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Isolation, purification and characterization of three lectins from ant eggs (*Oecophylla smaragdina* Fabr.)

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Abstract

Three lectins have been isolated from an extract of ant egg and purified by gel filtration on Sephadex G-75 of the 100% ammonium sulfate fraction from the crude extract, followed by ion-exchange chromatography on DEAE-cellulose. All the lectins were homogeneous as judged by polyacrylamide gel electrophoresis. The molecular weights of the lectins, AEL-I, AEL-II, and AEL-III, as estimated by gel filtration, were 195,000, 105,500, and 84,000. All the lectins were found to be composed of four sub-units of unequal sizes, and are glycoproteins with a neutral sugar content of 3.0–7.5%. The lectins agglutinated rat red blood-cells; in a hapten-inhibition study, D-galactose was found to be a better inhibitor for AEL-I and AEL-II whereas D-mannose was a better inhibitor for AEL-III.

Keywords: Ant-egg lectins; Molecular weight; Sub-unit structure; Biological property

1. Introduction

Lectins have been found in a wide variety of plants and animals. They are also found particularly in all of the approximately 30 phyla and subclasses of invertebrates [1,2] mainly in the hemolymph and sexual organs, for example, albumin glands and egg [3,4]. They are proteins which bind to specific oligosaccharide determinants on the cell surface and cause some cells to agglutinate. Recently, Ray and Chatterjee [5] purified a glycoprotein of molecular weight ca. 5000 from ant eggs.

In this paper we report the purification and characterization of three lectins from ant egg.

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2. Experimental

Ant eggs (*Oecophylla samaragdina* Fabr.), used as avian food and as bait for fish, were collected from their nests which were found in the leafy branches of mango trees at Charghat, Rajshahi, Bangladesh. All operations were carried out at 4°C unless otherwise stated. Sephadex G-75 and G-150 were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. DEAE-cellulose was purchased from Sigma Chemical Co., USA. Molecular weight markers were the products of Fluka Biochemica, Switzerland. All other reagents used were of analytical grade [6].

Preparation of crude extract.—Ant eggs (10 g) were homogenized in precooled aq 0.5% AcOH and the slurry was stirred slowly overnight at 4°C. The suspension was filtered through cheesecloth. The filtrate was collected and further clarified by centrifugation at 12,000 rpm for 10 min at 4°C. The clear supernatant was saturated to 100% by addition of solid $(NH_4)_2SO_4$ with gentle stirring at 4°C. The resulting precipitate was collected by centrifugation and dialyzed against distilled water for 24 h at 4°C. The dialyzed solution was then centrifuged at 8000 rpm for 5 min and the clear supernatant was used as crude extract.

Purification of the proteins.—Gel filtration. The crude extract after dialysis against 5 mM phosphate buffer, pH 7.6, was loaded onto the Sephadex G-75 column previously equilibrated with the same buffer and the protein was eluted with the same buffer.

DEAE-Cellulose chromatography. The protein fraction obtained after gel filtration was dialyzed against 10 mM Tris-HCl buffer, pH 8.5, and applied to a DEAE-cellulose column previously equilibrated with the same buffer. The protein was eluted from the column with the buffer containing NaCl.

Electrophoresis. The molecular weights of the proteins and their sub-units were determined by gel filtration on Sephadex G-150 and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, respectively. Molecular weight markers employed for gel filtration were catalase from bovine liver (M_r 240,000), β-galactosidase from *E. coli* (M_r 116,000), alpha-amylase from *Bacillus subtilis* (M_r 58,000), and trypsin inhibitor from corn kernels (M_r 12,028). SDS-polyacrylamide gel electrophoresis was conducted on 10% gel according to Weber and Osborn [7], and the marker proteins employed were β-galactosidase (M_r 116,000), bovine serum albumin (M_r 66,000), carbonic anhydrase (M_r 29,000), ovalbumin (M_r 43,000), and trypsin inhibitor from corn kernels (M_r 12,028). Dissociation and reduction of proteins were performed by heating for 5 min at 100° in 0.1% SDS with 0.1% of 2-mercaptoethanol and the proteins were stained with Amido Black.

