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Isolation and characterisation of a *Salvia bogotensis* seed lectin specific for the Tn antigen

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Abstract

A lectin was isolated and characterised from *Salvia bogotensis* seeds. Removal of the abundant pigments and polysaccharides, which are present in seeds, was an essential step in its purification. Several procedures were assayed and the best suited, including Pectinex treatment, DEAE-cellulose and affinity chromatography, led to a protein being obtained amounting to 18–20 mg/100 g seeds having high specific agglutination activity (SAA). The lectin specifically agglutinated human Tn erythrocytes and was inhibited by 37 mM GalNAc, 0.019 mM ovine submaxillary mucin (OSM) or 0.008 mM asialo bovine submaxillary mucin (aBSM). Enzyme-linked lectinosorbent assay (ELLSA) revealed strong binding to aOSM and aBSM, corroborating Tn specificity, whereas no binding to fetuin or asialo fetuin was observed. The lectin's monomer MW (38,702 Da), amino acid composition, p*I*, carbohydrate content, deglycosylated form MW, thermal stability and Ca^{2+} and Mn^{2+} requirements were determined. Evidence of the existence of two glycoforms was obtained. The lectin's specificity and high affinity for the Tn antigen, commonly found in tumour cells, makes this protein a useful tool for immunohistochemical and cellular studies.
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1. Introduction

In the course of their research into lectins, Bird's group (Bird and Wingham, 1974; Bird and Wingham, 1976, 1977) found lectins able to specifically recognise the Tn antigen (GalNAc-O-Ser/Thr) in several Old World species of Salvia (Lamiaceae); this antigen is responsible for the erythrocyte polyaggutinability shown by some individuals and has been identified as being a tumour cell marker (Springer, 1984; Lisowska, 1995). The latter is useful in diagnosis when following-up the evolution of several types of cancer. The Tn epitope has also been detected on human immunodeficiency virus gp160 and gp120 proteins (Hansen et al., 1991).

Detailed studies, using Lamiaceae lectins, have been carried out on a few species from the Northern hemisphere's temperate zone. The lectin from *Salvia sclarea* seeds (SSL) was the first to be isolated and partially characterised (Piller et al., 1986). This established its specific binding to

Abbreviations: ABTS, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); aBSM, asialo bovine submaxillary mucin; BCA, bicinchoninic acid; BSA, bovine serum albumin; BSM, mucin; ConA, Concanavalin A; DAB, diaminobenzamidine; DDCA, diethyldithiocarbamic acid; DTT, dithiothreitol; EGTA, ethylene glycol-*O*,-*O*′-bis (2-amino-ethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid; ELLSA, enzyme-linked lectinosorbent assay; EME, enzymatically modified erythrocytes; ERL, *Erythrina rubrinervia* lectin; SBoL, *Salvia bogotensis* lectin; SSL, *Salvia sclarea* lectin; MeCN, acetonitrile; MLL, *Moluccella laevis* lectin; GLL, *Galactia lindenii* lectin; DLL-II, *Dioclea lehmanni* lectin II; OSM, ovine submaxillary mucin; aOSM, asialo ovine submaxillary mucin; PVPP, polyvinylpolypyrrolidone; RBC, red blood cell; RT, room temperature; SAA, specific agglutination activity; SDS, sodium dodecyl sulphate; VVB4, *Vicia villosa* isolectin B4; TBS, Tris buffer saline; TFA, trifluoroacetic acid; Gleheda, *Glechoma hederacea* lectin.

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both native Tn red blood cells (RBCs) and enzyme-treated RBCs, as well as the inhibitory potential to a variety of carbohydrates, Synsorb-coupled synthetic glycopeptides, BSM, and aBSM. Several molecular features of SSL have been described, such as MW and N-linked oligosaccharide structures (Medeiros et al., 2000); competition binding studies with soluble synthetic glycopeptides have also helped to define the density requirements of Tn structures.

A lectin from *Moluccella laevis* (MLL) has been isolated (Lis et al., 1988). Besides recognising A^{MM} and O^{NN} erythrocytes, the lectin binds strongly to Tn-bearing glycoproteins (Duk et al., 1992), Tn-bearing lymphocytes (Thurnher et al., 1993) and glycosphingolipids (Teneberg et al., 1994). Analysis of the lectin's structural features revealed a unique subunit composition (Alperin et al., 1992), making it an unusual lectin. Wang et al. (2003a) have recently found a lectin (Gleheda) in *Glecoma hederacea* leaves which readily interacts with *O*-glycans linked to asialo mucin or asialo fetuin in which Gal/GalNAc are terminally exposed. The authors used sequence and molecular modelling studies to demonstrate that Gleheda is structurally and evolutionarily related to legume lectins. A potentially interesting development arises from the insecticidal properties presented by the lectin (Wang et al., 2003b).

