0014-4754/93/121098-03$1.50 + 0.20

A lectin from the liverwort *Marchantia polymorpha* L.*

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Received 16 July 1993; accepted 18 August 1993

Abstract. Bryophytes have been screened for lectins. From the liverwort *Marchantia polymorpha* (Marchiantiales) a lectin could be purified to homogeneity using a combination of ultrafiltration, size exclusion chromatography and ion exchange chromatography. SDS polyacrylamide gel electrophoresis, size exclusion chromatography and electrospray mass spectroscopy showed that the lectin is a monomeric protein with a M_r of 16,134.64 ± 2.93. *Marchantia polymorpha* lectin agglutinates erythrocytes of different mammalia and exhibits carbohydrate specifity against complex carbohydrate structures. This is the first report of a lectin isolated from liverworts.
Key words. Bryophyta; liverwort; *Marchantia polymorpha*; lectin; isolation; characterization.

Lectins are a heterogeneous group of (glyco-) proteins of non-immune origin sharing the ability to recognize and bind specific sugars or sugar-containing molecules. They can agglutinate selected carbohydrate-coated cell surfaces, e.g. red blood cells and bacteria[1]. Lectins are widely used as molecular tools and have contributed to our understanding of cellular processes, including the elucidation of the fundamental principles of immunology and of differences between malignant and normal cells[2, 3].

They have been identified in nature in species ranging from microorganisms to animals[4]. At present over 100 lectins of plant origin have been isolated, either from seeds or vegetative tissues, and their molecular structure, biochemical properties, and sugar binding specificity characterized in detail. Lectins have been described for all taxa of the plant kingdom except for bryophytes. In this paper we report the screening of bryophytes for lectins and the isolation and partial characterization of a lectin from a liverwort.

Materials and methods

Plant material. Plant material was available from sterile cultures[4] or from different natural habitats (field collection). Liverworts: *Conocephalum conicum. Diplophyllum albicans, Jamesionella autumnalis, Lepidozia reptans, Lophocolea cuspidata, Marchantia plicata, Marchantia polymorpha* (11 different origins), *Pellia epiphylla, Plagiochila adiantoides, Reboulia hemispherica, Ricciocarpos natans, Scapania nemorea, Symphyogyna brongniartii.* Hornworts: *Anthoceros agrestis.* Mosses: *Hypnum cupressiforme, Polytrichum commune, Mnium hornum, Rhytidiadelphus squarrosus, Scleropodium purum, 2 Sphagnum* species.

Reagents. All reagents used were analytical grade. Sepharose 4B, Sephacryl S-100 Hr and SP Sephadex C50 were obtained from Pharmacia (Uppsala, Sweden). IgG-Sepharose was prepared according to Franz and Ziska[5].

Extract preparation. For the screening, 1 g of plant material was homogenized in 10 ml phosphate buffered saline (PBS), pH 7.4. The supernatant of the centrifuged suspension was used for the hemagglutination assays.

Extraction and purification of the lectin. 20 g of lyophilized powdered gametophytes of *Marchantia polymorpha*, collected in a greenhouse, were extracted for 30 min with 400 ml PBS, pH 7.4, containing 0.05% thiourea to inhibit phenoloxidase activity. The suspension was passed through cheesecloth to remove debris and centrifuged at 30,000 g for 30 min in the cold. The supernatant was concentrated by ultrafiltration. The concentrate was chromatographed on Sepharose 4B to remove high molecular mass polysaccharides. The lectin-containing fractions were pooled and concentrated by ultrafiltration. This crude lectin fraction was chromatographed on Sephacryl S-100 HR. The lectin-positive protein peak was rechromatographed on a SP-Sephadex column, equilibrated with 0.01 M acetate buffer, pH 4.0. The column was eluted stepwise with acetate buffer and a linear salt gradient (0–10% NaCl).

Preparation of stabilized erythrocytes. Human (group-0), bovine, rat and rabbit erythrocytes were washed 3 times in PBS, pH 7.4, followed by centrifugation at 600 g for 10 min. 100 ml of 1% erythrocyte suspension were incubated with 0.4 ml glutardialdehyde for 1 h at room temperature. After washing with PBS the stabilized erthrocytes were resuspended in PBS to give 1% solutions.

