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Research Note

ISOLATION AND PARTIAL PURIFICATION OF A LECTIN FROM *SWARTZIA PICKELLII* KILLIP (WHITE JACARANDA)

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Lectins are molecules able to recognize carbohydrates in cells or solutions, through specific binding sites (I. J. Goldstein et al., 1980, *Nature, 258:* 66; S. H. Barondes, 1988, *Trends Biochem. Sci., 13:* 480-89). Most of the known lectins exhibit defined monosaccharide or oligosaccharide specificity, what is advantageous to reach high levels of purification by affinity chromatography, allowing its aplicability to cell surface characterization (A. M. Atta et al., 1989, *Brazilian J. Med. Biol. Res., 22:* 279-85; N. Kochibe & K. L. Matta, 1989, *J. Biol. Chem., 264:* 173). However, some partially purified lectins of non-defined specificities have also presented very attractive properties (L. R. Glasgow & R. L. Hill, 1980, *Infect. Immun., 30:* 353-61; D. J. Rogers & G. Blunden, 1980, *Bot. Mar., 23:* 459).

Seeds from *Swartzia pickellii* were collected from the Atlantic Forest in the state of Pernambuco. The seeds were macerated and submitted to an initial extraction (20% w/v) in 0.15M-NaCl. After 4 h at room temperature the material was centrifuged at 12,100 x g for 15 min and the supernatant (preparation 1) was used to assay the hemagglutination activity and to measure the proteins according to O. H. Lowry et al., 1951 (*J. Biol. Chem., 193:* 265-75). The hemagglutination assay was performed in microtitre plates by using 50 μl of the preparation in a serial dilution (1:1 in 0.15M-NaCl) and an equal volume of a 2.5% (v/v) saline erythrocytes suspension. The cells used were previously treated with glutaraldehyde according to D. H. Bing et al., 1967 (*Proc. Soc. Cyp. Biol. Med., 124:* 1166-70). The specific hemagglutination activities (SHA = titre/mg.ml^{-1}) for different erythrocyte species are shown in the Table.

TABLE

Specific hemagglutination activity of preparation 1

Glutaraldehyde treated erythrocytes	SHA[a]
Bovine	22
Dog	178
Sheep	44
Horse	89
Chicken	178
Human A	89
Human B	178
Human O	44
Human AB	178
Duck	44
Pig	178

[a]The specific hemagglutination activity (SHA) is defined by the titres (the inverse of the highest dilution showing total agglutination) divided by mg of protein.ml^{-1}.

The inhibition of the lectin activity was attemped by a serial dilution of 50 μl of diluted preparation 1 (1:1 in saline) in different carbohydrate solutions (50 μl). After a 20 min incubation, 50 μl of a 2.5% (v/v) suspension of chicken erythrocyte treated with glutaraldehyde were added. No pseudoagglutination was detected in the preparation's absence. The lectin activity was not inhibited by D(+) glucose, D(+) mannose, D(+)galactose, D(+) fucose, L(−)fucose, D(+)xylose, L(+)rhamnose, L(+)arabinose, D(+)ribose, D(+)fructose, α-trehalose, melibiose, sucrose, lactose, raffinose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, 1-O-methyl-α-D-galactopyranoside, 2-deoxy-D-galactose, 1-O-methyl-β-D-galactopyranoside, 1-O-methyl-β-arabinopyranoside, neuraminic acid, and

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D-glucuronic acid, in concentrations varying from 1.52 to 200 mM, or by solutions of starch (1% w/v), agarose (0.01% w/v), and inositol (0.02% w/v). Additionally, no inhibition was detected when different combinations of saccharides were attemped such as D-glucose/D-galactose, D-glucose/N-acetyl-glucosamine, D-glucose/D-fucose, D-galactose/N-acetyl-glucosamine and D-galactose/D-fucose. When erythrocytes from human (type AB) or rabbit were assayed, the saccharides also did not promote any inhibition.

The differences in the hemagglutination activity observed with erythrocytes from bovine, dog, sheep, horse, chicken, duck and pig, could not give us a clue on the target sugar. Contrarily to other assayed lectins, there was no difference in the activity of the *S. pickellii* lectin when erythocytes from several animals of the same species were used, however the referred erythrocytes were not previously submitted to a sub-group classification.

In a preliminary attempt to purify the lectin(s), an affinity adsorption was made by using formalinized erythrocytes from human, (type AB) and chicken. Very low protein recoveries (1%) and very low activities were obtained when desorption was made non-biospecifically by using a borate buffer (H. Horejsi et al., 1978, *Biochim. Biophys. Acta, 532:* 98-104) or low pH buffers (H. Lis & N. Sharon, 1981, *J. Chromatog., 215:* 361-72). The high specific hemagglutination activity obtained with erytrocytes from human type AB and B could suggest that matrices containing galactose, N-acetyl-galactosamine or sugars with a similar related configuration could succeed to purify the lectin(s) by affinity chromatography.

Following, a 10% (w/v) extract was made with 0.15M-NaCl; 0.15M-sodium phosphate buffer (pH 5.0 to 8.5) and 0.15M-acetate buffer (pH 3.0 to 5.5), both containing 0.15M-NaCl. The best extraction was obtained in the sodium phosphate buffer, pH 7.5 (preparation 2) with a SHA of 277; in 0.15M-NaCl the SHA was 23. The preparation 2 was then submitted to a fractionation with ammonium sulphate (A. A. Green & R. C. Hughes, 1955, *Meth. Enzymol., 1:* 67-90), for 4 h at room temperature. The fractions (F 0-20%; F 20-40%; F 40-60% and F 60-80%) and the final supernatant were exhaustively dialized against distilled water, followed by a 0.01 M-sodium phosphate buffer, pH 7.5, containing 0.15M-NaCl. Most of the lectin activity (78%) was obtained in the F 20-40%. A lack of inhibition by the saccharides was also observed in this partially purified preparation.

It is possible that more than one form of the *S. pickellii* lectin (isolectins?) are present in the crude preparations, a phenomenon that is very frequent in plants. In a preliminary chromatographic approach, DEAE- cellulose seems to be of value to purify the lectin.

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