# Isolation and Characterization of Cyanogen Bromide Fragments and a Glycopeptide from the *Dolichos biflorus* Lectin†

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ABSTRACT: The 110000 molecular weight *Dolichos biflorus* lectin is a glycoprotein composed of four subunits of approximately 27000 molecular weight with one methionine residue per subunit (Carter and Etzler, 1975b). Cyanogen bromide cleavage of the lectin yielded two fragments with approximate molecular weights of 15000 and 12000 as determined by electrophoresis on sodium dodecyl sulfate gels. Only the 15000 molecular weight fragment stained for carbohydrate with the periodic acid–Schiff stain. The two fragments were isolated, and their amino acid compositions were determined. The 15000 molecular weight fragment was identified as the amino terminal segment of the lectin subunits by $NH_2$-terminal amino acid analysis. A glycopeptide with a minimum molecular weight of 1100 was isolated from the lectin by exhaustive Pronase digestion. Complete acid hydrolysis of the glycopeptide yielded aspartic acid, mannose, and *N*-acetylglucosamine in the ratio of 1:4–5:1–2. Partial acid hydrolysis of the glycopeptide produced a component which had an identical mobility with commercial *N*-acetylglucosaminylasparagine in high voltage paper electrophoresis. The data indicate that the carbohydrate unit of the lectin is bound to the amino terminal half of the subunits by a glycosylamine linkage between *N*-acetylglucosamine and asparagine.

The seeds of the *Dolichos biflorus* plant contain a lectin that agglutinates type A erythrocytes (Bird, 1951) and precipitates blood group A substance (Boyd and Shapleigh, 1954; Bird, 1959). The ability of this lectin to combine with blood group A active material is due to its specificity for terminal nonreducing $\alpha$-linked *N*-acetyl-D-galactosaminyl residues (Etzler and Kabat, 1970). The lectin is a glycoprotein (Etzler and Kabat, 1970; Font et al., 1971) and has been fractionated into two electrophoretically distinguishable forms (A and B) by chromatography on concanavalin A-Sepharose. The A and B forms of the lectin have identical specificities and appear to differ only in their carbohydrate compositions. Form A can be further subdivided into several fractions with slightly different carbohydrate contents (Carter and Etzler, 1975a). Such carbohydrate heterogeneity has been observed in a number of glycoproteins (for review, see Spiro, 1972).

The molecular weights of the various forms of the *Dolichos biflorus* lectin range from 109000 for the minor form B which contains the least carbohydrate to 113000 for the predominant form A (Carter and Etzler, 1975a). Form A of the lectin is a tetramer composed of two types of subunits, IA (27700 g/mol) and IIA (27300 g.mol). Although these two types of subunits have differences at their carboxyl terminal ends, they have similar amino acid and carbohydrate compositions, have the same amino-terminal amino acid (alanine), and show reactions of identity when tested with antisera prepared against the native lectin or against either subunit. The subunits thus appear to represent alterations of a single type of subunit (Carter and Etzler, 1975b).

Each subunit of the lectin contains one methionine residue per mole (Carter and Etzler, 1975b). In the present paper we describe the isolation and characterization of the fragments produced by cyanogen bromide cleavage of the lectin. We also report the isolation and properties of a glycopeptide isolated from the lectin by Pronase digestion.

## Materials and Methods

*Isolation of Lectin.* The *Dolichos biflorus* lectin was isolated from seed extracts as previously described (Etzler and Kabat, 1970; Etzler, 1972) by adsorption onto insoluble polyleucyl hog blood group A + H substance (Kaplan and Kabat, 1966) and specific elution from this immunoadsorbent with 0.01 *M* *N*-acetyl-D-galactosamine.

*Analytical Methods.* Protein concentration was determined by nitrogen determination using a modified ninhydrin method (Schiffman et al., 1964).

Amino acid analyses were performed by AAA Laboratories (Seattle, Wash.) after hydrolysis of the samples in 6 *N* HCl for 25, 48, and 72 hr at 110°. Tryptophan was determined after a 48-hr alkaline hydrolysis at 135° (Hugli and Moore, 1972).

