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Biochemistry

Isolation of New Lectins from the Greater Celandine Plant (*Chelidonium majus* L.), Study of their Properties and Distribution within the Plant

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ABSTRACT. A high hemagglutinaton activity lectin CBL-1 has been isolated from seeds of the greater celandine (*Chelidonium majus* L.) - plant used in folk medicine in Georgia - using the salt aqueous solution (PBS). Minimum hemagglutination activity of lectin to trypsin-treated rabbit erythrocytes makes 0.00022 mg/ml. Lectin CBL-1 belongs to the class of chitin-specific homotypic hololectins. It was shown, from kinetics of hemagglutination process, in the control variant (erythrocytes without lectins) and experimental (erythrocytes in the presence of lectins) kinetically the rate of sedimentation of erythrocytes is of reciprocal character. In particular, in the experimental variant the conductivity of light beam increases rapidly because of speedy sedimentation of erythrocytes and reaches maximum (100%) already after 20 minutes, though in the control variant the same index is registered only after 90 minutes. It was shown that lectin isolated by PBS was detected only in seeds of the greater celandine and none of other parts of the plant (flowers, leaves, stems, roots and milky sap). Of special notice is the fact that hemagglutination titre (T=16 384) of lectin isolated from *Chelidonium majus* using our method 584-1168 times exceeds the hemagglutionation titre of lectin isolated from seed of *Chelidonium majus* by W. J. Peumane and co-authors, which fluctuated within the range of 20-40. It has been established that the lectin CBL-1 isolated using our methods differs from lectins isolated from the greater celandine plant by other authors. *© 2013 Bull. Georg. Natl. Acad. Sci.*

Key words: *greater celandine, lectins, distribution.*

As early as in 1985 Belgian scientists isolated the chitin-specific lectin (CMA) from seeds of *Chelidonium majus* [1], though Polish scientists have isolated heparin-specific lectins CMN1 and CMN2 from the plant milky sap of orange color [2]. On the basis of nondirect evidence the authors proposed an idea on the possible involvement of the greater celandine lectins in processes of plant growth and development and defense from pathogenic microbes [3]. Despite the mentioned facts the mechanisms of their action and role in the organism of the greater celandine remain unknown so far.

Materials and methods

Medicinal plant - greater celandine - *Chelidonium majus L.* (family Papaveraceae) - growing in the wild in Georgia was chosen a object of investigation and allocation of lectins in its different parts (flowers,

leaves, stems, roots, mature seeds and milky sap of orange color was investigated. All parts of the greater celandine plant were combined together (at an equal weight ratio) and isolation of soluble protein fraction was performed using the extracting solutions of different composition: 1. PBS (0.9% NaCl, 0.04M KH2PO4, pH7.4), 2. PBS (pH 5.0). 3. PBS + 0.5 mM PMSF (pH 7.4). 4.PBS + 0.5 mM β-mercaptoethanol pH 7.4). In all cases the ratio of raw material to the extracting agent was equal to 1:40 (g/ml). Homogenization was performed in china bowls and extraction of soluble proteins from the homogenized raw material was mixed on magnetic mixer during an hour at room temperature. The extract was filtered through a double cotton fabric and the filtrate centrifuged at 16 000 for 15 minutes. The supernatant was filtered through special filter (miracloth, Calbiochem, USA), and then, consequently in Whatman GF/c and Synpor-0.45 μ filters.

Lectin activity was determined visually using 96-well immunological micro titer U-plates using a hemagglutination test on rabbits, trypsinized erythrocytes with the method of Takatsy [5]. Lectin activity was determined by the minimum concentration of protein (mg/ml) which caused agglutination of trypsin-treated rabbit erythrocytes. For the assessment of lectin activity also the specific activity $SA=T^{-1}\cdot C^{-1}$ was applied, where T^{-1} (titer) is a degree of protein dilution in the last well of the titration slide, where hemagglutination is still visible ($T=2^n$, n – number of agglutination wells, C – protein concentration in mg/ml. Hemagglutination titer was determined according to the value reciprocal to the highest dilution, which still caused visual agglutination of erythrocytes. $T = 2^n$· n – number of wells, where hemagglutination is detectable).

The content of lectin was judged about by the ratio of total protein to lectin activity (conventional agglutinaton unit) HU (Hemagglutination Unit).

