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Lectin genes and their mature proteins: Still an exciting matter, as revealed by biochemistry and bioinformatics analyses of newly reported proteins

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ABSTRACT

Two new lectins were purified through affinity chromatography after crude extract preparation under high ionic strength. The hemagglutinating activity of these lectins from the seeds of the legumes *Dioclea bicolor* (DBL) and *Deguelia scandens* (DSL) was inhibited by galactose and glucose, respectively, and the molecular masses were estimated at 24 and 22 kDa (via SDS-PAGE), respectively. The alignment of internal peptides of DBL (MS/MS) with known protein sequences revealed similarity to other legume lectins. The N-terminal amino acid sequence of DSL also aligned with legume lectins. Cross-similarities among the two studied lectins were observed only after sequence permutation. More than a dozen lectins have been reported for the genus Dioclea but none that recognize galactose. DSL is the first lectin reported for the Deguelia genus in the tribe Millettieae. With the aid of bioinformatics tools and searches for genome/transcriptome information about closely related sequences, new lectin members of Millettieae were also identified. Electrophoresis profiling and amino acid sequence analysis suggested that DBL-Gal and DSL do not undergo post-transcriptional ConA-like circular permutation. Molecular modeling of the deduced amino acid sequences of the Millettieae lectins suggested that the overall folding of the monomeric structures of legume lectins is conserved. This and other recent studies highlight native plants of the Amazon as renewed sources of lectins.

1. Introduction

Lectins are primarily defined as carbohydrate-binding proteins (Wittmann and Pieters, 2013) that are thought to be ubiquitous in nature and display a broad range of cellular and physiological functions in different organisms (Ingale and Hivrale, 2013). Plant lectins, notably those belonging to legume seeds, have become suitable models for molecular and

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structural studies involving protein-carbohydrate interactions at the atomic level (de Bentzmann et al., 2014). Indeed, their ability to discriminate specific structural features of carbohydrate-containing molecules highlights them as interesting tools for a wide range of applications, including diagnostics and the treatment of metabolic diseases (Kontro et al., 2014). Accordingly, screening for new lectins is a still attractive approach (Nascimento et al., 2012). However, screenings are still based on the evaluation of the erythrocyte agglutinating activity of protein extracts, and the success of this strategy has become marginal. With new DNA sequences of plant species now available, the use of bioinformatics allows the prospecting of lectin genes *in silico*, substantially improving bio-prospecting. In this study, we report two new purified legume lectins and two new lectin genes (cDNAs). Based on the amino acid sequence information of the new proteins, interesting insight on their phylogenetic clustering and overall folding are reported.

2. Material and methods

2.1. Chemicals

Chemicals for electrophoresis, α-lactose-agarose, Sephadex G-50, and carbohydrates were purchased from Sigma Chemical (São Paulo, Brazil). Chemicals for amino acid sequencing were obtained from Waco (Tokyo, Japan). Molecular weight markers were obtained from PROMEGA (São Paulo, Brazil). All other chemicals were of analytical grade.

2.2. Plant material

Seeds of *Dioclea bicolor* Benth. (Fabaceae Lindl.)were obtained of plants located at the following coordinates (04° 22′ 59″ S and 70° 01′ 52″ W), locally named Benjamim Constant, Amazon, Brazil. Seeds of *Deguelia scandens* Aubl. (Fabaceae Lindl.) were harvested of plants found on Judge Island in the downstream region of the Black River, São Gabriel da Cachoeira, Amazon, Brazil, at the following coordinates (00° 08′ 49.0″ S and 67° 04′ 29.0″). The collections were performed in August 2009.

2.3. Protein extraction

Fine seed flour of *D. bicolor* was suspended in 250 mM glycine-HCl (pH 2.6)-buffered saline (150 mM) and maintained at 8 °C. The resulting mixture (1:10; v/w) was shaken for 2 min and centrifuged for 10 min at 10,000 × g and 4 °C. The soluble phase was stored at 8 °C, and the precipitated material was subjected to a new cycle of extraction. The new soluble phase was combined with the first and subjected to dialysis in distilled water for 48 h at 8 °C, with the water being renewed eight times.

Fine seed flour of *D. scandens* was mixed with 50 mM Tris–HCl (pH 7.5)-buffered saline (150 mM) and shaken for 2 h at 25 °C. The resulting extract (1:10; v/w) was centrifuged and dialyzed as stated for the first sample described. The materials were freeze-dried stored at 25 °C and used in all analyses.

2.4. Lectin activity

Lectin activity in both samples was first assessed by hemagglutinating assays and further confirmed by using glucose and galactose as lectin inhibitors. The overall procedure described by Moreira and Perrone (1977) was followed. The freeze-dried materials were dissolved in 100 mM Tris–HCl (pH 7.6)-buffered saline (150 mM) added to 5 mM $CaCl_2/MnCl_2$. Rabbit erythrocytes (2%) in 150 mM saline were used for agglutination. The inhibition of agglutination was investigated by first incubating the samples [extracts (10 mg/ml) or purified lectins (1 mg/ml)] with different concentration of the following carbohydrates prepared in 150 mM saline at 100 mM, prior to adding the red blood cells: N-acetyl-D-galactosamine; α-lactose; 2-nitrophenyl-ß-D-galactopyranoside; D-galactose; ß-methyl-D-galactopyranoside; α-methyl-D-galactopyranoside; D-mannose; N-acetyl-D-glucosamine; D-glucose; α-methyl-D-glucopyranoside; D-fructose; L-arabinose; sucrose and maltose.

2.5. Affinity chromatography

The choice of the matrices for lectin purification was according to the results of the hapten inhibition of the extracts by the monosaccharides glucose and galactose. Accordingly, the extract of *D. bicolor* was subjected to fractionation through an α-lactose-agarose matrix. The extract of *D. scandens* was applied to a Sephadex G-50 resin. Both gels were first washed with Tris–HCl buffer, and then the samples, prepared in the same buffer, were applied to the columns and first eluted with Tris–HCl buffer until the unbound proteins were recovered. The lectins, which were expected to be retained in the columns, were recovered by exchanging the Tris–HCl buffer for 100 mM glycine (pH 2.6)-buffered saline (150 mM) in the α-lactose-agarose column and 100 mM glucose in the Sephadex G-50 resin. The elution of the proteins was monitored at 280 nm. All runs were performed at 25 °C. The lectin-containing fractions were dialyzed as described above and freeze-dried.