$NH_2$-terminal amino acids were determined by labeling proteins and peptides with 2,4-dinitrofluorobenzene following the basic method of Phillips (1958). The 2,4-dinitrobenzene derivatives of amino acids (Dnp-AA)[1] were identified by cochromatography with standard Dnp-AA derivatives on silica gel G plates (Quanta 1 g Q1; Quantum Industries, Fairfield, N.J.). The plates were developed in benzene–pyridine–acetic acid (80:20:2) (Niederwieser, 1972) and spots were detected under an ultraviolet light at 365 nm.

Quantitation of free amino groups was performed utilizing trinitrobenzenesulfonic acid (Habeeb, 1966) with calibration curves of aspartic acid and commercial Asn-GlcNAc.

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[1] Abbreviations used are: Dnp, 2,4-dinitrobenzene; GlcNAc, *N*-acetyl-D-glucosamine.

Carbohydrate analyses were performed as previously described (Carter and Etzler, 1975a) by gas–liquid chromatography of the alditol acetate derivatives of sugars released after acid hydrolysis. The glycopeptide was hydrolyzed in 3 *N* HCl for 3 hr at 100°, a condition found to give maximal sugar release from the glycopeptide with minimal sugar destruction. Colorimetric methods also employed to analyze carbohydrate content and to monitor fractionation of the glycopeptide included the orcinol procedure to detect hexoses using mannose and glucose as standards and a modified Elson–Morgan method for hexosamine as described by Kabat (1961).

Hemagglutination inhibition was performed with a Takatsy microtitrator using 0.025-ml loops and 2.0% human type A erythrocyte suspensions. Inhibition of agglutination was recorded after 1 hr incubation at room temperature.

*Physicochemical Methods.* Polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate was performed by the method of Weber and Osborn (1969). The gels were stained with either Coomassie Brilliant Blue (Weber and Osborn, 1969) or by the periodic acid–Schiff reaction (Segrest and Jackson, 1972). Molecular weights of the peptides produced by cyanogen bromide cleavage of the lectin were estimated from their mobilities on gels of 10, 12, 14, and 16% polyacrylamide concentrations in 0.1% sodium dodecyl sulfate as described by Segrest and Jackson (1972).

Protein samples were also run on a pH 9.7 glycine gel system in the presence of 0.1% sodium dodecyl sulfate and 8.0 *M* urea (Wu and Bruening, 1971).

Charge to friction ratios of the peptides were determined by measurements of mobility retardation with increasing gel concentrations (Hedrick and Smith, 1968) on pH 9.7 glycine gels in the presence of 8.0 *M* cyanate free urea (Wu and Bruening, 1971).

*Partial Acid Hydrolysis of Glycopeptide.* Identical samples of isolated *Dolichos biflorus* glycopeptide (75.4 nmol) were hydrolyzed in 200-μl aliquots of 1 *N* HCl at 100° for 0-, 5-, 15-, 25-, and 40-min intervals. Samples (180.4 nmol) of commercial 2-acetamido-1-β-(L-β-aspartamido)-1,2-dideoxy-D-glucose (Bachem Inc., Marina Del Ray, Calif.) were subjected to the same hydrolysis conditions. Immediately after hydrolysis the samples were frozen, lyophilized, and dissolved in 50 μl of $H_2O$, and aliquots were spotted on Whatman No. 1 chromatography paper and subjected to high voltage paper electrophoresis, color development, and spot quantitation as described by Dreyer and Bynum (1967).

## Results

*CNBr Cleavage of Dolichos biflorus Lectin.* Preliminary experiments, using detergents and various concentrations of formic acid as solvents, established that maximal CNBr cleavage of the *Dolichos biflorus* lectin occurred in 90% formic acid. Following the general method of Gross (1967), 350 mg of CNBr was added to 60–80 $OD_{280}$ units of lectin dissolved in 10 ml of 90% formic acid. The reaction mixture was incubated at room temperature for 24 hr and then dialyzed against $H_2O$ and lyophilized. The cyanogen bromide cleavage produced two peptides with different mobilities in electrophoresis on sodium dodecyl sulfate polyacrylamide gels (Figure 1, gels 1 and 2).

