Man_5GlcNAc_1$-size oligosaccharides were not bound, whereas the remainder of the oligomannosyl cores ($Man_{5-7}GlcNAc_1$) were efficiently recovered from the concanavalin A–agarose column in the α-methyl mannoside-eluted fractions (Fig. 6).

Although most of the radiolabelled $Man_5GlcNAc_1$-size oligosaccharide from both the CHO-Pha^R-cell and CHO-Pha^RConA^R-cell protein was bound to concanavalin A–agarose columns (Figs. 5*b* and 6*b*), the structures of the two oligosaccharides were different, since the five mannose oligosaccharides from the CHO-Pha^R cells were mostly sensitive to endo-β-*N*-acetylglucosaminidase D, whereas those from the CHO-Pha^RConA^R cells were mostly resistant to this enzyme (Hunt, 1980*b*). The concanavalin A–agarose chromatographic properties of the $Man_4GlcNAc_1$ structure indicated that the presence of at least two α-linked mannose residues with free hydroxy groups at C-3, C-4 and C-6 was not sufficient for binding of this oligosaccharide to concanavalin A, as suggested by Ogata *et al.* (1975). Harpaz & Schachter (1980) have reported that a glycopeptide with a similar four-mannose-residue core was also not bound to concanavalin A–Sepharose columns, and they concluded that the two interacting mannose residues must be attached to a single residue for tight binding (as seen for the $Man_5GlcNAc_1$ structure in Fig. 1*a*).

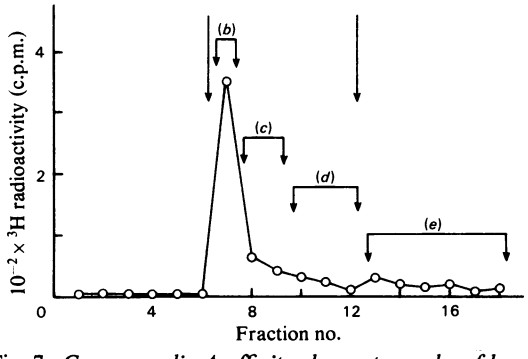


Fig. 7. *Concanavalin A affinity chromatography of large oligomannosyl cores from [^3H]mannose-pulse-labelled CHO-Pha^R and CHO-Pha^RConA^R cells*

$Man_9GlcNAc_1$ and $Man_7GlcNAc_1$ oligosaccharides were obtained by preparative gel filtration of endo-β-*N*-acetylglucosaminidase-H-digested glycopeptides from 30min-radiolabelled CHO-Pha^R and CHO-Pha^RConA^R cells respectively (Hunt, 1980*b*). Approximately equal radiolabelled amounts of the two oligosaccharides (total of approx. 16000c.p.m.) were mixed and then fractionated on a concanavalin A–agarose column. Fractions 7–12 were collected while the column was washed with 50mM-α-methyl mannoside, and fractions 13–18 were collected while it was washed with 100mM-α-methyl mannoside. The total amount of radiolabel in the 0.1ml portions of fractions 1–18 that were assayed for radioactivity was 798c.p.m. The bracketed arrows indicate the fractions pooled for further analysis, with (*b*)–(*e*) corresponding to gel-filtration profiles of these samples in Figs. 8(*b*)–8(*e*).

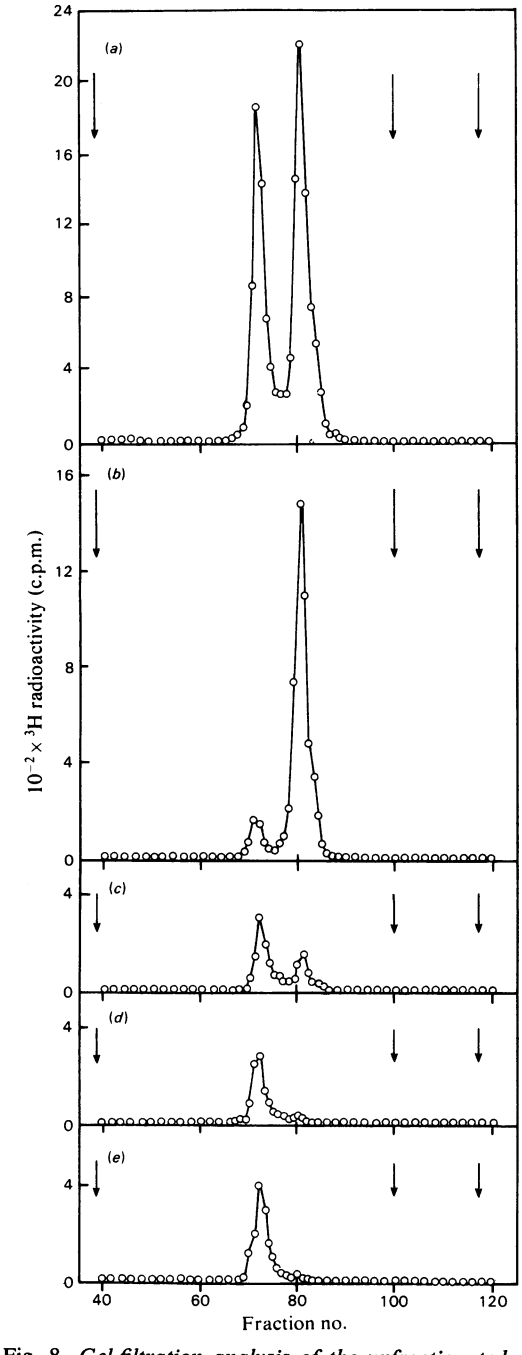


Fig. 8. *Gel-filtration analysis of the unfractionated and concanavalin-A–agarose-fractionated $Man_9GlcNAc_1$ and $Man_7GlcNAc_1$ oligosaccharides*

The conditions of gel filtration were identical with those in Fig. 3. (*a*) Profile of unfractionated mixture of $Man_9GlcNAc_1$ from CHO-Pha^R cells and $Man_7GlcNAc_1$ from CHO-Pha^RConA^R cells; (*b*)–(*e*) profiles of various concanavalin-A-bound and eluted fractions from the chromatography profile shown in Fig. 7.

