Mitogenic properties of two carbohydrate binding proteins from the *Dolichos biflorus* plant

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Dolichos biflorus *Seed lectin* *Cross-reactive material* *Stem and leaf lectin* *Mitogen*

1. INTRODUCTION

Most lectins described to this date have been isolated from the seeds of leguminous plants. One such lectin is the glycoprotein isolated from the seeds of the *Dolichos biflorus* plant expressing an activity for terminal non-reducing α-linked *N*-acetyl-D-galactosamine residues [1]. In addition to this seed lectin the stems and leaves of the *Dolichos biflorus* plant contain another glycoprotein that crossreacts with antibodies against the lectin from the seeds of this plant. This cross-reactive material (CRM) was isolated and characterized [2] and was shown to have an amino acid and carbohydrate composition very similar to that of the seed lectin [2,3].

CRM and the seed lectin both contain apparently equal amounts of two types of subunits; one CRM subunit is identical in electrophoretic mobility to subunit I of the seed lectin [2]. Both CRM subunits have a NH_2-terminal amino acid sequence identical to the sequence of the seed lectin subunits with the exception of an aspartate in place of an asparagine at the second residue [2,4]. It has also recently been shown that CRM, at low ionic strength, has carbohydrate binding activity [5] although its specificity is somewhat broader that that of the seed lectin.

Despite this close structural resemblance between these two carbohydrate binding proteins from the *Dolichos biflorus* plant we now report a distinct in vitro difference in mitogenic activity.

2. MATERIALS AND METHODS

2.1. *Materials and methods*

Dolichos biflorus seeds were obtained from F.W. Schumacher Co., Sandwich, MA. Blood group A+H substance (BGS) was isolated by ethanol precipitation [6] from hog gastric mucin (Wilson laboratories, Chicago, IL).

2.2. *Purification of seed lectin and CRM*

The *Dolichos biflorus* seed lectin was isolated from seed extract as previously described [3] by adsorption of the lectin onto insoluble polyleucyl hog blood group A+H substance and specific elution with 0.01 M *N*-acetyl-D-galactosamine. After removal of the hapten by chromatography on Bio-Gel P-10, the lectin was concentrated by ultrafiltration in a Diaflow ultrafiltration device using a PM-10 filter. CRM was also isolated by affinity chromatography using BGS-Sepharose as previously described [5] and eluted by raising the ionic strength.

2.3. *Neuraminidase treatment of isolated lymphocytes*

Venous blood obtained from a healthy human donor was heparin treated and the lymphocytes were prepared by Ficoll-Isopaque (Pharmacia Fine Chemicals, Uppsala, Sweden) centrifugation. 20×10^6, cells were incubated at 37°C for 45 min with 60 μg neuraminidase (*Clostridium perfringens*, type VI 5 U/mg protein NAN-lactose substrate,

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Sigma St. Louis, MO) in 1.5 ml Tris-buffered Hanks solution (pH 7.4) [7].

2.4. *Mitogenic assay*

Activation of the neuraminidase-treated human peripheral lymphocytes was measured as the incorporation of [*methyl*-^{3}H]thymidine (2.0 Ci/mmol) (New England Nuclear) into the cells. One ml lymphocytes (0.5×10^6 cell/ml) in RPMI 1640 (Gibco Biocult Ltd.) supplemented with 10% fetal calf-serum, 5 mM L-glutamine, streptomycin (50 μg/ml) and penicillin (50 IU/ml), and 1% 100× non-essential amino acids (Flow Laboratories, Inc.) were incubated for 96 h in 15 mm Linbro wells in the presence of CRM (2.5–200 μg/ml) or seed lectin (3–300 μg/ml). After 72 h at 37°C, the cells were pulsed for 24 h with 2 μCi [*methyl*-^{3}H]thymidine/well. The cells were then transferred to 0.45 μm Millipore filters and washed twice with 2 ml cold 0.01 M phosphate-buffered saline (pH 7.2) and twice with 5 ml 10% trichloroacetic acid. After a final wash with 3 × 2 ml 95% ethanol the filters were dried and the radioactivity of the insoluble material was determined by liquid scintillation counting in a toluene based scintillation cocktail.

3. RESULTS AND DISCUSSION

The two carbohydrate binding proteins from the *Dolichos biflorus* plant were tested in their ability to stimulate human peripheral lymphocytes to incorporate radiolabelled thymidine. Despite the structural resemblance between these two proteins a distinct difference appeared in their induction of in vitro mitogenic activity. The two carbohydrate-binding proteins were initially tested using human peripheral lymphocytes isolated by density centrifugation. Under these circumstances neither of the two lectins was able to induce cell proliferation which in the case of the *Dolichos biflorus* seed lectin is in agreement with other reports concerning mitogenic activity [7]. However, when terminal sialic acid residues were removed from glycoconjugates on the surface of the isolated lymphocytes using neuraminidase a significant difference in the biological activity of the two lectins was found. CRM was able to induce proliferation of the neuraminidase-treated peripheral lymphocytes whereas the seed lectin did not show any mitogenic activity.

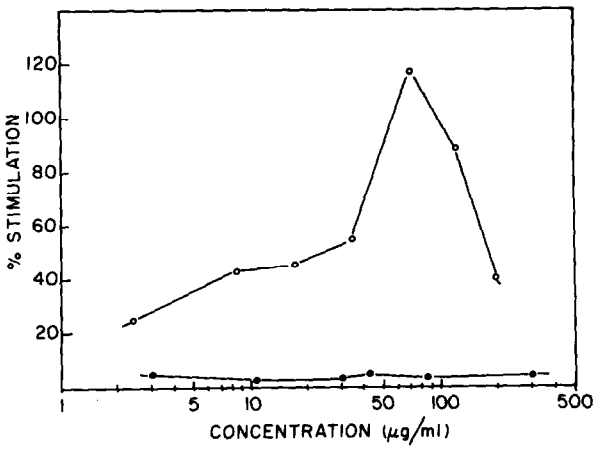

Fig.1. Stimulation of neuraminidase-treated human peripheral lymphocytes by CRM (○—○) and the seed lectin (●—●). % Stimulation = ((cpm in presence of lectin/cpm in absence of lectin) − 1)×100. All samples were run in duplicates.

As can be seen from fig.1 the *Dolichos biflorus* seed lectin did not activate the cells when tested in the range of 3–300 μg lectin/mg whereas CRM stimulated the growth of the lymphocytes with a dose response maximum at 70 μg CRM/ml when tested in the range of 2.5–200 μg CRM/ml.

The dose–response maximum of different mitogenic lectins varies over a wide range; a maximum of 5 μg/ml has been reported for phytohemagglutinin (PHA), the seed lectin from *Phaseolus vulgaris* whereas over 100 μg/ml of soy bean agglutinin (SBA) is required for maximum stimulation [8]. This difference seems partly due to parameters such as binding affinity, valence and carbohydrate binding specificity of the lectins. The state of aggregation and consequently the valence of lectins have been shown to be closely related to their mitogenic activity [8,9]. The somewhat elevated dose response maximum of CRM might be explained by the fact that this stem and leaf lectin is a dimeric protein consisting of two different types of subunits most probably giving it a low valence. The binding affinity of CRM to carbohydrate containing cell-surface structures, its content of tightly bound divalent cations [10] and the somewhat different carbohydrate binding specificity [5] compared to the *Dolichos biflorus* seed lectin might also contribute to the above difference in mitogenic activity.

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