*Isolation and Characterization of CNBr Fragments.* Molecular weights of the lectin subunits and the peptides produced by CNBr cleavage were estimated on sodium dodecyl sulfate polyacrylamide gels by the method of Segrest and Jackson (1972). By this method, molecular weights of 15000 and 12000 were obtained for the two CNBr fragments and a molecular weight of 27000 was obtained for the intact lectin subunits; this latter value is in agreement with the molecular weights previously determined by sedimentation equilibrium for the isolated subunits (Carter and Etzler, 1975b).

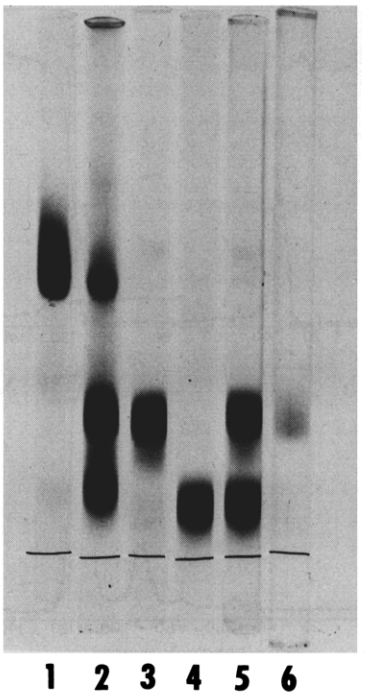


FIGURE 1: Polyacrylamide gel electrophoresis of cyanogen bromide cleaved *Dolichos biflorus* lectin on pH 7.2 phosphate gels in the presence of 0.1% sodium dodecyl sulfate (Weber and Osborn, 1969). (1) *Dolichos biflorus* lectin (27000 mol wt subunit); (2) *Dolichos biflorus* lectin after cleavage with CNBr (27000 mol wt subunit, 15000, and 12000 mol wt CNBr peptides); (3) peak 1, Figure 2A (15000 mol wt CNBr peptide); (4) peak 2b, Figure 2B (12000 mol wt CNBr peptide); (5) peak 1, Figure 2A + peak 2b, Figure 2B. Gels 1–5 were stained with Coomassie Brilliant Blue (Weber and Osborn, 1969). (6) Peak 1, Figure 2A + peak 2b, Figure 2B stained with periodic acid–Schiff stain (Segrest and Jackson, 1972). Direction of migration is from top (−) to bottom (+).

Mobility retardation experiments with increasing gel concentrations (Hedrick and Smith, 1968) on discontinuous polyacrylamide glycine gels (pH 9.7) in the presence of 8.0 *M* cyanate free urea (Wu and Bruening, 1971) indicated similar molecular weights for the two CNBr peptides but differentiable charge to friction ratios. This apparent difference in charge was used to separate the CNBr peptides by passage of the peptide mixture (CNBr fragments + small quantity of intact subunit) over a DEAE-cellulose column in 8.0 *M* cyanate-free urea (Figure 2A). Under these conditions, the 15000 molecular weight peptide (peak 1) did not bind to the column while the 12000 molecular weight peptide and intact subunit were eluted together in a high salt wash (peak 2). The pooled fraction (peak 2) subunit and 12000 molecular weight peptide were dialyzed against $H_2O$, lyophilized, and then redissolved in 1% dodecyl sulfate and fractionated on a Sephadex G-200 column in 1% sodium dodecyl sulfate (Figure 2B). As can be seen in Figure 1 (gels 3, 4, and 5), the combination of ion-exchange and molecular sieve chromatography fractionated the two CNBr fragments to electrophoretic homogeneity. Occasionally, trace quantities of 15000 molecular weight peptide re-

