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CARBOHYDRATE BINDING ACTIVITY OF A LECTIN-LIKE GLYCOPROTEIN FROM STEMS AND LEAVES OF DOLICHOS BIFLORUS

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SUMMARY

A glycoprotein from the stems and leaves of the Dolichos biflorus plant that cross reacts with antibodies to the seed lectin has been found to bind to affinity columns of blood group A + H substance covalently linked to Sepharose. This binding of the cross reactive material to the affinity resin differs from that of the seed lectin in that it is easily dissociated with 0.15 M NaCl. Affinity electrophoresis using entrapped blood group A + H substance shows that the carbohydrate binding activity of the cross reactive material is weakly inhibited with N-acetyl-D-galactosamine and N-acetyl-D-glucosamine. Glucose, mannose and galactose gave no inhibition when tested at concentrations of 50 mM. These data indicate that the specificity of the cross reactive material is somewhat different from the N-acetyl-D-galactosamine specificity of the seed lectin. The significance of these findings is discussed in relation to the structural similarities of the cross reactive material and the seed lectin.

INTRODUCTION

The stems and leaves of the Dolichos biflorus plant contain a glycoprotein that cross reacts with antibodies to the lectin from the seeds of this plant (1). This cross reactive material (CRM*) is composed of two subunits which have identical NH_2-terminal amino acid sequences (2). The seed lectin also contains two types of subunits (I and II) with identical NH_2-terminal amino acid sequences (3,4). With the exception of an asparagine instead of an aspartate at residue 2, these sequences are identical to the NH_2-terminal sequences of the CRM subunits.

Structural studies on the seed lectin subunits indicate that they differ from one another only at their COOH-terminal ends where subunit I appears to

ABBREVIATIONS

CRM, cross reactive material to antibodies against the seed lectin; SDS, sodium dodecyl sulfate; PBS, 0.15 M NaCl in 0.01 M phosphate, pH 7.2, containing 0.02% NaN_3; PEG, polyethylene glycol; BGS, blood group A + H substance.

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be slightly longer than subunit II (3-5). A comparison of the CRM and seed lectin subunits by polyacrylamide electrophoresis in SDS and urea shows that one of the CRM subunits has an identical mobility to subunit I of the seed lectin wherease the second CRM subunit has a higher molecular weight than either of the seed lectin subunits (2). Since the NH_2 terminal ends of these subunits are the same, both the CRM and the seed lectin subunits may represent different degrees of completion or modification of a common polypeptide chain.

The seed lectin can agglutinate type A erythrocytes and precipitate blood group A substance (6,7) due to its specificity for terminal nonreducing α-N-acetyl-D-galactosamine (8,9). When tested under similar conditions as the seed lectin, the CRM did not agglutinate erythrocytes nor did it bind to blood group A + H substance (2).

The present study shows that the CRM is capable of binding to blood group A + H substance under conditions of low ionic strength but that it may have a somewhat different specificity than the seed lectin. The significance of this finding in relation to the structural similarities of these molecules is discussed.

MATERIALS AND METHODS

CRM was isolated from extracts of the stems and leaves of the Dolichos biflorus plant by ammonium sulfate precipitation and ion exchange chromatography as previously described (2). The CRM was iodinated with [^{125}I] sodium iodide (New England Nuclear) using the iodine monochloride method (10). A specific activity of 3.7 x 10^5 cpm/μg protein was achieved.

Rabbit antiserum against isolated CRM was produced as previously described (1) and passed over a column of Sepharose covalently linked to the Dolichos biflorus seed lectin (11). This chromatographic step removed those antibodies that cross react with the seed lectin and rendered the antiserum specific for the CRM. Using this antiserum a competitive radioimmunoassay was developed as previously described (1) in which CRM was estimated by its ability to inhibit the interaction of ^{125}I CRM with the antiserum. The sample, ^{125}I CRM and the antiserum were combined in a final volume of 175 μl containing 2.3% bovine serum albumin and PBS. After incubation for 30 minutes at 37°C, 25 μl of goat anti-rabbit IgG (Miles laboratories) at a concentration of 7.5 mg/ml were added. Following incubation for 30 minutes at 37°C, 200 μl of cold 6% PEG in PBS were added and the samples were kept for 10 minutes in an ice bath. The tubes were centrifuged at 1700 x g for 10 minutes and the pellets were washed two times with 400 μl of 3% PEG in PBS. The pellets were counted in a γ-scintillation counter and the amount of CRM in the unknowns was calculated from a standard curve run with known amounts of CRM.

Blood group A + H substance (BGS) was obtained from hog gastric mucin (Wilson Laboratories) by ethanol precipitation (12). A 2 mg/ml solution of this material was covalently coupled to CNBr-activated Sepharose 4B (Pharmacia) following the procedure of Pereira and Kabat (13).

Affinity electrophoresis was run on 5% polyacrylamide gels as previously described (14) in the presence and absence of 0.5 mg/ml entrapped BGS.

RESULTS

Preliminary experiments showed that ^{125}I CRM does not bind to BGS-Sepharose columns equilibrated with PBS. However, when the NaCl is eliminated from this buffer the ^{125}I CRM binds to the column. Elution of the columns with 0.01 M N-acetyl-D-galactosamine failed to remove any significant amount of ^{125}I CRM, but the CRM was dissociated from the column by elution with BGS. Stepwise elutions of the columns with increasing amounts of NaCl also resulted in the removal of ^{125}I CRM at a NaCl concentration of 0.15 M.

