JERZY PETRYNIAK, MARIA JANUSZ, ELŻBIETA MARKOWSKA and ELWIRA LISOWSKA

PURIFICATION OF THE EUONYMUS EUROPÆUS LECTIN BY AFFINITY CHROMATOGRAPHY ON THE DESIALIZED MN BLOOD GROUP GLYCOPROTEIN, AND LECTIN NH₂-TERMINAL ANALYSIS *

Department of Immunochemistry, Ludwik Hirschfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Czerska 12; 53-114 Wroclaw, Poland

A new method for purification of the Euonymus europaeus lectin by affinity chromatography is presented. Desialized MN glycoprotein coupled to Sepharose 2B was used as immunoadsorbent and lectin was eluted with lactose. NH₂-Terminal amino acid analysis of lectin showed only the presence of alanine.

The major sialoglycoprotein from human erythrocytes (MN blood group glycoprotein) contains one alkali-stable complex and about 16 alkali-labile oligosaccharide chains (Adamany & Kathan, 1969; Thomas & Winzler, 1969; Krotkiewski & Lisowska, 1978; Tomita et al., 1978; Lisowska et al., 1980). Since native and desialized oligosaccharides (both alkali-labile and alkali-stable) of this glycoprotein are receptors for many lectins (Lis & Sharon, 1977; Lisowska, 1977), insolubilized MN glycoprotein may present a convenient specific adsorbent for purification of lectins (Lisowska et al., 1976) and carbohydrate-specific antibodies (Kim & Prakash, 1979).

The E. europaeus lectin, which is specific for B and H blood group determinants, has been purified, both by the conventional technique (Pacák & Kocourek, 1975) and by affinity chromatography on the insoluble derivative of purified A+H-blood group active hog gastric mucin (Petryniak et al., 1977).

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The specific interaction between the *E. europaeus* lectin and desialized alkali-stable oligosaccharide chain of MN glycoprotein was described by Petryniak *et al.* (1978, 1980). In this report we present purification of the *E. europaeus* lectin on the desialized MN glycoprotein coupled to Sepharose 2B. This technique enables rapid purification of the lectin on a preparative scale. NH₂-Terminal amino acid analysis of the *E. europaeus* lectin showed only the presence of alanine.

**MATERIAL AND METHODS**

*Euonymus europaeus* extract. Since the *E. europaeus* lectin was reported to be located exclusively in the arils (Schmidt, 1954), they were used as a source of lectin. Seeds were collected from wild growing shrubs in the neighbourhood of Wroclaw in September/October. Arils were obtained by pressing seeds in hands, followed by shaking on a Petri dish. Fifty grams of arils were defatted by treatment with 150 ml of cold methanol at 4°C for 1 h with occasional stirring. The defatted arils were extracted with NaCl/Pi¹, as described earlier (Petryniak *et al.*, 1977). The initial four extracts, containing over 90% of haemagglutinating activity, were combined and used for lectin purification. The haemagglutinating titer of the combined extracts varied from 1:64 to 1:512.

Preparation of desialized MN glycoprotein-Sepharose 2B conjugate. MN glycoprotein was obtained from human blood group 0 erythrocyte membranes by phenol-water extraction (Baranowski *et al.*, 1959). Sialic acid was released by hydrolysis in 0.025 M-H₂SO₄ at 80°C for 40 min (Lisowska & Duk, 1976) and removed by dialysis. Sepharose 2B (Pharmacia Fine Chemicals, Uppsala, Sweden) was activated by the cyanogen bromide technique (Cuatrecasas, 1970). To the activated Sepharose (150 ml) was added 150 mg of the desialized MN glycoprotein. The coupling procedure was carried out according to the instructions of Pharmacia Fine Chemicals; 92% of the desialized MN glycoprotein was coupled to the gel. The conjugate stored at 4°C was stable over 5 months.

Affinity chromatography. All procedures were performed at 4°C. Before each run, the column containing the conjugate was washed consecutively with 0.15 M-NaCl/0.01 M-NaHCO₃, 0.15 M-NaCl/0.01 M-acetate buffer, pH 4, and with NaCl/Pi. The *E. europaeus* extract was centrifuged for 1 h at 2000 r.p.m. directly before application to the affinity column. After exhaustive washing of the column with NaCl/Pi, the bound lectin was eluted with 0.1 M-lactose (Sigma). Active fractions showing the haemagglutinating titer not lower than 1:2 were pooled, concentrated and

¹ Abbreviations: NaCl/Pi, 0.01 M-phosphate in 0.15 M-NaCl, pH 7.1, containing 0.02% sodium azide; Dan, 1-dimethylaminonaphthalene-5-sulfonyl residue; Dan-Cl, 1-dimethylaminonaphthalene-5-sulfonyl chloride.
dialysed in Diaflo chamber, using UM10 membrane Amicon, Holland, until no periodate-positive material (Kabat, 1961) was found in the ultrafiltrate.