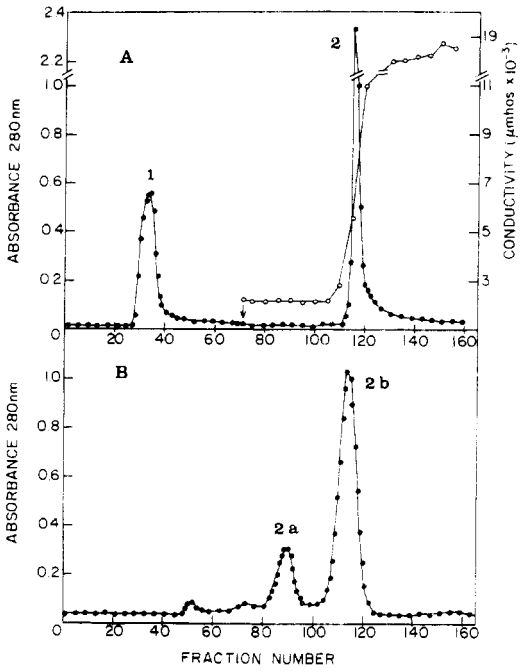


FIGURE 2: (A) Ion exchange chromatography of cyanogen bromide cleaved *Dolichos biflorus* lectin on DEAE-cellulose in 8.0 *M* urea. Ion exchange chromatography was carried out on DEAE-cellulose (Whatman DE52) columns, 1.7 × 57 cm, in the presence of 8.0 *M* cyanate free urea and 0.06 *M* Tris-HCl buffer (pH 7.3) at room temperature. The dialyzed and lyophilized CNBr-cleaved *Dolichos biflorus* sample was dissolved in 10 *M* urea–0.06 *M* Tris-HCl buffer (pH 7.4) at room temperature (10.0 ml total, $OD_{280}$ = 6.3), then incubated at 65° for 1 hr. The sample was then applied to the DEAE-cellulose column and the column washed with 1 bed volume of 0.06 *M* Tris-HCl buffer (pH 7.3) containing 8.0 *M* urea. At tube 71 (↓) a 0.5 *M* NaCl wash in 0.06 *M* Tris-HCl buffer (pH 7.3) containing 8.0 *M* urea was applied (3.0-ml fractions were collected). (●) Absorbance, 280 nm, (○) conductivity, μmhos × $10^{-3}$. The pooled fractions from peaks 1 and 2 were dialyzed against $H_2O$ and lyophilized. (B) Gel filtration chromatography of cyanogen bromide cleaved *Dolichos biflorus* lectin in 1% sodium dodecyl sulfate. The dialyzed and lyophilized peak 2 of Figure 2A was dissolved in 1% dodecyl sulfate (4.2 ml total, $OD_{280}$ = 6.6), heated in a boiling water bath for 5 min, and applied to a Sephadex G-200 column (40–120-μ bead size, 1.9 × 105 cm) in 1.0% sodium dodecyl sulfate. The column was developed with 1% sodium dodecyl sulfate and 2-ml fractions were collected. (○) Absorbance, 280 nm. Pooled fractions from peaks 2a and 2b were dialyzed against 80% acetone–0.001 *M* NaCl, 80% acetone, and $H_2O$ in succession to remove sodium dodecyl sulfate as described by Rice (1974).

Table I: Amino Acid Analyses[a] of *Dolichos biflorus* Lectin Subunits and Cyanogen Bromide Derived Peptides.

