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Lectins from squash (*Cucurbita ficifolia*) seedlings

Irena Lorenc-Kubis, Agnieszka Klimczak and Bronisława Morawiecka

Institute of Biochemistry, University of Wrocław, Tamka 2, 50-137 Wrocław, Poland

Lectins are proteins or glycoproteins of non-immune origin, which interact with free saccharides or glycosylated macromolecules in soluble or membrane bound form [1, 2].

Most legumes and grass seeds are a rich source of lectins which have been isolated and characterized with respect to their molecular structure and carbohydrate-binding specificity. Plant lectins do not occur exclusively in seeds but are also present in all of vegetative tissues, i.e. roots, bulbs, tubers, leaves, stems and fruits.

Very little is known about the presence of lectins in germinating seeds and developing seedlings of *Cucurbita ficifolia*. Sabnis & Hart [3] found hemagglutination activity in phloem exudates of three members of the *Cucurbitaceae* family: pumpkin *(Cucurbita maxima)*, cucumber (*Cucumis sativus)* and melon *(Cucumis melo)*, but no lectin was detected in seeds of these three species or in the developing plants until the seedlings were at least 5 days old.

The aim of this investigation was to test cotyledons from squash *(C. ficifolia)* for their hemagglutination activity during a germination period of 22 days, as well as the activity of dry seeds.

It was found that the agglutination activity in resting seeds and in cotyledons at the onset of germination was rather low but it was increased to values 10 to 12 times as high on the

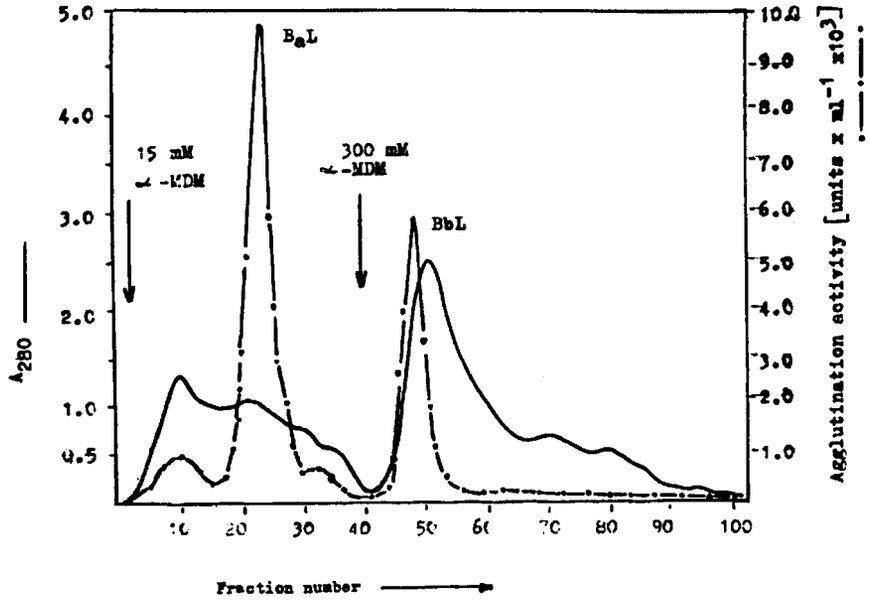

Fig. 1. Affinity chromatography on Con A-Sepharose 4B of lectins from cotyledons of C. ficifolia.
The proteins were applied onto the column equilibrated with 0.1 M sodium acetate buffer, pH 5.6, containing 0.1 M NaCl and $CaCl_2$, $MgCl_2$, $MnCl_2$ (1 mM each). Elution was carried out first with the above buffer (peak A), then the proteins bound to the column (peak B) were eluted with buffer containing: 15 mM (BaL) and 300 mM α-methyl-D-mannopyranoside (α-MDM)-(BbL)

6th day and then was sharply decreased on the 10th day (not shown). Therefore lectins were isolated only from 6 day-old seedlings.

The cotyledons were homogenized in 0.1 M acetate buffer, pH 5.1, at 4°C and extracted for 1 h with constant mechanical stirring. After centrifugation, the supernatant was used for further purification of the lectins by affinity chromatography on Con A-Sepharose (Fig.1).

About 50% of the total protein applied on the column was washed out with 0.1 M acetate buffer, pH 5.6. These proteins (fraction AL) showed agglutination activity.

The Con A-bound proteins were eluted from the column with 15 mM α-methyl-D-mannopyranoside (fraction BaL) and then with 300 mM solution of this sugar (fraction BbL).

