Purification and characterization of the gonad lectin of *Aplysia depilans*

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Extracts of gonads and fertilized eggs of *Aplysia depilans* contain a D-galacturonic and D-galactose-binding lectin. This lectin reacts strongly with rabbit and human erythrocytes independent of ABO blood groups, weakly with dog, mouse, rat, and chick erythrocytes and not at all or very weakly with sheep erythrocytes. Purification of the gonad lectin was easily achieved, with a high yield, by heating to 70°C, precipitation with ammonium sulfate and affinity chromatography on Sepharose 4B. The purified lectin was found to be a glucoprotein of molecular mass around 55–60 kDa; it stimulates mitogenesis of human peripheral lymphocytes.

Aplysia *Gonad lectin* *Lectin purification* *Mitogenic lectin*

1. INTRODUCTION

The sexual organs of various molluscs are rich in lectin activity [1,2]. A prominent example is the *N*-acetyl D-galactosamine-specific lectin from the albumin gland of *Helix pomatia* (HPL), which detects the human blood group A antigen [3–6]. When purified [7,8], this lectin resembles plant lectins in its properties and is employed for detecting the presence of sugars in various macromolecules and cells and for detection of alterations in the cell surface due to physiological events or malignant (viral) transformations [1,6]. However, in contrast to many plant and animal lectins [9], HPL does not stimulate lymphocytes [10].

We have demonstrated the presence of galactophilic lectin in extracts from gonads and fertilized eggs from four species of *Aplysia* [11]. The activity of the *Aplysia* gonad lectin (AGL) increases with gonad maturation, is resistant to heating to 70°C, but is sensitive to pronase and EDTA treatment [11]. Kamiya and Shimizu described similar lectins in fertilized eggs of three Japanese species of *Aplysia* and showed that the three lectins also exhibit a strong affinity for D-galacturonic acid [12].

The aim of the present research was: (i) to purify AGL; (ii) to examine its reactivity with D-galacturonic acid and related sugars; (iii) to examine its reaction with erythrocytes from different animals and different human blood groups; and (iv) to assay its ability to stimulate DNA synthesis in human peripheral lymphocytes.

2. MATERIALS AND METHODS

A. depilans were collected along the Mediterranean coast of Israel. Gonads were removed from freshly dissected animals and eggs were gathered within 24 h of being laid. The gonads (20 g of wet weight per 100 ml) and eggs were homogenized in a glass tissue grinder, and the homogenate was suspended in 0.85% NaCl solution (saline). The suspension was then exposed to ultrasonic vibrations for 10–30 s and centrifugation at 30000 × *g* for 10 min. The protein concentration in the supernatant was adjusted to 10–20 mg/ml.

Human (from A, B, AB and O blood groups), sheep, dog, rabbit, rat, mouse and chicken bloods from normal subjects were drawn into heparin.

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The hemagglutinating activity of AGP was assayed using untreated and papain-treated erythrocytes as in [13].

The binding of sugars to the lectin was examined by incubating 0.2 ml of the lectin preparation with 0.2 ml of the examined sugar solution at room temperature for 1 h before titration of residual hemagglutinating activity.

The lectin was purified by heating the crude extract (10–20 mg protein/ml) to 70°C for 15 min, and centrifugation. The lectin was then precipitated from the supernatant in a 60%-saturated solution of ammonium sulfate. The precipitate was resuspended in PBS, dialysed overnight at pH 7.2 and applied to a column of Sepharose 4B. The column was washed with PBS and the lectin was eluted from the column with 0.3 M D-galactose solution [13]. The fractions were examined for hemagglutinating activity toward papain-treated human erythrocytes and protein was determined by the method of Lowry et al. [14], using bovine serum albumin as standard.

Molecular mass was estimated by filtering the lectin through various Bio-Gels, using hemoglobin as a marker for around 65 kDa and wheat germ agglutinin as a marker for 36 kDa. The Bio-Gels employed were: P-30, P-60, P-100 and P-300 (Bio-Rad Labs) in columns (15 × 1 cm) prepared according to the directions of Bio-Rad Labs (Richmond, CA). One-ml samples were introduced onto the columns and 2-ml fractions of the effluent were collected. Hemoglobin-containing fractions were examined spectrophotometrically at 540 nm and those containing lectins were examined for hemagglutinating ability using papain-treated human erythrocytes.

Presence of sugar in the lectin was examined by the anthrone [15] and phenol sulfuric acid reactions [16] using glucose as standard, and by precipitation with concanavalin A. The precipitation was performed by introducing 0.1 ml of Con A (Miles Yeda, Rehovot) with 0.1 ml of the purified AGL (each at a concentration of 1 mg protein/ml) into a narrow glass tube. Precipitation was assayed after 2 and 24 h at room temperature.