Hemagglutination assay. Hemagglutination was assayed in microtiter plates with U-shaped bottoms by serially diluting a 50 µl sample into 50 µl of PBS. 50 µl of 1% suspension of glutardialdehyde-stabilized human or animal erythrocytes in the same buffer were added to

each well. Hemagglutination was determined after 1 h incubation at room temperature as the reciprocal of the greatest dilution that gave visible aggregation. Effects of simple sugars and glycoproteins were examined by preincubation of the lectin with the test substance at room temperature for 30 min, followed by incubation at room temperature for 1 h after addition of erythrocyte suspension.

Protein determination. Protein was determined by the method of Lowry et al.[6], with bovine serum albumin as standard.

SDS polyacylamide-gel electrophoresis. Lectin preparations were analyzed by SDS polyacrylamide-gel electrophoresis by using a discontinuous system[7] on 10% (w/v)-acrylamide gels. The molecular mass of the lectin treated with SDS was determined in the presence and absence of 2-mercaptoethanol by using standard M_r markers. Glycoprotein staining was done according to the method of Zacharius[8].

Ultrafiltration. Ultrafiltration was carried out using a stirred cell (Amicon 52) and Diaflo PM10 ultrafiltration membranes, both Grace & Co. Conn. Amicon Division, Beverly (USA).

Molecular mass determination. The molecular weight of the lectin was determined by size exclusion chromatography (SEC) on a Sephacryl S-100 HR column (70 × 1.6 cm) calibrated with BSA (M_r 66,000) and horseradish peroxidase (M_r 40,000), and by electrospray mass spectroscopy (ES-MS) on a VG BioQ mass spectrometer, 3 kV, (VG BioTech, Altricham, England).

Results

Screening. Standardized extracts of 13 liverwort species, one hornwort and 7 moss species were screened for hemagglutination. Plant lectins often exhibit species specificity for erythrocyte agglutination. Therefore we used red blood cells of several vertebrates in the hemagglutination assays. Among the tested plants only *Marchantia polymorpha* showed activity. One of the 11 investigated *M. polymorpha* origins was negative in the assay.

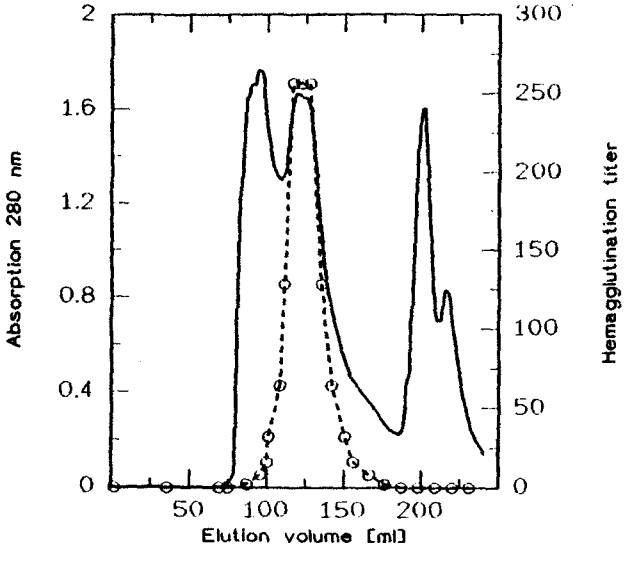

Figure 1. Purification of the lectin by ion size exclusion chromatography on a Sephacryl S-100 HR column 16 mm × 700 mm. Flow rate 40 ml/ h. [——] Absorption at 280 nm; [O----O] Hemagglutination titer.

