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*Biochemistry*

# Isolation and Biochemical Characterization of Mannose-Specific Lectin from Georgian Endemic Plant *Polygonatum obtusifolium* Miscz. rhizomes

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**ABSTRACT. From the Georgian endemic medicinal plant *Polygonatum obtusifolium* Miscz. rhizomes hemagglutinin (lectin SABA-1) was separated and partially purified. It was revealed that minimal hemagglutinating activity of lectin is 0.01 mg/ml. SABA-1 is a thermostable protein and maintains hemagglutinating activity during 15 min incubation at $60^{\circ}$C. Hemagglutinating activity of lectin SABA-1 is inhibited by mannose, $\alpha$-methylmannopyranozide and N-acethyl-D-glucosamine. Lectin SABA-1 belongs to the mannose-specific lectins class.** *© 2012 Bull. Georg. Natl. Acad. Sci.*

***Key words:*** *lectins, Polygonatum obtusifolium* Miscz. *rhizomes, mannose-specific lectin.*

**Introduction**. It is well known that biologically active compounds are widely used in biology, medicine, agriculture and in other fields of science. The available literature shows that most important class of these compounds is information carrier and information decoding – carbohydrate contains biomolecules (glycoproteins, glycolipids, glycolipoproteins, etc.) and carbohydrate binding proteins – lectins [1].

The first plant lectin was discovered in 1888 by H. Stillmark. To date hundreds of plant lectins have been isolated and biochemically characterized. Because of the unique property (specifically binding to carbohydrates and carbohydrates containing biomolecules) of lectins they are successfully used as exploration tools, affinity adsorbents for the isolation of various glycoproteins, cells and organelles. Lectins as specific biological probes have found much use in studies of the membrane carbohydrate topography, in medicine and criminalistics as a diagnostic means [1]. Special interest of scientists was stirred by the ability of lectins for cell proliferation, having hormonal, toxic, immunomodulatory, antitumor, antiviral and other functions [2-4]. Thus, isolation of new plant lectins and ascertaining their biological role still remains one of the topical bio-medical problems. Therefore, we carried out experiments to identify lectins from the Georgian endemic medicinal plant *Polygonatum obtusifolium* Miscz. and study some of their biochemical properties.

## Material and Methods.

The underground parts of rhizomes of *Polygonatum obtusifolium* Miscz., which is extensively used in folk

medicine due to their essential medicinal properties, served as the object of this study. With the aim of lectin extraction the plants rhizomes were homogenized. For extraction PBS solutions of different composition were used:

1) 0.9% NaCl, 40 mM $K^+$-phosphate buffer, pH 7.4

2) 0.9% NaCl, 40mM $K^+$- phosphate buffer 0.1% b-mercaptoethanol (β-M), pH 7.4

3) 0.9% NaCl, 40mM $K^+$- phosphate buffer, 1% polyvinylpyrolidon (PVP), pH 7.4;

4) 0.9% NaCl, 40mM $K^+$- phosphate buffer, 0.5 mM phenylmethylsulfanylfluoride (PMSF),H pH 7.4.

To choose the optimal extraction conditions we took into account the ratio of crude material and extraction solution (w/v=1/5; 1/10; 1/20), pH (5.0; 6.0; 7.0; 7.4; 8.0; 9.0) and incubation time (15; 30; 45; 60 min). The homogenate was placed on magnetic stirrer in accordance with the extraction time at room temperature. The mixture was filtered using cheese cloths and the filtrate was centrifuged at 16 000 g/min for 15 min. For partial purification the supernatant was fractionated with ammonium sulfate under 0-20, 20-40, 40-60, 60-80, 80-100% saturation. The separate suspension was centrifuged at 10 000 g for 20 min at +4$^0$C ("Beckman" SW-27 rotor). The precipitate was dissolved in a minimal volume of extraction solution, was homogenized and centrifuged at 5 000 g (centrifuge TY5.375-4172-78, rotor PY180) for 15 min. The supernatant was filtered through Whatman CF/C and sinpor-0.45-0.22 mkm filter and ammonium sulfate was removed with dialysis on G-10 Sephadex column (50x2.7 cm). Extracts were stored at +4$^0$C.

