

PURIFICATION OF *VICIA GRAMINEA* ANTI-N LECTIN BY AFFINITY CHROMATOGRAPHY

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1. Introduction

The lectin of *V. graminea* seeds specifically agglutinates blood group N-positive (NN and MN) erythrocytes [1,2] and is inhibited by glycoproteins obtained from N-positive red cells [3–5]. The lectin is a useful tool in studies on M and N blood group specific structures, as it shows a different specificity than rabbit and human anti-N antibodies. The release of sialic acid from M and N glycoproteins results in a marked increase of activity toward *V. graminea* lectin (N_{Vg} activity), and makes M and N glycoproteins almost equally potent inhibitors of this lectin [3,4,6–8]. Mild alkaline degradation destroys the N_{Vg} activity of red cell glycoproteins [9,10]. These results indicate that N_{Vg} specificity is confined to desialylated alkali-labile oligosaccharide chains of erythrocyte glycoproteins, namely to the structure β -galactosyl(1–3)*N*-acetylgalactosamine [9,11]. However, *V. graminea* lectin is not inhibited, either by the free disaccharide [8] or by the 'anti-freeze' glycoprotein containing multiple disaccharide chains of this structure [12]. On the other hand, *V. graminea* lectin reacts with some other glycoproteins that do not show blood group N activity [8,13–16].

Purification of *V. graminea* lectin should be helpful in the elucidation of its interesting specificity. However, it is difficult to obtain a sufficient amount of the seeds, due to difficulties with the cultivation of *Vicia graminea*. Recently, Prigent and Bourrillon [17] purified *V. graminea* lectin by chemical methods. They found that this lectin is an acidic glycoprotein of molecular weight 100 000, containing 7.3% carbohydrates (mainly galactose and *N*-acetylglucosamine). We report here the purification

of *V. graminea* lectin by affinity chromatography, using desialylated erythrocyte glycoprotein as an immobilized ligand.

2. Materials and methods

2.1. *Vicia graminea* extract

Vicia graminea was cultivated in the garden of one of authors (M.D.) from seeds offered by Professor G. F. Springer (Evanston, Ill.).

Three grams of ground seeds were treated with 60 ml phosphate buffer (0.1 M phosphate, pH 6.5, in 0.15 M NaCl; this buffer was also used in other experiments and will be referred to as phosphate buffer). The fine suspension was decanted and the coarse residue was treated again with 30 ml of the buffer. The two extracts were combined, incubated at 37°C for 2 h and centrifuged at 6000 $\times g$ for 10 min. The agglutinating titer of the supernatant fluid was 1:16 – 1:32.

2.2. Desialylated erythrocyte glycoprotein

Glycoprotein was prepared from human blood group O erythrocytes by the phenol–water extraction of membranes [18]. Sialic acid was released by a mild acid hydrolysis in 0.05 N sulfuric acid, at 60°C for 4 h [7].

2.3. Affinity chromatography

Sephacrose 4-B was activated with CNBr, according to the method of Cuatrecasas [19]. The activated Sepharose (20 ml) was treated with 20 mg of desialylated red cell glycoprotein at 4°C for 20 h. Approximately 70% of the glycoprotein was coupled to the

gel, which was subsequently equilibrated with the phosphate buffer. The adsorbent is stable for several months when stored at 4°C.

V. graminea extract (65 ml), dialyzed against phosphate buffer, was filtrated through the glyco-protein-Sephadex column (20 ml), which was then washed with the phosphate buffer, until the absorbance at 280 nm was at background. The column was then eluted with 3 M KCNS and 2 ml fractions were collected. The fractions showing absorbance at 280 nm (as one sharp peak) were pooled and exhaustively dialyzed against phosphate buffer.

The maximal lectin-binding capacity of the column was checked in a separate experiment: 1 ml column can totally bind the lectin from 10 ml of the extract.

2.4. Polyacrylamide gel electrophoresis

The electrophoresis was performed according to the conventional method [20], using 7.5% polyacrylamide gel in 0.1 M phosphate buffer, pH 7.2. The size of gels was 0.6 × 7.0 cm, and the electrophoresis was carried out for 4 h at the current 8 mA/gel.