Hemagglutination and hemagglutination-inhibition assays.—Hemagglutinating activity was determined by using albino-rat and human 'O'- and 'B'- type red blood-cells as described by Lin et al. [8]: 0.2 mL of protein solution in 5 mM phosphate buffer, pH 7.2, was mixed with 0.2 mL of 4% red blood-cells and incubated at 34°C for 1 h. The degree of hemagglutination was observed under a microscope. The agglutinating activity was expressed as the titre, the reciprocal of the greatest dilution at which agglutination could be detected. The specific activity was expressed as titre/mg of protein. The hemagglutination-inhibition test was performed in the presence of different sugars as described above.

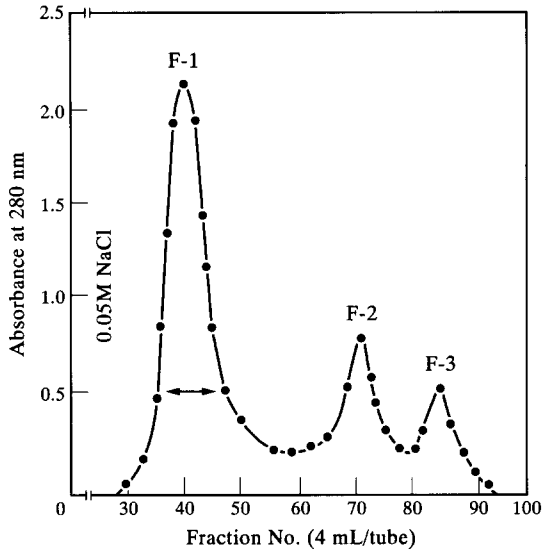

Fig. 1. Gel filtration of 100% $(NH_4)_2SO_4$-saturated crude extract on Sephadex G-75. The crude extract was applied to the column (2.5 × 100 cm) preequilibrated with phosphate buffer, pH 7.6, at 4°C and developed with the same buffer.

Protein and carbohydrate analyses.—The protein concentration of different fractions was measured by the method of Lowry et al. [9] using bovine serum albumin as the standard. The total neutral carbohydrate contents of the proteins were determined by the phenol–sulfuric acid method of Dubois et al. [10], with D-glucose as the standard. For identification of neutral sugars, the proteins were hydrolyzed with 1 M HCl for 4 h at 100°C. The sugar components of the proteins were determined by the one-dimensional TLC method of Joseph and Murrell [11] using D-galactose, D-mannose, D-glucose, D-arabinose, *N*-acetyl-D-galactosamine, and *N*-acetyl-D-glucosamine as the standard sugars. The chromatogram was developed with 3:1:1 2-PrOH–AcOH–water, and the spots were identified by spraying with aniline-phthalate solution.

3. Results

Purification of ant-egg lectins.—The crude extract from ant eggs was applied to a Sephadex G-75 column at 4°C previously equilibrated with 5 mM phosphate buffer, pH 7.6, and eluted with the same buffer. As shown in Fig. 1, the components of the crude extract were separated as a major peak (F-1 fraction) and two minor peaks (F-2 and F-3 fractions). Only F-1, as indicated by the solid line, was pooled, precipitated by ammonium sulfate, and purified further by ion-exchange chromatography. Fractions F-2 and F-3 were not used for further study as they contained mostly colored materials and small amounts of low molecular weight proteins.

