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THE PURIFICATION OF POTATO LECTIN BY AFFINITY CHROMATOGRAPHY ON A FETUIN–SEPHAROSE MATRIX

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato lectin; affinity chromatography; chitotriose; fetuin.

INTRODUCTION

Lectins are proteins and glycoproteins that specifically bind carbohydrate residues and are characterized by a common ability to agglutinate red blood cells. A lectin that binds *N*-acetylglucosamine oligomers has been purified from potato tubers. It is a basic glycoprotein consisting of approximately 50% by weight of carbohydrate and is rich in arabinose and hydroxyproline. It is also notably rich in cystine (11.5% half-cystine) [1]. It has been shown that the hydroxyproline residues in the polypeptide are glycosylated by tri- and tetra-arabinoside residues [2], and that the arabinose residues are β-linked in the furanose form [3]. Additionally most of the serine residues are substituted with single α-galactosyl residues. Chemically, therefore, potato lectin resembles the glycoprotein component of plant cell walls [2, 4, 5].

The lectin was first purified from an aqueous extract of potato tubers by ion-exchange chromatography and gel filtration [1]. Subsequently two methods of purification by affinity chromatography on Sepharose substituted by a synthetic ligand (*p*-aminobenzyl-1-thio-*N*-acetylglucosaminide) [6] and tri-*N*-acetylchitotrioside-Sepharose [7] have been reported. This paper describes the purification of potato lectin by affinity chromatography on a fetuin–Sepharose matrix by a procedure similar to that used for the purification of a lectin from seeds of *Datura stramonium* [8]. This latter lectin also binds oligomers of *N*-acetylglucosamine.

RESULTS AND DISCUSSION

Sela *et al.* [9] have suggested that lectins generally can be eluted from fetuin–Sepharose with 0.5 M NaCl/50 mM glycine HCl, pH 3.0. However the potato lectin could not be eluted from the column by this method (Fig. 1). Additionally in contrast to Delmotte *et al.* [6] and Desai and Allen [7], the lectin could not be removed by lowering the pH with 0.1 M acetic acid. It was eluted as a single symmetrical peak with chitotriose (0.1 mM). The binding of potato lectin to fetuin presumably depends upon the *N*-acetylglucosamine residues in the carbohydrate side chains of the glycoprotein. Although recently it has been shown that the binding of wheat germ agglutinin to fetuin depends upon the presence of terminal sialic acid residues [10], wheat germ agglutinin has a sugar binding specificity similar to that of potato lectin.

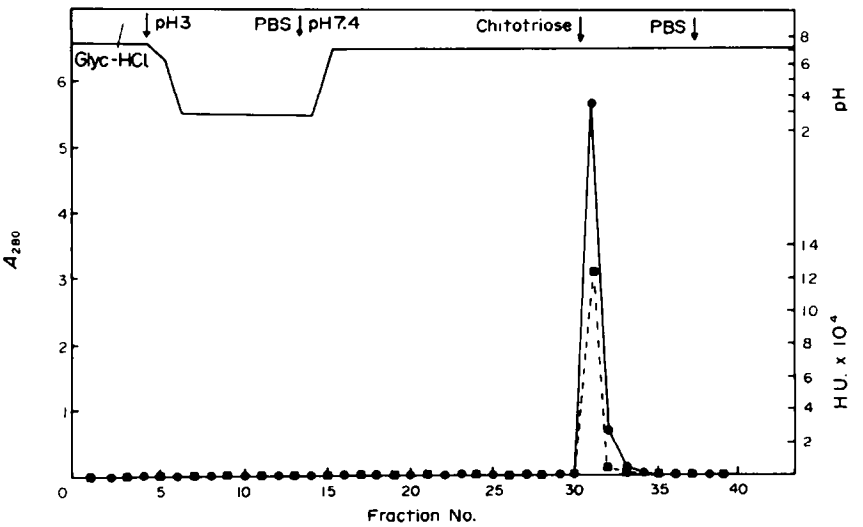

Fig. 1. Elution profile of potato extract from fetuin–Sepharose. Fraction volume, 6 ml. A_{280} ●–●; haemagglutination, ■–■; H.U., haemogglutinating unit—the minimum amount of lectin required to cause agglutination.