2.5. Agglutination assay

Two-fold serial dilutions of the lectin were incubated with an equal volume of 20% suspension of red cells for 2 h at 20°C. The agglutinating activity is expressed in units: the reciprocal of the titer is taken as the number of units per milliliter.

3. Results and discussion

The affinity chromatography gives substantial purification of the lectin (table 1) but as shown by polyacrylamide gel electrophoresis (fig.1) the obtained preparation contains an aggregated protein, which does not enter the gel. The final purification was achieved by gel filtration on Sephadex G-200 (fig.2). The agglutinating activity was recovered in fractions of the eluate following the unretarded protein peak. The active fractions were pooled and concentrated by ultrafiltration.

The purified lectin is homogeneous in polyacrylamide gel electrophoresis (fig.1), no protein migrating to cathode at pH 7.2 was found. The concentra-

Table 1
Scheme of purification of *V. graminea* lectin

Purification step	Volume (ml)	A_{280}	Total hemagglutinating activity (units)	Recovery of material absorbing at 280 nm (%)	Recovery of activity (%)	Purification factor ^a
1. Dialyzed extract	65	3.7	1040	100	100	
2. Affinity chromatography						
(i) fraction eluted with the phosphate buffer	70	3.2	not active	93	0	
(ii) fraction eluted with KCNS	24	0.58	768	5.8	74	13
3. Sephadex G-200 chromatography, active eluate concentrated by ultrafiltration	4.8	0.135	614	0.3	60	200

^aCalculated from the recovery of activity and absorbance at 280 nm

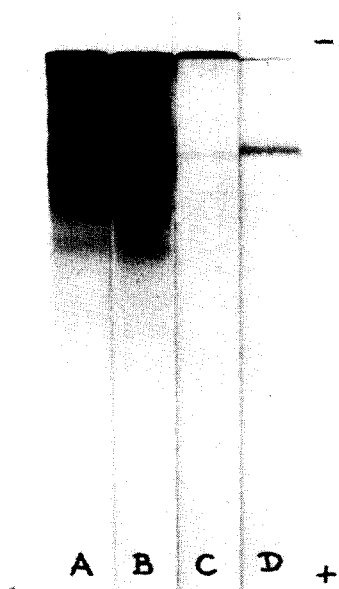


Fig.1. Polyacrylamide gel electrophoresis of (A) *V. graminea* extract (10 μ l) (B) inactive fraction from affinity chromatography (15 μ l) (C) lectin after affinity chromatography (70 μ l) (D) lectin after gel filtration (140 μ l). Gels were stained with Coomassie Brilliant Blue.

tion of protein in the solution of purified lectin is of the range 100 μ g/ml, taking into account the coefficients of absorbance at 280 nm for other lectins [21]. The agglutinating titer of this solution is 1:128, the agglutination is specific for N erythrocytes. The results obtained are comparable with those of Prigent and Bourrillon [17], regarding the purification factor, the electrophoretic mobility, and the specific agglutinating activity of the purified lectin.

The use of desialylated glycoprotein for affinity chromatography has the following advantages in comparison with untreated glycoprotein:

(1) Desialylated glycoprotein has the higher lectin-binding capacity.

(2) It can be obtained from erythrocytes, regardless their MN phenotype.

(3) The unspecific ionic binding of protein to the acidic sialoglycoprotein adsorbent is avoided.

The simple two-step procedure, involving affinity chromatography, allows purification of *V. graminea*

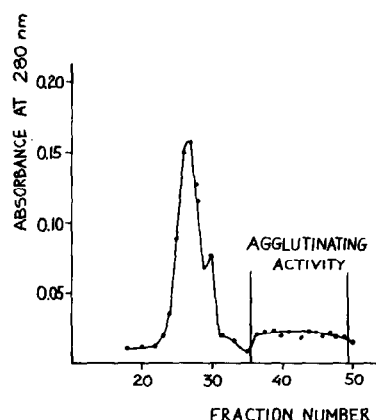


Fig.2. Gel filtration of *V. graminea* lectin. Solution obtained from affinity chromatography (24 ml, concentrated by ultrafiltration to 6 ml) was applied to the column of Sephadex G-200 (2.7 \times 60 cm) in the phosphate buffer. The flow rate was 16 ml/h, 4 ml fractions were collected.

anti-N agglutinin with a good yield, even from a small sample of the seed extract.

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