| Amino Acid | μmol of AA/mg of Protein (Peptide)[b] | | | μmol of AA/μmol of Protein (Peptide)[b] | | |
|---|---|---|---|---|---|---|
| | 27000 Mol wt Subunit | 15000 Mol wt Peptide | 12000 Mol wt Peptide | 27000 Mol wt Subunit | 15000 Mol wt Peptide | 12000 Mol wt Peptide |
| Ala | 0.793 | 0.815 | 0.585 | 20.6 | 11.3 | 7.0 |
| Arg | 0.224 | 0.183 | 0.217 | 5.8 | 2.5 | 2.6 |
| Asp | 0.963 | 0.919 | 0.756 | 25.0 | 12.8 | 9.1 |
| Glu | 0.573 | 0.511 | 0.532 | 14.9 | 7.1 | 6.4 |
| Gly | 0.559 | 0.686 | 0.351 | 14.5 | 9.5 | 4.2 |
| His | 0.095 | 0.000 | 0.156 | 2.5 | 0.0 | 1.9 |
| Ile | 0.530 | 0.419 | 0.496 | 13.7 | 5.8 | 6.0 |
| Leu | 0.651 | 0.414 | 0.722 | 16.9 | 5.8 | 8.7 |
| Lys | 0.338 | 0.398 | 0.150 | 8.8 | 5.5 | 1.8 |
| Met | 0.043 | 0.000 | 0.000 | 1.1 | 0.0 | 0.0 |
| Phe | 0.434 | 0.651 | 0.120 | 11.2 | 9.1 | 1.4 |
| Pro | 0.415 | 0.473 | 0.257 | 10.8 | 6.6 | 3.1 |
| Ser[c] | 1.181 | 1.334 | 0.901 | 30.6 | 18.6 | 10.8 |
| Thr[c] | 0.583 | 0.495 | 0.549 | 15.1 | 6.9 | 6.6 |
| Trp[d] | 0.191 | 0.109 | 0.092 | 5.0 | 1.5 | 1.1 |
| Tyr | 0.291 | 0.217 | 0.233 | 7.5 | 3.0 | 2.8 |
| Val | 0.680 | 0.644 | 0.610 | 17.6 | 9.0 | 7.3 |
| Cys[e] | 0.000 | | | | | |

[a] Amino acid analyses were performed by AAA Laboratories after hydrolysis in 6 *N* HCl for 25, 48, and 72 hr at 110°. [b] The milligrams of protein (peptide) was based on nitrogen determinations assuming 15.0% nitrogen per mg of protein, this required that the molecular weight of 27,000 and 15,000 for the subunit and CNBr peptide be corrected for a 4.0 and 7.2% carbohydrate content, respectively, for calculations. [c] The serine and threonine values were obtained by linear extrapolation to zero hydrolysis time. [d] Tryptophan was determined after a 48-hr alkaline hydrolysis at 135° (Hugli and Moore, 1972). [e] Prior work (Carter and Etzler, 1975a,b) indicated no detectable cysteine or cystine residues in the intact lectin or isolated subunits after performic acid oxidation.

mained with the 12000 molecular weight peptide through the molecular sieve step. These traces could be removed by dialysis of the mixture against 80% acetone to remove all sodium dodecyl sulfate (Rice, 1974) and rechromatography on DEAE-cellulose in 8.0 *M* urea under the conditions described above.

As seen in Figure 1 (gel 6), only the larger of the two isolated CNBr fragments stained with the periodic acid–Schiff reaction thus indicating that all of the subunit carbohydrate may be on the larger fragment. As suggested by Segrest and Jackson (1972) for glycoproteins, the asymptotic minimum molecular weight of 15000 for the larger CNBr fragment may in reality approximate 14000 due to its carbohydrate content.

Amino acid analyses of the lectin and the isolated CNBr peptides are shown in Table I.

Derivatization of the isolated intact subunits and the CNBr peptides with 2,4-dinitrofluorobenzene and subsequent chromatography of the isolated Dnp-amino acid derivatives on silica gel G plates resulted in cochromatography of Dnp-alanine standard with both the intact subunit and 15000 molecular weight CNBr peptide amino terminal amino acid residue. No amino terminal amino acid residue was detected from the 12000 molecular weight peptide indicating a probable $NH_2$-terminal blockage. These data indicate that the 15000 molecular weight peptide originated from the $NH_2$-terminal of the intact subunit and the 12000 molecular weight peptide from the COOH-terminal end of the intact subunit. The amino terminal amino acid residue of the *Dolichos biflorus* lectin was previously shown to be alanine in other solvent systems (Carter and Etzler, 1975a,b; Pere et al., 1974).

Electrophoresis of the isolated 12000 molecular weight carboxyl terminal CNBr fragment on pH 9.7 glycine gels in the presence of 0.1% sodium dodecyl sulfate and 8.0 *M* urea (Wu and Bruening, 1971) resolved two bands of equal intensity, whereas the 15000 molecular weight N-terminal fragment migrated as a single band on this gel system. This same gel system was previously found to resolve the IA and IIA subunits of the A form of the *Dolichos biflorus* lectin (Carter and Etzler, 1975b).