Lectins from fractions AL were further purified by ion-exchange chromatography on SP-Sephadex C-50, pH 4.8 and next on QAE-Sephadex pH 8.6. The adsorbed lectins were eluted from the column with 0.1 M Tris/HCl buffer, pH 8.0 containing 0.25 M NaCl (lectin AL_I) and 1 M NaCl (lectin AL_{II}).

The lectins present in fractions BaL and BbL were further purified by ion-exchange chromatography on SP-Sephadex C-50 and again applied on Con A-Sepharose. Lectin BaL was washed out from this Con A column with 0.1 M acetate buffer, pH 5.6, while lectin BbL was eluted with 50 mM α-methyl-D-mannopyranoside.

The purified lectins were analysed by the double immunodiffusion test, using antibodies raised against Con A (Fig. 2). Each of the lectins formed an immunoprecipitate which suggests that squash cotyledon lectins and Con A have common antigenic sites. Immunological evidence for structural similarity among lectins has been provided for *Leguminosaceae* [4], *Solanaceae* [5] and *Gramineae* [6].

Lectins from *C. ficifolia* interact with free Con A and form affinity precipitates indicating that they are glycoproteins with exposed mannose or glucose units. The highest agglutination activity of lectins was observed for A and B human erythrocytes and sheep erythrocytes.

Three of the lectins isolated from squash cotyledons Al_I, BaL and BbL were examined for the sugar specificity. Among the monosaccharides tested as inhibitors of agglutination, D-GalNAc and L-Ara were most active with lectin BaL, while L-Fuc and D-Ara were most potent inhibitors of lectin BbL. In the case of lectin AL_I the hemagglutination was inhibited by D-GlcNAc and D-Glc. Mannose, xylose and lactose were less effective inhibitors of all lectins.

These results seem to suggest that in squash cotyledons very similar but not identical lectins are present.

The occurrence of lectins differing in sugar specificity within the same plant has also been reported for several legumes [7, 8].

Earlier we have shown that cotyledons of *C. ficifolia* contain two glycoprotein acid phosphatases which differ in binding to Con A [9]. To check whether the purified lectins were able to

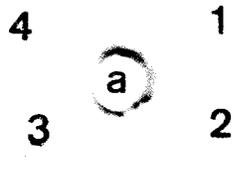

Fig. 2. Ouchterlony immunodiffusion of lectins from cotyledons of C. ficifolia against Con A antibodies.
Antibodies were applied in the central well (a). The peripheral wells were filled with cotyledon lectins (20 μg): 1, Al_I; 2, Al_{II}; 3, BaL; 4, BbL

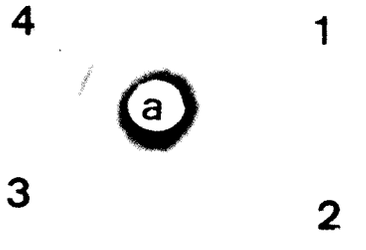

Fig. 3. Double diffusion in 10 mg/ml agarose gel (pH 8.6) of lectins from cotyledons of C. ficifolia and acid phosphatase Bb_1.
The enzyme (20 μg) was placed in the centre well (a), the lectins (20 - 30 μg) in peripheral wells: 1, AL_L; 2, BbL; 3, AL_{II}; 4, BaL

bind the endogenous acid phosphatase (AcPase) Bb_1, the double diffusion test was performed (Fig. 3). It was found that both the GlcNAc/Glc lectin AL and the Fuc specific lectin BbL formed an affinity precipitate with AcPase Bb_1; this may indicate that the AcPase contains exposed GlcNAc/Glc and fucose units.

It has been found earlier that some plant glycoprotein acid phosphatases are activated by binding to Con A [10, 11]. Con A caused a decrease of the apparent K_m value [12], changed the pH optimum and increased thermostability and resistance to proteolysis [13].

We have found that squash cotyledon lectins (Al and BbL) also stimulate the activity of endogenous AcPase Bb_1 and Bb_2 by 60 - 90% and change their resistance to heat denaturation.

These results suggest a protective action of lectins consisting in stabilization of the enzyme conformation.

The molecular interaction between lectins and glycoprotein enzymes, especially those isolated from the same tissue, like acid phosphatases and lectin from potato [13], rye germ [14] and squash seedling cotyledons may suggest a possible role of lectins as transmitters and effectors involved in the metabolism of the plant cell.

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