The maximal lectin binding capacity of the adsorbent was about 4 ml of the extract with haemagglutinating titer 1:256, per 1 ml of the conjugate.

\[ \text{NH}_2 \text{-Terminal analysis.} \] Dan-Cl and dimethylformamide were from B.D.H. (England), standards of Dan-amino acids were from Serva (F.R.G.). Trypsin inhibitor from soybean, type I-S, used as a reference protein was purchased from Sigma (U.S.A.) \textit{E. europaeus} lectin (560 µg) and trypsin inhibitor (200 µg) were dansylated as described by Zanetta et al., (1970), except that the hydrolysis was run at 110°C for 4 and 18 h. Dan-amino acids were identified by two dimensional thin-layer chromatography on silica gel plates (Kieselgel 60, Merck A.G., F.R.G.) and on micropolyamide sheets F 1700 (Schleicher and Schüll, F.R.G.). The solvent systems used for silica gel plates were: I — benzene/pyridine/acetic acid, 80:20:5, by vol.; II — toluene/chloro-2-ethanol/25% ammonia, 6:10:0.5, by vol. (Gos & Labouesse, 1969). The micropolyamide sheets were developed with: I — 1.5% formic acid; II — benzene/glacial acetic acid, 9:1, v/v, followed by ethyl acetate/methanol/acetic acid, 10:1:1, by vol. (Woods & Wang, 1967; Dévényi & Gergely, 1974).

\[ \text{Other materials and methods.} \] The blood group H hog 59 10% precipitate was purified from individual hog stomach extract (numbered 59) by precipitation with ethanol (Kabat, 1956) and was a gift of Dr. Elvin A. Kabat. Haemagglutination, quantitative precipitin assay, immunoelectrophoresis and disc electrophoresis in polyacrylamide gel were carried out as described earlier (Petryniak et al., 1977). The agglutinating activity is expressed in units: the reciprocal of the titer is taken as the number of units per 1 ml.

Rabbit sera against the crude extract of \textit{E. europaeus} seeds were obtained according to the procedure described by Petryniak et al. (1977). Double diffusion test was performed in 1.5% agar in 0.05 m-sodium barbital buffer, pH 8.2 (Ouchterlony, 1948). Protein was estimated by the method of Lowry et al. (1951).

\[ \text{RESULTS AND DISCUSSION} \]

\[ \text{Purification of lectin.} \] The elution pattern of \textit{E. europaeus} extract from the affinity column is shown in Fig. 1, and results of purification are presented in Table 1. The yield of the purified \textit{E. europaeus} lectin from one run on the column was 22 - 26 mg; 50 g of arils yielded about 58 mg of purified lectin.

In previous studies evidence was obtained for immunochemical homogeneity of the \textit{E. europaeus} lectin purified on the insoluble polylycyl hog blood group A+H substance (Petryniak et al., 1977). The \textit{E. europaeus} lectin purified by the method described in this paper was identical with the lectin purified previously (Petryniak et al., 1977), as judged by immuno-
Fig. 1. Affinity chromatography of the *E. europaeus* lectin on a MN glycoprotein-Sepharose 2B column. The crude extract of lectin (400 ml, haemagglutinating titer 1:64) was applied to the column, which was then washed with NaCl/Pi until the absorbance at 280 nm was less than 0.005; flow rate was 56 ml/h, 14 ml fractions were collected. The lectin was eluted with 0.1 M lactose in NaCl/Pi at the flow rate 30 ml/h; 7.5 ml fractions were collected. The arrow shows the point of application of the lactose solution. For determination of haemagglutinating activity the aliquots of fractions eluted with lactose were dialysed against NaCl/Pi. Agglutination score was counted as follows: 4+ =10. 3+ =8. 2+ =5. 1+ =3.

Table 1

Results of purification of the *E. europaeus* lectin on the MN glycoprotein-Sepharose 2B column

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Volume (ml)</th>
<th>Total protein (mg)</th>
<th>Total haemagglutinating activity (units)</th>
<th>Specific activity (units/mg protein)</th>
<th>Recovery of activity (%)</th>
<th>Purification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>400</td>
<td>820</td>
<td>25 600</td>
<td>31</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Lactose eluate *</td>
<td>18</td>
<td>22</td>
<td>18 400</td>
<td>836</td>
<td>72</td>
<td>27</td>
</tr>
</tbody>
</table>

* After dialysis and concentration.

electrophoresis (Fig. 2), double diffusion (not shown), and disc electrophoresis in polyacrylamide gel (not shown). Similarly, there was no substantial difference in the quantitative precipitin test between the two preparations of the lectin in the assay with the desialized M glycoprotein and blood group H substance hog 59 10% precipitate (Petryniak *et al.*, 1977).

The advantage of the purification technique presented here is its rapidity and convenience, resulting from the use of the column, instead of the batch procedure used previously (Petryniak *et al.*, 1977), and low lactose concentration...
Fig. 2. Immuno-electrophoresis of the purified *E. europaeus* lectin. Troughs contained rabbit antiserum against the crude extract of *E. europaeus*. The lectin preparations (2.5% solutions) purified on the desialized MN glycoprotein-Sepharose 2B conjugate and on the polyleucyl hog A+H blood group substance were placed in wells 1 and 2, respectively.

required for lectin elution. The lower lactose concentration sufficient for elution of lectin in the new procedure is due to the weaker binding of the *E. europaeus* lectin to the desialized MN glycoprotein than to the blood group H substance (Petryniak et al., 1977; 1980).

**NH₂-Terminal analysis.** Alanine was found to be the only NH₂-terminal amino acid in the *E. europaeus* lectin preparations purified either on the insoluble polyleucyl hog blood group A+H substance, or on the desialized MN glycoprotein-Sepharose 2B conjugate. We could not confirm the presence of aspartic acid and tyrosine at the NH₂-terminal end of the *E. europaeus* lectin, reported by Pacák & Kocourek (1975). In our preparations only a trace of aspartic acid was found. The loss of Dan-aspartic acid during hydrolysis is unlikely, since soybean trypsin inhibitor, having aspartic acid at the NH₂-terminal end, gave a distinct spot of Dan-aspartic acid when its dansylation and hydrolysis was run in parallel with the *E. europaeus* lectin. The presence of alanine at the NH₂-terminal end of the *E. europaeus* lectin is consistent with the homology of amino acids at position 1 of the polypeptide chain, observed in most lectins of plant origin (Etzler et al., 1977).
REFERENCES