*Pronase Digestion of Dolichos biflorus Lectin and Isolation of Glycopeptide.* The lectin (233 mg containing 8.4 mg of hexose) was dissolved in 17 ml of 0.05 *M* Tris-HCl buffer (pH 7.5) containing 0.01 *M* $CaCl_2$, titrated to pH 7.5 with 0.1 *N* NaOH, then heated in a boiling water bath for 10 min. After cooling, 2.0 mg of Pronase (45000 P.U.K./g) was dissolved in 1.0 ml of 0.05 *M* Tris-HCl buffer (pH

7.5)–0.01 *M* $CaCl_2$ and immediately added to the denatured lectin.

The digestion mixture was stoppered in a toluene atmosphere and incubated at 40°. Successive 2.0-mg additions of Pronase were made after 24, 48, and 72 hr of incubation. After 4 days, the reaction mixture was centrifuged and the small quantity of washed precipitate found to contain no orcinol positive material.

The digested lectin supernatant and precipitate wash were pooled, lyophilized, and chromatographed on Bio-Gel P-4 in 0.1 *M* pyridine acetate buffer (pH 5.0) as described by Spiro (1972). As seen in Figure 3A, most of the orcinol positive material was eluted in the first peak (peak 1); this material represented 91.4% of the original lectin hexose.

A small aliquot of the pooled material from peak 1 was subjected to acid hydrolysis (6 *N* constant boiling HCl for 21 hr at 115°) labeled with 1-dimethylaminonaphthalene-5-sulfonyl chloride (Zanetta et al., 1970), and chromatographed on silica gel G plates in toluene–pyridine–HOAc (150:50:3.5). The chromatogram produced multiple amino acid spots, indicating incomplete digestion of the peptide portion of the glycoprotein.

The pooled peak 1 mixture was subjected to a second Pronase digestion (total of 1.5 mg of Pronase added in three aliquots) over a 3-day period. The digestion mixture was fractionated on a Bio-Gel P-4 column in $H_2O$. Two orcinol positive peaks were detected (Figure 3B); the smaller peak (peak 1a) represented 6.3% and the larger peak (peak 1b) represented 89.8% of the original lectin hexose. The smaller of these peaks (peak 1a) was not subjected to further analysis and possibly contained the hexose contaminant from the Pronase. The total Pronase additions added 0.34 mg or 4.0% hexose to the original lectin hexose.

The major hexose peak (peak 1b) was lyophilized and dried in vacuo over $P_2O_5$ to a constant weight of 11.51 mg representing 4.94% of the original dry weight of the lectin (233 mg). This isolated glycopeptide was homogeneous on high-voltage paper electrophoresis and thin-layer chromatography on silica gel G plates run in phenol–$H_2O$ (3:1).

*Characterization of Glycopeptide.* Amino acid analyses of the isolated glycopeptide showed the presence of both aspartic acid and serine residues in a ratio of 5:1. Other amino acids were detected in only trace quantities. Carbohydrate analyses of the glycopeptide by the colorimetric orcinol and Elson–Morgan methods detected 5.3 mol of hexose and 1.4 mol of hexosamine per mol of aspartic acid. Gas–liquid chromatography of alditol acetate derivatives of acid-hydrolyzed glycopeptide detected 3.9 mol of mannose and 1.5 mol of *N*-acetyl-D-glucosamine per mol of aspartic acid. A total of 1.1 mol of glucosamine was found per mol of aspartic acid by amino acid analyses after 24-hr hydrolysis in 6 *N* HCl at 110°.

A minimum molecular weight of 1140 was calculated for the glycopeptide by summing the total weights of the individual glycopeptide components.

Attempts at quantitation of the number of free amino groups per aspartic acid residue utilizing trinitrobenzensulfonic acid (Habeeb, 1966) indicated approximately one amino terminus per three aspartic acids indicating that the true molecular weight of the carbohydrate unit is possibly a multiple of the minimum molecular weight of 1000.