Lectin activity was determined visually using 96-well immunological microtiter U-plates using a hemagglutination test on rabbits trypsinized erythrocytes with the method of Takatsy [5]. Lectin hemagglutinating activity (HA) was estimated according to that minimal protein concentration (mg/ml) which shows full agglutination of rabbits trypsinized erythrocytes. In order to estimate lectin activity we used also a specific activity (ml/mg) which is its inverse value and reflects the minimal concentration which still causes agglutination: $SA=T^{-1}xC^{-1}$, where $T^{-1}$ (titer) is the degree of minimal protein concentration in which agglutination is still noticeable (6), C is protein concentration expressed in mg/ml. Lectins content was judged by the ratio of overall protein content to lectin activity (conventionally agglutination unit, hemagglutination unit – HU) or by the formula $GAA=1/Tm$ ($T$ - titer of lectin hemagglutinating activity; $m$ - mass of the used fragment of *Polygonatum obtusifolium*.

Lectin activity kinetics was measured in hemagglutination medium by the photocolorimetric method, at 670 nm light, a 200 $\mu$l 2% suspension of trypsinized erythrocytes was introduced into the control and testing cuvettes. Lectin fraction was added into the testing cuvette and PBS of the same volume to the control one. In the control cuvette of light transmission at 670 nm was considered as 100% and variation of this index was fixed with an automatic recorder connected with a photo colorimeter (      -3). The influence of $H^+$ ions concentration on lectins hemagglutinating activity was studied in the pH 2.0 – 10.0 ranges PBS, at 1 unit intervals. The extracted protein was titered according to a decreasing concentration on immunological plates, in the relevant pH of PBS. Agglutination was studied in 2% suspension of rabbit's trypsinized erythrocytes that had been prepared on pH changed buffer.

To study lectin thermostability the purified protein fractions were incubated in a water bath at the temperature 20, 40, 60, 80and 100$^0$C for 10 min. The thermally treated samples were cooled in the ice bath (15min) and centrifuged in order to remove the denatured protein sediment (1500 g/15min). Hemagglutinating activity was measured in supernatants at room temperature.

Lectin specificity in relation to carbohydrates was studied by means of the hapten-inhibitory method [7]. For the analysis we used 0.6 M solution of monosaccharide prepared on PBS. In the experiments 18 different carbohydrates were used: D-galactose, methyl-D-galactose, α-methylmannopyranoside, D-

**Table 1. The influence of extraction time and different extraction solutions on the protein concentration and hemagglutinating activity of *Polygonatum obtusifolium* rhizome extracts**

| Composition of PBS | Time (min) | Titer | Protein concentration (mg/ml) | Lectin activity (mg/ml) | Specific activity (ml/mg) |
|---|---|---|---|---|---|
| PBS | 15 | $2^3$ | 0.720 | 0.023 | 11.1 |
| 0.9%NaCl + 40mM | 30 | $2^3$ | 0.752 | 0.024 | 10.6 |
| $KH_2PO_4$ | 45 | $2^3$ | 0.816 | 0.026 | 9.8 |
| pH 7.4 | 60 | $2^3$ | 0.832 | 0.026 | 9.6 |
| PBS | 15 | $2^4$ | 0.976 | 0.015 | 16.4 |
| 0.9%NaCl + 40mM | 30 | $2^5$ | 1.264 | 0.010 | 25.3 |
| $KH_2PO_4$ | 45 | $2^5$ | 1.408 | 0.011 | 22.7 |
| 0.1% -M pH 7.4 | 60 | $2^5$ | 1.648 | 0.013 | 19.4 |
| PBS | 15 | $2^4$ | 0.992 | 0.016 | 16.1 |
| 0.9%NaCl + 40mM | 30 | $2^5$ | 1.336 | 0.010 | 24.0 |
| $KH_2PO_4$ | 45 | $2^5$ | 1.352 | 0.011 | 23.6 |
| 1% PVP pH 7.4 | 60 | $2^5$ | 1.344 | 0.010 | 23.8 |
| PBS | 15 | $2^4$ | 0.872 | 0.013 | 18.3 |
| 0.9%NaCl + 40mM | 30 | $2^4$ | 1.072 | 0.016 | 14.9 |
| $KH_2PO_4$ | 45 | $2^4$ | 1.160 | 0.018 | 13.8 |
| 0.5mMPMSF pH7.4 | 60 | $2^4$ | 1.208 | 0.019 | 13.2 |

$p<0.01$

mannose, D-raffinose, D-glucose, D-ramnose, N-acetyl-D-glucosamine, N-acetyl-D-galactoseamine, D-galacturonic acid, D-fructose, L-inosite, D-arabinose, L-ribose, Melibiose, D-lactose, D-cellobiose, Saccharose.