Botanical studies have revealed the presence of around 190 Lamiaceae species in Colombia, Salvia being the most diverse genus as it has 75 species (Wood and Harley, 1989; Fernández-Alonso, 2003). We have recently carried out an extensive survey of six genera and 40 taxa as no data was available concerning the presence of lectins in Colombian species of Lamiaceae (Fernández-Alonso et al., 2003). This study revealed both the presence of lectins able to recognise the Tn antigen in more than 80% of the studied species and remarkable differences in lectin activity within a given genus (i.e. eight out of 19 *Salvia* species had more than 80% activity levels). Considering the potential applications of anti-Tn lectins, we chose the *Salvia bogotensis* (Benth.) species for this study, taking its endemic character into account (wide distribution throughout the eastern Colombian Cordillera), the availability of substantial amounts of seeds and its high lectin activity (98%). This work describes isolating and characterising *S. bogotensis* lectin (SBoL) as a first step in studying its interaction with Tn-bearing cells.

2. Results and discussion

2.1. Lectin extraction

Removing troublesome abundant pigments which usually appear in Lamiaceae seed protein extracts was an essential purification step when obtaining lectin. The problems pertained to reduced protein solubility, inadequate assessment of elution profiles if followed by absorption at 280 nm and inaccurate determination of $A^{280}_{1\%}$ values which could be used for calculating lectin content in seeds.

Treatment with 0.1 M ascorbic acid, 2% polyvinylpolypyrrolidone (PVPP) or 0.5% diethyldithiocarbamic acid (DDCA) appreciably reduced the lectin's activity (data not shown) with no significant reduction of pigment in the extract. This loss of activity, particularly with PVPP, has been observed in our laboratory with other proteins (*Salvia palifolia* or *Hyptis mutabilis* lectins); it is likely that this was due to protein adsorption on PVPP. The total loss of activity when including dithiotreitol (DTT) indicated the presence of disulphide bridges in the protein.

The best results were obtained by including 5 mM thiourea in PBS pH 7.2 and in dialysis solutions, which reduced the amount of polyphenols by inhibiting polyphenol oxidases (Van Driessche et al., 1983) whilst keeping the lectin's activity unaltered (79%). Most remaining pigments were effectively removed by DEAE-cellulose and DEAE-Sephadex during subsequent purification steps. This approach has proved very effective in our hands when working with highly pigmented extracts from several Lamiaceae species. It is likely that improved yields and higher specific agglutination activities than those obtained with previously described Lamiaceae seed lectins (Piller et al., 1986; Alperin et al., 1992) have been due to the isolation procedure described in this work.

There was 2.09% nitrogen content in seeds, amounting to 13.1% crude protein which is lower than that of most legume seeds; with the exception of *S. palifolia* (15.7%) and *S. rubescens* (19.6%) (Filgueira and Aldana, personal communication), no data is currently available for other *Salvia* seeds. Non-protein nitrogen accounted for 0.11%; net protein content in seeds was thus 12.3%.

2.2. Lectin purification

Precipitation assays using PBS extracts showed that the lectin precipitated at 50% ethanol presenting 70–80% activity as determined by ELLSA. Higher ethanol concentrations did not precipitate the lectin further as opposed to SSL behaviour which precipitated at 80% ethanol (Piller et al., 1986). Dissolving the resulting precipitate led to a very viscous solution being formed, due to pectin-like polysaccharides which are present in nearly all *Salvia* species and which usually hamper detecting lectin (Fernández-Alonso et al., 2003); viscosity diminished after digestion with Pectinex and chromatography over DEAE-Sephadex (at an improved flow) yielded a non-retained peak in which lectin activity was readily detected (52%), even at low protein concentrations.

Affinity chromatography on aBSM-Sepharose 4B of the non-retained DEAE-Sephadex peak yielded two fractions (Fig. 1a); the first (I) was devoid of lectin activity (6.7%, 1.9 mg protein/ml) and the second (II), eluted by pH 11.4, presented 71–78% activity (0.4–0.5 mg protein/ml) after dialysis, as well as high Tn-specific agglutination activity (Table 1). The minor peak eluting before fraction II was devoid of lectin activity and was therefore discarded.

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