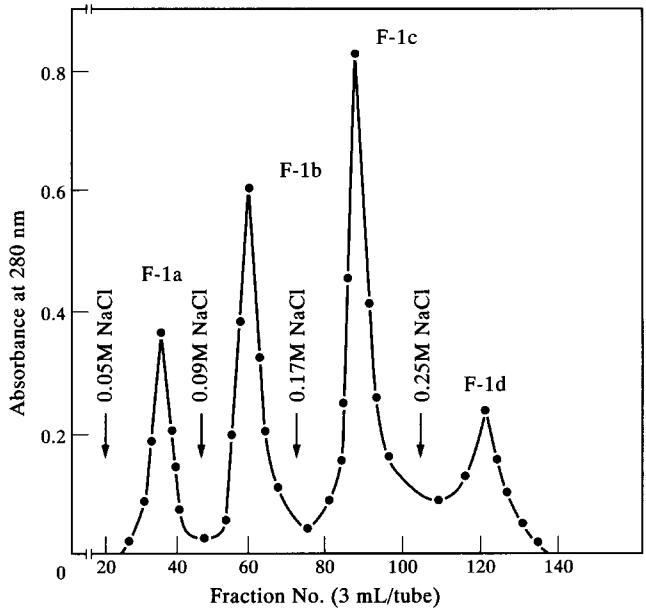

Fig. 2. Ion-exchange chromatography of fraction F-1 on DEAE-cellulose. F-1 (87 mg) obtained by gel filtration was applied to the column (2.1 × 24 cm) prewashed with 10 mM Tris-HCl buffer, pH. 8.5, at 4°C and eluted by stepwise increases of NaCl concentrations in the same buffer.

The ammonium sulfate precipitate of F-1 was dissolved in the minimum volume of distilled water and dialyzed against 10 mM Tris-HCl buffer, pH 8.5, at 4°C for 24 h. After removal of the insoluble material, the clear supernatant was applied to a DEAE-cellulose column at 4°C, previously equilibrated with 10 mM Tris-HCl buffer, pH 8.5 and eluted by a linear gradient of NaCl from 0 to 0.3 M in the same buffer. The proteins were eluted as a single but broad peak, indicating the presence of more than one component. In order to separate these components, the elution was carried out stepwise with increasing concentrations of NaCl in the same buffer. As shown in Fig. 2, the components of F-1, i.e., F-1a, F-1b, and F-1c were eluted with the buffer containing 0.05, 0.09, and 0.17 M NaCl, respectively. The fractions F-1a, F-1b, and F-1c were pooled separately and their homogeneity was checked by polyacrylamide gel electrophoresis. All these fractions contained pure protein as they gave single bands on the gel (Fig. 3).

Table 1 summarizes the data pertaining to the purification of ant-egg lectins. The bulk of the hemagglutinating activity was obtained in fraction F-1c with a 21.3-fold increase in hemagglutinating activity over the crude extract, while F-1a and F-1b showed 3.26- and 7.6-fold increases in the hemagglutinating activity.

Molecular weight of the proteins and their sub-units.—The molecular weights of the proteins, as determined by gel filtration, were estimated to be 195,000, 105,500, and 84,000 for F-1a (ant-egg lectin-I, i.e., AEL-I), F-1b (AEL-II), and F-1c (AEL-III), respectively. As shown in Fig. 4, in the presence of 0.1% SDS, all the proteins gave four

Fig. 3. Polyacrylamide gel electrophoresis of the proteins at room temperature on 7.5% gel. A = Crude protein, B = F-1a, C = F-1b, and D = F-1c.

distinct bands on SDS-polyacrylamide gel electrophoresis. Similar results were observed when SDS-polyacrylamide gel electrophoresis was carried out in the presence of 2-mercaptoethanol. The molecular weights of the sub-units of AEL-I, AEL-II, and AEL-III, as determined by SDS-polyacrylamide gel electrophoresis, were estimated to be 78,000, 44,500, 39,400, and 22,600; 39,000, 37,200, 21,000, and 14,000; and 37,500, 21,500, 14,000, and 9950; respectively.