The glycopeptide was subjected to partial acid hydrolysis in 1 *N* HCl at 100° for time periods up to 40 min. Comparison of the various hydrolysates in high-voltage paper electrophoresis with commercial standards showed a component

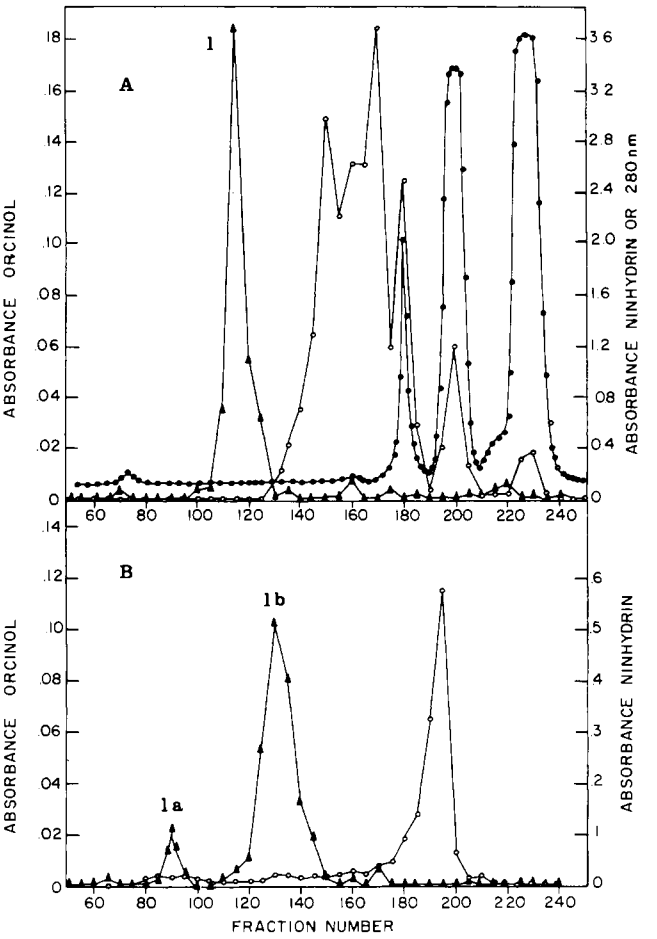


FIGURE 3: Gel filtration chromatography of *Dolichos biflorus* lectin after exhaustive Pronase digestion. (A) A total of 233 mg (46.6 μmol of hexose) of *Dolichos biflorus* lectin was subject to exhaustive Pronase digestion in 0.05 *M* Tris-HCl buffer (pH 7.5)–0.01 *M* $CaCl_2$ at 40° for 4 days. The digest sample was then lyophilized, redissolved in 12.4 ml of 0.1 *M* pyridine acetate (pH 5.0), and chromatographed on a Bio-Gel P-4 column (mesh 200–400, 108 × 2.2 cm) equilibrated and developed in 0.1 *M* pyridine acetate buffer (pH 5.0); 2-ml fractions were collected. The fractions from peak 1 were pooled and lyophilized and found to contain 91.4% (42.9 μmol of hexose) of the original *Dolichos biflorus* orcinol positive material. (▲) Absorbance, orcinol, (○) absorbance, ninhydrin, and (●) absorbance, 280 nm. (B) The lyophilized peak 1 from Figure 3A was subjected to a second Pronase digestion under similar conditions as above and chromatographed on a second Bio-Gel P-4 column (mesh 200–400, 1.75 × 185 cm) equilibrated and developed in $H_2O$; 2-ml fractions were collected. Two orcinol positive peaks were resolved. One containing 6.3% (peak 1a) and the other 89.8% (peak 1b) of the original *Dolichos biflorus* orcinol positive material. Peak 1b contained 41.9 nmol of hexose and was subjected to further analyses. (▲) Absorbance, orcinol, and (○) absorbance, ninhydrin.

with an identical electrophoretic mobility to commercial *N*-aspartyl-*N*-acetylglucosamine was present at a maximum concentration after 25 min of hydrolysis.

The isolated *Dolichos biflorus* glycopeptide inhibited agglutination of human type A red blood cells by concanavalin A.