The apparent concanavalin A binding of the $Man_{5-7}GlcNAc_1$-size structures from the CHO-Pha^RConA^R cells (all presumably lacking the Man-α1,2-Man-α1,6- disaccharide structure present in the nine-mannose-residue precursor oligosaccharide shown in Fig. 1*c*) suggested that the two necessary mannose residues had to be adjacent, but not necessarily attached to the same residue. A fraction of the oligosaccharides from the CHO-Pha^RConA^R-cell protein seemed to be strongly retarded rather than actually bound to the concanavalin A–agarose column (fractions 4–6, Fig. 4*b*), and gel filtration indicated that minor amounts of the total $Man_{4-7}GlcNAc_1$-size oligosaccharides were present in this retarded fraction (result not shown).

Differential concanavalin A binding of CHO-Pha^R and CHO-Pha^RConA^R precursor oligosaccharides

In order to elucidate possible differences in the binding of the largest precursor oligomannosyl core structures (Fig. 1*c*) from the CHO-Parent and CHO-Pha^R cells (nine mannose residues) and the CHO-Pha^RConA^R cells (seven mannose residues), a mixture of the $Man_9GlcNAc_1$ and $Man_7GlcNAc_1$ oligosaccharides was subjected to concanavalin A–agarose chromatography, followed by gel filtration of individual eluted fractions from the column. Essentially all of the radiolabel was bound to the column and subsequently eluted by extensive washing with 50 mM- and 100 mM-α-methyl mannoside (Fig. 7). The radiolabel in the first eluted fraction (fraction 7, Fig. 7) was greatly enriched in the smaller $Man_7GlcNAc_1$ species (Fig. 8*b*). The ratio of radiolabel in $Man_9GlcNAc_1$ to that in $Man_7GlcNAc_1$ was 0.1:1.0 for this fraction (Fig. 8*b*), compared with a ratio of 0.8:1.0 in the original unfractionated oligosaccharides shown in Fig. 8(*a*). In contrast, the later-eluted fractions from the concanavalin A–agarose column were greatly enriched for the larger $Man_9GlcNAc_1$ structure (Figs. 8*d* and 8*e*).

In summary. the present studies have demonstrated a differential specificity and relative affinity of concanavalin A–agarose for a series of neutral oligomannosyl core structures ($Man_nGlcNAc_1$) with increasing mannose content and branching complexity. The major [^{3}H]mannose-labelled oligosaccharide from the CHO-Pha^RConA^R-cell protein was not bound to concanavalin A, and the large neutral oligosaccharides ($Man_{8-9}GlcNAc_1$) that were not present in the glycopeptides of these 'double-mutant' cells were very tightly bound to concanavalin A–agarose. Although the relative amounts of [^{3}H]mannose-labelled glycopeptides and oligosaccharides that were bound to the concanavalin A–agarose and lentil lectin–Sepharose columns were consistent with the relative lectin-sensitivity and lectin binding of the intact cells of the wild-type and mutant CHO cell lines (Stanley *et al.*, 1975*a*; Stanley & Carver, 1977), no direct comparison is possible because the studies with intact cells were performed under different ionic conditions and presumably involved only those oligosaccharides present on cell-surface proteins.

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References

Baenziger, J. U. & Fiete, D. (1979) *J. Biol. Chem.* **254**, 2400–2407

Etchison, J. R. & Summers, D. F. (1979) *J. Supramol. Struct. Suppl.* **3**, 205 (Abstr.)

Harpaz, N. & Schachter, H. (1980) *J. Biol. Chem.* **255**, 4894–4902

Hunt, L. A. (1980*a*) *J. Virol.* **35**, 362–370

Hunt, L. A. (1980*b*) *Cell* **21**, 407–415

Hunt, L. A. (1981) *Virology* **113**, 534–543

Hunt, L. A. (1982) *J. Virol. Methods* **4**, 283–295

Hunt, L. A. & Summers, D. F. (1976) *J. Virol.* **20**, 646–657

Hunt, L. A., Etchison, J. R. & Summers, D. F. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 754–758

Hunt, L. A., Lamph, W. & Wright, S. E. (1981) *J. Virol.* **37**, 207–215

Kornfeld, S., Rogers, J. & Gregory, W. (1971) *J. Biol. Chem.* **246**, 6581–6586

Kornfeld, K., Reitman, M. L. & Kornfeld, R. (1981) *J. Biol. Chem.* **256**, 6633–6640

Li, E. & Kornfeld, S. (1978) *J. Biol. Chem.* **253**, 7762–7770

Ogata, S., Muramatsu, T. & Kobata, A. (1975) *J. Biochem. (Tokyo)* **78**, 687–696

Robertson, M. A., Etchison, J. R., Robertson, J. S., Summers, D. F. & Stanley, P. (1978) *Cell* **13**, 515–526

Stanley, P. & Carver, J. P. (1977) *Adv. Exp. Med. Biol.* **84**, 265–284

Stanley, P. & Siminovitch, L. (1977) *Somatic Cell Genet.* **3**, 391–405

Stanley, P., Callibot, V. & Siminovitch, L. (1975*a*) *Cell* **6**, 121–128

Stanley, P., Narasimhan, S., Siminovitch, L. & Schachter, H. (1975*b*) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3323–3327