Hemagglutinating properties of the lectins.—All the proteins agglutinated albino-rat red blood-cells whereas those of human 'O'- and 'B'- type red blood-cells were not agglutinated by any of the proteins. The minimum protein absorbances at 280 nm of AEL-I, AEL-II, and AEL-III needed for visible agglutination were 0.06, 0.031, and 0.015, respectively. The results of the hemagglutination (rat red blood-cells) inhibition test of ant-egg lectins with haptenic sugars are presented in Table 2. It is evident from

Table 1
Purification of ant-egg lectins

Fraction	Total protein (mg)	Hemagglutination activity (titre)	Specific activity (titre/mg)	Yield (%)	Purification fold
Crude extract	580	3500	6.03	100	100
100% $(NH_4)_2SO_4$ saturated	240	700	2.90	—	—
After gel filtration	78	1600	20.50	45	3.40
DEAE-cellulose fractions					
F-1a	10.2	201	19.70	5.70	3.26
F-1b	8.2	380	46.34	10.85	7.60
F-1c	6.4	823	128.59	23.00	21.30

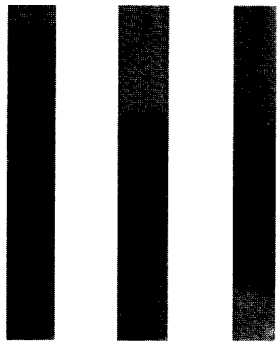

Fig. 4. SDS-polyacrylamide gel electrophoresis of the proteins at room temperature on 10% gel. A = F-1a, B = F-1b, and C = F-1c.

the results that AEL-I and AEL-II displayed high specificity for D-galactose, whereas AEL-III displayed high specificity for D-mannose. Further, methyl β-D-galactopyranoside is more effective than methyl α-D-galactopyranoside for AEL-I and AEL-II, whereas methyl α-D-mannopyranoside is more effective than methyl β-D-mannopyranoside for AEL-III. D-Glucose was also found to be a poor inhibitor for AEL-I and AEL-II, whereas D-galactose was found to be a poor inhibitor for AEL-III.

Table 2
Hemagglutinating inhibition assay [a] of ant-egg lectins

Saccharides	Concentration that inhibits hemagglutination by 50% (mM)		
	AEL-I	AEL-II	AEL-III
D-Mannose	66.0	55.0	5.0
Methyl α-D-mannopyranoside	—	70.0	11.0
Methyl ß-D-mannopyranoside	—	—	18.0
D-Glucose	58.0	90.0	—
D-Glucosamine HCl	60.0	90.0	—
Methyl α-D-glucopyranoside	60.0	100	—
Methyl ß-D-glucopyranoside	55.0	88.0	—
D-Galactose	5.0	10.0	44.0
N-Acetyl-D-galactosamine	11.8	12.0	55.0
Methyl α-D-galactopyranoside	5.5	9.0	55.0
Methyl ß-D-galactopyranoside	3.5	5.0	50.0
Lactose	12.0	20.0	44.0
Melibiose	29.0	—	—

[a] The following sugars were noninhibitory up to 110 mM concentration: raffinose, D-arabinose, D-ribose, D-fructose, *N*-acetyl-D-glucosamine

Protein concentration and carbohydrate composition of the lectins.—The purified proteins in aqueous solution gave absorption maxima around 274–279 nm and minima around 239–242 nm. The absorbance of 1.0 at 280 nm for AEL-I, AEL-II, and AEL-III corresponded to 0.92, 0.94, and 0.98 mg of proteins, respectively, determined by drying the protein under vacuum. Almost similar results were obtained when the concentrations of the proteins were measured by the Lowry method.

The neutral sugar contents of AEL-I, AEL-II, and AEL-III were found to be 3.1, 4.3, and 7.5%, respectively. It is not clear at this moment why AEL-III contained a so much higher percentage of sugar. One reason may be the presence of traces of coloring material in AEL-III. The sugar compositions of the lectins, as determined by TLC, were found to be glucose and galactose for AEL-I, galactose for AEL-II, and galactose and mannose for AEL-III.