Mitogenic stimulation by the lectin was assayed with untreated and sialidase-treated human mononuclear cells separated from heparinized (0.3 mg/ml) blood and treated according to Novogrodsky and Ashwell [17].

3. RESULTS

AGL exhibited a strong agglutinating activity with rabbit and human erythrocytes, irrespective of their ABO blood group. The agglutination was considerably stronger when papain-treated cells were used. Erythrocytes from dog, rat, mouse and chicken (both untreated or papain-treated) were weakly agglutinated by AGL. Sheep erythrocytes were not agglutinated or agglutinated very weakly (table 1). The lectin of the fertilized eggs laid by the animals exhibited similar patterns of affinity towards the erythrocytes from the different sources.

The purification of AGL (by a factor of approx. 125) is shown in table 2. The purified lectin was found to behave in Bio-Gel filtration as a molecule of about 55–60 kDa. The presence of sugar in the lectin (7%) was shown by anthrone and phenol sulfuric acid reactions and by precipitation with Con A (inhibited by D-mannose). In polyacrylamide gel electrophoretic analysis of the purified lectin preparations, one main band was obtained (fig.1).

The AGL hemagglutinating activity was strongly inhibited by D-galacturonic acid (minimal inhibitory concentration for 2 hemagglutinating units, 0.2 μM) followed by methyl-β-D-galactoside (0.05 mM), D-galactose (0.15 mM) and methyl-α-D-galactoside (0.4 mM), but not by D-glucuronic acid or *N*-acetyl-D-galactosamine (at 0.3 M concentration).

The stimulating effect of AGL upon DNA synthesis of lymphocytes was investigated over a dose

Table 1

Hemagglutination of untreated and papain-treated animal erythrocytes by AGL preparation (0.5 mg/ml)

Erythrocytes	Hemagglutinin titer (dilution^{-1})	
	Untreated	Papain-treated
Mouse	2–4	2–4
Rat	4–8	8–16
Sheep	0	2–4
Dog	4–8	8–16
Rabbit	256	512
Human	8–16	256–512
Chicken	0–2	2–4

Table 2

Steps in the purification of AGL

Preparation	Hemagglutination titer (dilution^{-1})	Total protein (mg/ml)	Specific activity[a]	Fold purification
Crude extract	512	10.0	51.2	–
70°C supernatant	512	5.0	102.4	2
Ammonium sulfate	512	4.0	128	2.5
Affinity chromatography	512	0.08	6400	125

[a] The specific activity is expressed as the ratio of the hemagglutination titer (using papain-treated human erythrocytes) per mg/ml protein. After each step of purification the lectin-containing fraction was diluted so as to give a titer of 512^{-1} prior to protein determination. From 10 g (wet wt) of gonads, about 5 mg purified lectin preparation was obtained

Fig.1. Polyacrylamide disc gel electrophoresis of the purified lectin preparation.

Table 3

Incorporation of [^{3}H]thymidine into DNA in sialidase-treated human blood lymphocytes stimulated by PHA, Con A, SBA or AGL

Lectin	μg/ml	cpm/1 × 10^6 lymphocytes ± SE
PHA	25	115539 ± 10753
Con A	3	81817 ± 21223
SBA	50	26850 ± 520
AGL	50	79709 ± 8100

range of 10–150 μg/ml and 1 × 10^6 cells. As can be seen in table 3, AGL stimulated sialidase-treated lymphocytes, as do PHA, Con A and soybean agglutinin [18]. It also induced significant stimulation of untreated lymphocytes.

4. DISCUSSION

AGL from *Aplysia*, the largest of all gastropods [19], was found to exhibit a high affinity for rabbit and human erythrocytes compared with those of mouse, rat, chicken, dog and sheep (table 1). The similarity between AGL and the fertilized egg lectin [11,12] may be regarded as another indication of a common source of the two lectins and suggests that the egg lectin is produced in the gonad [11]. Although AGL binds D-galacturonic acid and D-galactose, it is not specific for blood group B antigen. Therefore, unlike the HPL [3,6] or the lectin of *Aplysia kurodai* fertilized eggs [12], it cannot be used for blood group typing. In this respect, it

resembles the lectins from *Aplysia juliana* and *Aplysia dactylomela* eggs, which exhibit a strong affinity for D-galacturonic acid and bind D-galactose, but do not exhibit blood group B specificity [12]. As may be seen from table 2 and fig.1, AGL can be easily purified 125-fold, by affinity chromatography on Sepharose 4B. Like the HPL [7], this lectin is a glycoprotein. However, in contrast to HPL which lacks mitogenic activity [10], AGL stimulates DNA synthesis in peripheral human lymphocytes (table 3).

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