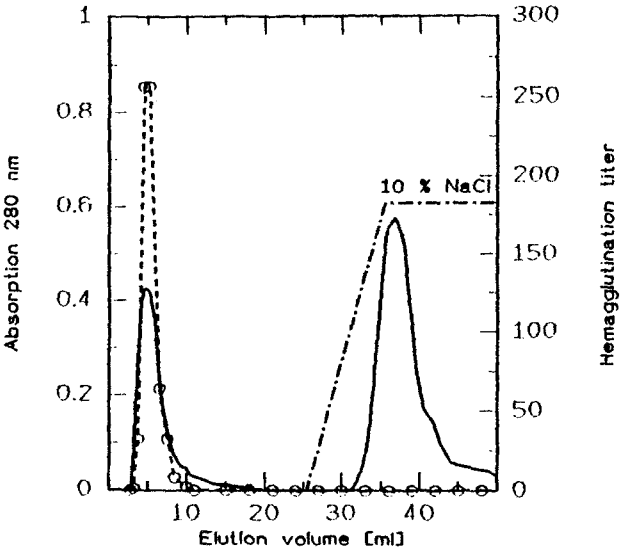

Figure 2. Purification of the lectin by ion exchange chromatography on a SP Sephadex C50 column 16 mm × 150 mm. Flow rate 1 ml/ min. [——] Absorption at 280 nm; [O----O] Hemagglutination titer [·—·—·] NaCl gradient.

Purification of the lectin. Preliminary experiments showed that the agglutination of the crude extract of *M. polymorpha* was inhibited by IgG, but the lectin could not be isolated by affinity chromatography using a IgG-Sephrose column because it was irreversibly bound to the affinity matrix. Isolation of the lectin could be achieved by subsequent ultrafiltration, size exclusion (fig. 1) and ion exchange chromatography (fig. 2). In the latter step, the lectin was purified by binding the contaminants to the cation exchanger. The lectin eluted with the dead volume of the column. The overall yield was 1 mg lectin /20 g dry weight of gametophytes.

Characterization of the purified lectin.

Molecular mass. Homogeneity and molecular mass of the purified lectin were confirmed by several methods. On SDS–PAGE it gave a single protein band both in the presence and absence of mercaptoethanol. The M_r value was calculated to be approximately 18, 000. The native M_r as estimated by size exclusion chromatography on Sephacryl S-100 HR resulted in a single peak at approximately 20,000. By ES-MS the precise mass of the lectin was found to be 16,134.64 ± 2.93 D.

Agglutination properties and carbohydrate specificity. The *M. polymorpha* lectin agglutinated all types of erythrocytes tested, although there were some quantitative differences. Human blood showed the highest sensi-

tivity. The carbohydrate specificity of the lectin was determined using a series of simple sugars and molecules with complex carbohydrate structure. Hemagglutination of human erythrocytes could not be inhibited using 0.2 M solutions of any of the following carbohydrates: glucose, galactose, fructose, rhamnose, fucose, xylose, mannose, lactose, N-acetylglucosamine and N-acetylgalactosamine. Hemagglutination was inhibited by blood group substance A and B, IgG, and lactalbumin. A 0.1% solution of the purified lectin showed an agglutination titer of 1024 with human erythrocytes.

Discussion

M. polymorpha contains a lectin which exhibits carbohydrate binding specificity for complex carbohydrate structures. Similar binding specificities have been described e.g. for lectins from *Vicia graminea*[9], *Lepidum sativum*[10] and *Agaricus edulis*[11]. SDS-PAGE, size exclusion chromatography and ES-MS studies show the lectin to be a monomeric protein without intramolecular disulfide bonds and with no covalently bound carbohydrate. The transformed mass spectrum gives a single peak implying that the lectin contains no carbohydrate[12]. Furthermore, the lectin band in SDS-PAGE did not stain for glycoprotein. The *M. polymorpha* lectin is the first lectin described from bryophytes.

Acknowledgements. We thank Prof. Dr. H. Franz (†) and Dr. P. Ziska, Staatliches Institut für Immunpräparate, Berlin, for their support and Dr. A. van Dorsselaer, Faculté de Chimie, Université de Strasbourg, for the measurement of the ES-MS spectrum.

* This article forms Publication No. 71 of the 'Arbeitskreis Biologie und Chemie der Moose'.

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