4. Discussion

Three proteins have been isolated from the crude extract of ant egg. All these proteins are glycoprotein in nature as they gave a yellow–orange color in presence of phenol–sulfuric acid. The agglutination of rat red blood-cells by the lectins AEL-I and AEL-II were inhibited in presence of D-galactose while that of the lectin AEL-III was inhibited in presence of D-mannose. It might be concluded from the results that ant egg contained two lectins that are specific for D-galactose and one lectin which is specific for D-mannose. The lectins differ in their molecular weights also.

The three lectins moved as a single band with similar mobilities on polyacrylamide disc gel electrophoresis but SDS-polyacrylamide gel electrophoresis results clearly indicated that the lectins were composed of four non-identical sub-units with different molecular weights. Furthermore, the glycoproteins were transformed into four sub-units in the presence of SDS only, indicating that the sub-units are held together by non-ionic hydrophobic interactions. The lectins of ant eggs are therefore quite different in molecular composition from the lectins purified from other sources. The lectins purified from other sources contained mostly four sub-units of two identical pairs, viz., Indian bean (*Dolichos lablab* L.) [12], *Arbus precatorius* [13], and *Ricinus communis* agglutinin [14], and very few contained four sub-units of identical molecular weight, viz., *Phaseolus vulgaris* [15].

Recently, Ray and Chatterjee [5] reported that ant-egg glycoprotein contained, galactose, glucose, mannose, *N*-acetylglucosamine, and *N*-acetylgalactosamine. However, our results showed that ant-egg glycoprotein contained only galactose, mannose and glucose. It should be pointed out that Ray and Chatterjee worked on a low molecular weight glycoprotein whereas we determined the sugar compositions of three high molecular weight glycoproteins.

References

[1] E. Cohen (Ed.), *Prog. Clin. Biol. Res.*, 157 (1984) 207 pp.

[2] E. R. Gold and P. Balding, *Receptor-specific Proteins: Plant and Animal Lectins*, Excerpta Medica, Amsterdam, 1975, 440 pp.

[3] R. W. Yeaton, *Dev. Comp. Immunol.*, 5 (1981) 391–402.
[4] N. Gilboa-Garber, A. J. Susswein, L. Mizrahi, and D. Avichezer, *FEBS Lett.*, 181 (1985) 267–270.
[5] S. Ray And B. P. Chatterjee, *Carbohydr. Res.*, 191 (1989) 304–314.
[6] L. Ornstein, *Ann. N.Y. Acad. Sci.*, 121 (1964) 321–349.
[7] K. Weber and M. Osborn, *J. Biol. Chem.*, 244 (1969) 4406–4412.
[8] J.-Y. Lin, T.-C. Lee, S.-T. Hu, and T.-C. Tung, *Toxicon*, 19 (1981) 41–51.
[9] O. H. Lowry, N. Rosebrough, N. Farr, and R. J. Randell, *J. Biol. Chem.*, 193 (1951) 265–275.
[10] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
[11] J. C. Touchstone and M. F. Dobbins, *Practice of TLC*, 1st ed., Wiley-Interscience, New York, 1978, pp 173 and 212.
[12] A. Guran, M. Ticha, K. Filka, and J. Kocourek, *Biochem. J.*, 209 (1983) 653–657.
[13] N. Absar and G. Funatsu, *J. Fac. Agric., Kyushu Univ.*, 29 (1984) 103–115.
[14] S. Olsnes, E. Saltvedt, and P. Alexander, *J. Biol. Chem.*, 249 (1974) 803–810.
[15] M. Itoh, K. Kondo, H. Komoda, K. Izutsu, Y. Shimabayashi, and T. Takahashi, *Agric. Biol. Chem.*, 44 (1980) 125–133.