The above findings were used to design an affinity chromatographic procedure for the isolation of the CRM from the leaves and stems of the Dolichos biflorus plant. The stems and leaves were extracted as previously described (2), and the 80% ammonium sulfate pellet was dialyzed against 0.01 M phosphate, pH 7.2 containing 0.02% NaN_3. This dialyzed fraction was then applied in the cold to a BGS-Sepharose column equilibrated with the same buffer. The column was washed and then eluted with increasing concentrations of NaCl in the above buffer. Radioimmunoassays of the column fractions (Figure 1) showed that most of the CRM was bound to the column and could be eluted with 0.15 M NaCl. Analysis of the column eluates by SDS-urea gel electrophoresis confirmed the presence of the CRM in the 0.15 NaCl peak. The other peaks eluted with 0.1 M and 0.5 M NaCl contained other proteins that may represent other lectin-like material in the stems and leaves.

Affinity electrophoresis using entrapped BGS was used in an effort to verify the ability of the CRM to bind to carbohydrate. The electrophoretic mobility of the CRM was reduced when BGS was entrapped in the gels. Various monosaccharides were tested for their ability to inhibit the binding of the CRM to blood group substance. Inhibition was obtained with both N-acetyl-D-

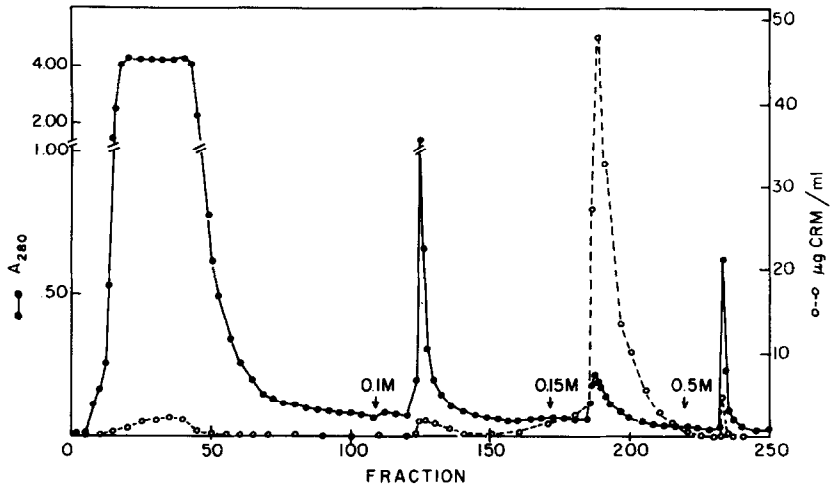

Figure 1. Isolation of CRM from stem and leaf extracts by affinity chromatography on BGS-Sepharose. 133 mls of extract were applied to a BGS-Sepharose column (1 x 47 cm) equilibrated with 0.01 M PO_4, pH 7.2, containing 0.02% NaN_3. After washing, the column was stepwise eluted with increasing concentrations of NaCl as shown by the arrows. Fractions of 5.4 mls were collected.

galactosamine and N-acetyl-D-glucosamine at concentrations of 50 mM; no inhibition was obtained with mannose, glucose or galactose at concentrations of 50 mM (Table 1) or 100 mM.

Discussion

The similarities in composition and structure between the CRM and the seed lectin from Dolichos biflorus have suggested that these two glycoproteins may be functionally related to one another (2). The affinity chromatographic and electrophoretic data presented above now confirm this functional relationship by showing that the CRM has the ability to bind to carbohydrate. This carbohydrate binding activity of the CRM differs from that of the seed lectin in that it is easily disrupted with 0.15 M NaCl. Since this concentration of NaCl is routinely used in assays for seed lectin activity (15), the previous failure to detect carbohydrate binding activity of the CRM when tested by the seed lectin assay procedures (2) is now readily understood.

The binding of CRM to the BGS is dissociated with N-acetyl-D-galactosamine and N-acetyl-D-glucosamine. These results suggest that the specificity of the

Table I. Monosaccharide Inhibition of CRM-BGS Interaction in Affinity Electrophoresis

Free Ligand (50 mM)	% Increase in Mobility of CRM
N-Acetyl-D-galactosamine	8.0
N-Acetyl-D-glucosamine	7.5
D-Mannose	0
D-Glucose	0
D-Galactose	0

The electrophoretic mobility of CRM in gels containing entrapped BGS was compared in the presence and absence of various monosaccharides. An increase in electrophoretic mobility denotes the ability of the free ligand to inhibit the interaction of CRM with BGS. The mobility of unretarded CRM in gels containing no BGS is 27% more than when CRM binds to BGS entrapped in the gels.

CRM may be somewhat different from the specificity of the seed lectin which is inhibited by N-acetyl-D-galactosamine but not by N-acetyl-D-glucosamine (8,9).

It is of interest to note that both the CRM and the seed lectin appear to have one subunit in common (2). Recent studies in our laboratory (Etzler, Borrebaeck and Gupta, unpublished) indicate that it is this subunit (subunit I of the seed lectin) that is primarily responsible for the carbohydrate binding activity of the seed lectin. The difference in carbohydrate binding abilities between the CRM and the seed lectin may thus be due to some modifying influence conferred by the presence of the other subunit.

Although the carbohydrate binding ability of the CRM technically qualifies it to be considered as a lectin in its own right, we as yet hesitate to identify it by that term. We note that the CRM differs from the classical plant lectins by its failure to agglutinate erythrocytes or to bind to carbohydrates in the presence of physiological saline. Since the CRM is present in the stems and leaves of the plant, it may serve a different physiological role than the lectins found in the seeds, and this role may possibly be regulated by variations in salt content. Further studies now in progress on the struc-

ture, specificity and localization of the CRM should help to clarify the physiological relationship of this "lectin-like" glycoprotein to the seed lectin.

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