Sugar solution was titered from 200 mM with decreasing concentration, on the immunological plates. Equal concentration of 1:4 titer lectin solutions was introduced in all cells of the plate. Hapten specificity was estimated by that minimal concentration of sugar (mM) which resulted in inhibition of lectin hemagglutinating activity. Protein concentration was measured by the method of Lowry, et al. [8].

## Results and Discussion

In the first series of experiments we studied the effect of different solutions, widely varying the composition and extraction time on hemagglutination activity of extracts from *Polygonatum obtusifolium* rhizome lectin - SABA-1. As seen from Table 1, hemagglutinating activity of lectin -SABA-1 is completely extracted by PBS (0.9% NaCl, 40 mM $K^+$- phosphate buffer 0.1% β-mercaptoethanol (β-M), pH 7.4), extraction time was 30 min, the minimal concentration of protein causing agglutination was 0.01 mg/ml, its specific activity index was highest 25.3 ml/mg. Introduction of PMSF into extraction solution did not alter the extract's hemagglutination and specific activity, suggesting stability of SABA-1. Introduction of PVP into extraction solution did not change hemagglutinating activity of SABA-1.

As seen from Table 1, in the case of PBS use of (0.9%NaCl + 40mM $KH_2PO_4$ 0.1% β-M pH 7.4) as extraction solution (extraction time 30 min) there occurs maximal separation of the protein with lectin activity.

It is known from the literature that during visual determination of lectin activity, by the Takatsy microtitration method, apparent hemagglutination is frequently recorded. Namely, it is known that this kind of artefacts may be elicited by polysaccharides, phenol compounds and also by damaged immunological plates. In order to avoid this, we have used the photocolorimetric method to determine the kinetics of the hemagglutination process.

Fig. 1 shows that in control (erythrocytes without lectin) and in testing (erythrocytes in the presence of lectin) erythrocytes sedimentation kinetics is of reciprocal character. In particular, in testing

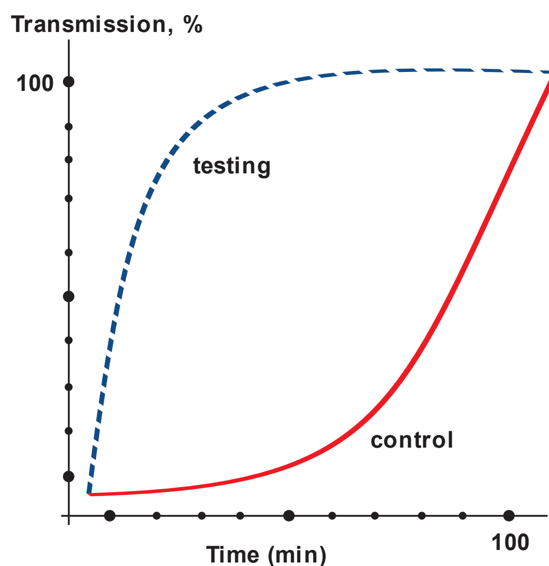


**Fig. 1.** Kinetics of SABA-1 lectin hemagglutinating activity.
Control: typsinized erythrocytes without lectin;
Testing: trypsinized erythrocytes in presence of lectin.