Potato lectin prepared by the method reported here gave a single band following electrophoresis in a 10% SDS polyacrylamide gel at pH 8.8. It was stained for protein with Coomassie blue and carbohydrate with periodic acid-Schiff reagent. The lectin eluted as a single symmetrical peak from Sephadex G100 in the presence of β-mercaptoethanol (100 mM) in the position expected for a globular protein of MW 105 000 ± 20 000. The results of amino acid and neutral sugar analyses were generally consistent with those previously reported [1, 2]. According to these criteria, this lectin is homogeneous and identical with that previously purified from potato tubers.

EXPERIMENTAL

CL Sepharose 4B, Sephadex G15 and G100 were obtained from Pharmacia (Uppsala, Sweden), fetuin from GIB Co. (New York, U.S.A.) and chitin (practical grade) from Sigma Chemicals (Poole, Dorset, U.K.). All other chemicals were purchased from BDH Chemicals (Poole, Dorset, U.K.) Analar grade.

Preparation of affinity matrix. CL Sepharose 4B was reacted with cyanogen bromide by the method of ref. [11]. The CNBr-activated Sepharose was then added to an equal vol. of $NaHCO_3$ (0.1 M) containing 200 mg fetuin and continually mixed overnight at 4°. The coupled gel was then washed successively with $NaHCO_3$ (0.1 M), NaCl (0.5 M), glacial HOAc (0.1 M) and $NaHCO_3$ (0.1 M). Unreacted imidocarbonate groups were blocked with glucosamine (glucosamine 0.2 M in 0.1 M $NaHCO_3$ shaken at 4° for 4 hr). Finally the gel was washed again with $NaHCO_3$ (0.1 M) and packed into a column (3 × 2 cm equilibriated with Pi-buffered saline (PBS; Na_2HPO_4 1.48 g/l. KH_2PO_4 0.5 g/l., NaCl 7.2 g/l.) pH 7.4.

Preparation of N-acetylglucosamine oligomers. Chitin from crabshells was deproteinized by a modification of the method of ref. [12]. Chitin (20 g) was then dissolved in conc. HCl (340 ml) and incubated at 40° for 2.5 hr (partial hydrolysis conditions of Rupley [13]). Oligosaccharides were separated by gel filtration on a column of Sephadex G15. (110 × 3 cm).

Purification of potato lectin. Peeled potato tubers (cv King Edwards) (2 kg) were homogenized in a total vol. of 2 l. Pi-buffered saline to which sodium metabisulphite (2 mM) had been added as an anti-oxidant. The pulp was then filtered through 4 layers of muslin and centrifuged for 30 min at 2000 g. The residues were combined and re-extracted. The combined supernatants were brought to 60% saturation by the addition of solid $(NH_4)_2SO_4$ and the ppt. allowed to settle overnight. The ppt. was recovered by centrifugation (9000 g for 30 min) and resuspended in distilled H_2O. This was dialysed against two changes of distilled water and Pi-buffered saline. The dialysate was then applied directly to the fetuin–Sepharose column, at a flow rate of 30 ml/hr (10 mm/hr) and eluted with PBS pH 7.4 until A_{280} of the eluant was <0.02. The column was then eluted with *N*-acetylglucosamine oligomers (0.1 mM) and fractions (6 ml) monitored for A_{280} and lectin activity by the agglutination of trypsin-treated human (A, D+) erythrocytes using a microtitre system. Active fractions were combined, dialysed against 2 changes of PBS, then 2 changes of distilled water. The dialysate was lyophilized and stored at −20°.

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