## Discussion

The predominant form A (113000 g/mol) of the *Dolichos biflorus* lectin is composed of two types of very similar subunits. These subunits, IA (27700 g/mol) and IIA (27300 g/mol), each contain one methionine residue and appear to differ from one another only at their COOH-terminal ends (Carter and Etzler, 1975b). Cyanogen bromide cleavage of the lectin produced two fragments with approxi-

mate molecular weights of 15000 and 12000, as determined by sodium dodecyl sulfate gel electrophoresis, thus indicating that the methionine residue of each subunit is located in the central portion of each peptide chain. The larger fragment (15,000 g/mol), which contains most of the phenylalanine and lysine, has the same $NH_2$-terminal residue (alanine) as the lectin subunits and has, therefore, been identified as the $NH_2$-terminal fragment. The COOH-terminal (12000 g/mol) fragment contained all of the lectin histidine and was resolved into two bands of equal intensities after electrophoresis on discontinuous polyacrylamide gels in 0.1% sodium dodecyl sulfate and 8.0 *M* urea. Such heterogeneity of the COOH-terminal fragment was expected in light of our previous observation of differences between the COOH-terminal ends of the IA and IIA subunits (Carter and Etzler, 1975b).

Previous studies have shown that the *Dolichos biflorus* lectin is a glycoprotein containing 2.4% mannose and 1.6% *N*-acetylglucosamine (Etzler and Kabat, 1970; Font et al., 1971; Carter and Etzler, 1975a,b). These two sugars have also been reported to be the major carbohydrate constituents of a number of other lectins (for review see Sharon and Lis, 1972).

The data presented above show that at least 90% of the carbohydrate can be isolated from the *Dolichos biflorus* lectin as a glycopeptide fraction with a minimal molecular weight of 1100 containing mannose, *N*-acetylglucosamine, and aspartic acid in a ratio of 4-5:1-2:1. If this carbohydrate unit were present on each of the four subunits of the intact lectin it would account for all of the carbohydrate present in the molecule. It is possible, however, that the carbohydrate unit may be a multimer of the minimum molecular weight of 1000 as suggested by the presence of only one detectable free amino group per three asparagine residues of the glycopeptide. The carbohydrate unit may thus not be present on all subunit chains although previous work has shown that it is associated with both types (IA and IIA) of subunits of the lectin (Carter and Etzler, 1975b).

The identification of a product of partial acid hydrolysis of the isolated glycopeptide as *N*-acetylglucosaminylasparagine suggests that the carbohydrate is bound to the protein by a glycosylamine linkage between *N*-acetylglucosamine and asparagine. This type of linkage of carbohydrate to protein is common for those glycoproteins containing small amounts of carbohydrate (Spiro, 1970; Marshall, 1972) and has been found to be the linkage involved in the soybean lectin (Lis and Sharon, 1973) and *Ricinus communis* lectin (Funatsu et al., 1971). The detection of carbohydrate only on the amino terminal CNBr fragment of the lectin indicates that the carbohydrate unit is localized on the amino-terminal half of the *Dolichos biflorus* lectin subunits. Amino acid sequence data on the *Phaseolus vulgaris* lectin indicate that the 12th amino acid residue from the amino terminal end may be a glycosylated asparagine (Miller et al., 1973).

The above data on the glycopeptide indicate that the carbohydrate unit of the *Dolichos biflorus* lectin may resemble the tentative structure proposed for the glycopeptide of the soybean lectin (Lis and Sharon, 1973) of $(Man)_{4-5}$-GlcNAc-$(Man)_{4-5}$-$(GlcNAc)_{1-2}$-Asn. Similar structures have also been proposed for the three glycopeptides isolated from the *Ricinus communis* toxin (Funatsu et al., 1971). These structures are quite similar to those proposed for the carbohydrate units of some animal glycoproteins such as thyroglobulin (Arima et al., 1971), ovalbumin, and ribonuclease B (Spiro, 1970; Marshall, 1972). The structures also resemble the carbohydrate core region, involved in glycoprotein biosynthesis in mouse myeloma cells as described by Hsu et al. (1974).

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