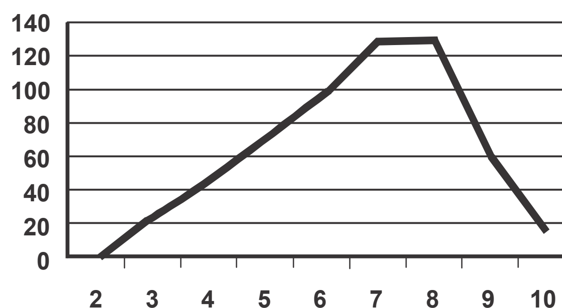


**Fig. 2.** The influence of $H^{+}$-ion concentration on lectin SABA-1 hemagglutinating activity. On ordinate – hemagglutination titer. On abscissa – pH

transimission rises rapidly and achieves maximum (100%) after 40 min, in control, the same index is recorded only after 90 min, this is induced by a fast agglutination of erythrocytes by SABA-1, as a result sedimentation and trasmission are rapidly increased.

In the next series of experimemts we studied the influence of different extraction solution volumes (crude material/PBS – 1/5, 1/10, 1/20) on the lectin SABA-1 content in *Polygonatum obtusifolium* rhizome extracts (Table 2).

The results presented in Table 2 show that the most optimal ratio between the weight of *Polygonatum obtusifolium* rhizome and extraction solution volume is 1/20 (g/ml). With the use of extraction solution of this ratio one can fix the highest indices of lectin content in extracts of plant tissue (0.0156).

Lectin activity is changed depending on a wide range of pH. We have experimentally studied the effect of various pH on SABA-1 lectins hemagglutinating activity within 2.0-10.0 range of pH (Fig. 2).

As seen from Fig.2, at pH 7.0-8.0 range the highest hemagglutinating activity of lectin SABA-1 is recorded.

In parallel the effect of temperature on lectin SABA-1 specific activity was investigated (Fig. 3). As reported in the literature, as a rule, most lectins are thermostabile and they are used for their purification because lectin stability facilitates their separation from the relatively thermolabile proteins.

As evident from Fig. 3 SABA-1 maintains activity at +60$^0$C under 15 min incubation. With a further rise in temperature there occurs a gradual inactivation of lectin, while at 100$^0$C its hemagglutinating activity becomes completely inhibited (Fig.3). Thus, the results obtained indicate that SABA-1 belongs to the thermostable class of proteins.

Next series of experiments was aimed at partial purification of SABA-1 by ammonium sulfate under varying saturation (Fig.4).

As seen from Fig. 4, the highest specific activity of SABA-1 is recorded in the fractions obtained by

**Table 2. The effect of *Polygonatum obtusifolium* rhizome weight and extraction solution volume ratio on the lectin content in plant tissue extracts**

| Crude material/PBS (g/ml) | Titer | m – mass fragment of the used Polygonatum obtusifolium | Lectin content (GAA=1/Tm) |
|---|---|---|---|
| 1:5 | $2^8$ | 1g | 0.0039 |
| 1:10 | $2^8$ | 1g | 0.0078 |
| 1:20 | $2^8$ | 1g | 0.0156 |

$p<0.01$

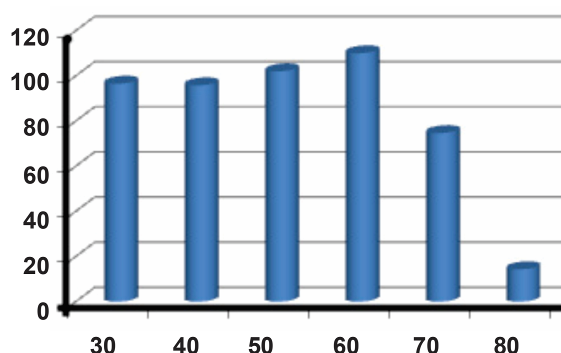


**Fig. 3.** The effect of temperature on lectin SABA-1 hemagglutination specific activity. On ordinate – specific activity. On abscissa – temperature in $^0$C.

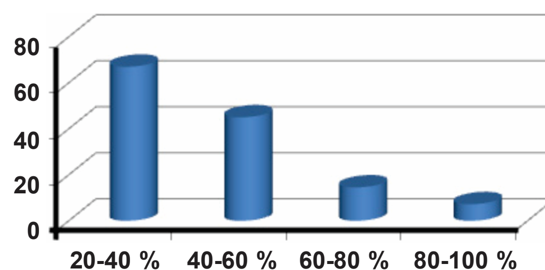


**Fig. 4.** Fractionation of the extracts from *Polygonatum obtusifolium* rhizome with ammonium sulfate under different saturation. On ordinate – specific activity of SABA-1. On abscissa – ammonium sulfate saturation

ammonium sulfate saturation from 20-40 to 40-60%. The results indicate that maximal sedimentation of lectin SABA-1 from the extracts of *Polygonatum obtusifolium* rhizome occurs under 0-60% saturation of ammonium sulfate.

In a special series of experiments we studied SABA-1 specificity in relation to carbohydrates (Table 3). To obtain full-value characteristics of lectin it is necessary to ascertain lectin specificity to carbohydrates. Lectins are known to bind with carbohydrates specifically and inhibit lectin-induced hemagglutination. As shown in Table 3, 18 different carbohydrates were tested in an initial concentration of 200 mM.

The data presented in the table demonstrate that lectin SABA-1 hemagglutinating activity is inhibited only in the presence of mannose, α-methylmannopyranoside and N-acetyl-D-glucosamine. The data obtained indicate that lectin SABA-1 isolated from *Polygonatum obtusifolium* rhizome is attributed to the mannose-specific lectin class.

## Conclusion

Lectin SABA-1 has been isolated from the Georgian endemic medicinal plant *Polygonatum obtusifolium*

**Table 3. Specificity of lectin SABA-1 to carbohydrates**

| Carbohydrates (Initial concentration 200 mM) | Inhibition of hemagglutinating activity | Minimal inhibiting concentration (mM) of a carbohydrate |
|---|---|---|
| D -galactose | – | |
| α -methyl-D-galactose | – | |
| α-methylmannopyranoside | + | 25 |
| D-mannose | + | 50 |
| D-raffinose | – | |
| D-glucose | – | |
| D-ramnose | – | |
| N-acetyl-D-glucosamine | + | 75 |
| N-acetyl-D-galactoseamine | – | |
| D-galacturonic acid | – | |
| D-fructose | – | |
| L-inosite | – | |
| D-arabinose | – | |
| L-ribose | – | |
| Melibiose | – | |
| D-lactose | – | |
| D-cellobiose | – | |
| Saccharose | – | |

+ Inhibition of hemagglutinating activity.

– Noninhibition of hemagglutinating activity.

rhizome and has been partially purified. Minimal hemagglutinating activity of lectin SABA-1 is 0.01mg/ml. SABA-1 belongs to the thermostable class of proteins, its specific inhibitors of hemagglutination activity are mannose, α-methylmannopyranoside and N-acetyl-D-glucosamine. Lectin SABA-1 belongs to the mannose specific class of lectins.

*ბიოქიმია*

# საქართველოს ენდემური მცენარე სვინტრიდან (*Polygonatum obtusifolium* Miscz.) მანოზა-სპეციფიკური ლექტინის გამოყოფა და ბიოქიმიური დახასიათება

**ნ. დუმბაძე*, ნ. ალექსიძე**, გ. ალექსიძე***

** საქართველოს საპატრიარქოს წმიდა ანდრია პირველწოდებულის სახ. ქართული უნივერსიტეტი, თბილისი*
*** აკადემიის წევრი; საქართველოს საპატრიარქოს წმიდა ანდრია პირველწოდებულის სახ. ქართული უნივერსიტეტი, თბილისი*

**საქართველოს ენდემური მცენარე *Polygonatum obtusifolium* Miscz.-დან პირველადაა გამოყოფილი და ბიოქიმიურად დახასიათებული მანოზა-სპეციფიკური ლექტინი SABA-1. ლექტინი SABA-1-ის მინიმალური ჰემაგლუტინაციური აქტივობა შეადგენს 0.01 მგ/მლ-ს. SABA-1. თერმოსტაბილური ცილაა და ლექტინურ აქტივობას ინარჩუნებს 15 წუთის განმავლობაში 60 $^0$C–ზე თერმული დამუშავების პირობებში. SABA-1-ის ჰემაგლუტინაციური აქტივობა ინჰიბირდება მანოზათი, α-მეთილ-მანოპირანოზიდით და N-აცეტილ-D-გლუკოზამინით. გაკეთებულია დასკვნა, რომ SABA-1 განეკუთვნება მანოზა-სპეციფიკური ლექტინების კლასს.**

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