Kinetics of lectin activity was studied using the photoelectrocolorimeter at 670 nm by the index of light conductivity through the hemagglutination incubation medium according to the sedimentation rate of erythrocytes. With this aim 200 μl suspension of trypsin-treated erythrocytes was introduced into control and experimental cuvettes. Certain concentration of lectin fraction was added to the analytical cuvette, though the same volume of buffer was added to the control cuvette. Index of conductivity of 670 nm vawelength light through the control cuvette was ascertained as 100% and change of this index in the experimental (analytical) cuvette was registered by the self-recorder connected to the photocolorimeter (ФК-3) [4].

Specificity of lectins to carbohydrates was studied using hapten-inhibitory method [5]. 0.6 M solutions of oligosaccharides on the basis of PBS were used for analyzes. 20 carbohydrates were used in experiments: D-galactose, methyl-D-galactose, N-acetyl-D-galactosamine, D-mannose, methyl-D-mannose, D-glucose, methyl-D-glucose, L-ramnose, N-acetyl-D-glucosamine, L-fucose, D-galacturonic acid, fructose, D-arabinose, L-ribose, D-melibiose, D-lactose, D-maltose, D-trehalose, saccharose as well as oligomers of N-acetyl glucosamine (partially purified hydrolysate of chitin). Carbohydrate solution was titrated at decreasing concentration from 200 mM, on the immunologic titration slide. In all wells of a slide equal concentration of a lectin solution at a titer 1:4 was introduced. Hapten-specificity was judged by the minimum concentration of a carbohydrate (mM), which caused inhibition of hemagglutination activity of a lectin.

Hydrolysis of colloidal chitin (Chitin practical grade powder obtained from Sigma Chemical Co.) was performed in HCl of 7 normality at 40°C for 15 hours. HCl was removed by evaporation and the hydrolyzate was dissolved in the PBS.

Protein concentration was determined according to the method by Lowry and others [6]. Protein concentration was determined using the graph calibrated on the basis of bovine serum albumin (BSA, Sigma).

Results and Discussion

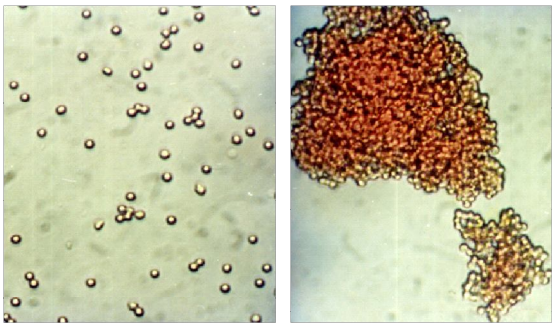
At the first stage of experiments optimum conditions were established for the extraction of soluble proteins of lectin activity from all organs of adult plant of the greater celandine. With this aim extracting solutions of different composition were used (Table 1).

Data presented in Table 1 indicate that using salt solutions of different composition (PBS) soluble proteins - lectins of a new type are extracted from tissues of the greater celandine, which differ from those presented in the literature. The supposition is based on the fact that for the extraction of lectins from tissues of the greater celandine they used 0.1 M HCl - extracting agent which sharply differs from the salt solutions of neutral pH applied by us.

It is known from the literature that mostly lectins of two or more types occur in plants and their extraction is mainly conditioned by the type of the applied extracting agent.

As seen from Table 1, the highest titre (32768), hemaggutination activity (0.028) (Fig. 1) and specific activity (24094.1) was registered in the case when extraction was performed using the PBS+0.5mM â-mercaptoethanolamine (pH 7.4) solution being of 10 times higher efficiency as compared with the efficiency of other extracting agents.

Thus the highest content and hemagglutination activity of lectins (Fig. 2) was found in the extract of soluble proteins isolated from greater celandine using the fourth extracting agent PBS+0.5mM β-mercaptoethanolamine (pH 7.4), which might be conditioned by the effect of β-mercaptoethanolamine.



a. Control b. Experimental

Fig. 1. Visual fixation by the microscope of hemagglutinating activity of *Chelidonium majus* extracts at x40 magnification)
a. Diffusely scattered red blood cells without lectins
b. Agglutionation of erythrocytes caused by lectins

This substance is known as an inhibitor of phenol oxidizing enzymes as well as reducing agent of sulfhydril (-SH) groups and correspondingly protects the proteins from oxidation and inactivation.

It is known from the literature that at visual determination of lectin activity by the Takachi microtitration method very often hemagglutination seem to take place. In particular, it is knows that such artefacts might be caused by polysaccharides, phenolic compounds and even invisibly damaged immunologic slides. This shows that there exists a threat of drawing a false conclusion on the presence of lectins in the object under study. In order to avoid the above mentioned, besides the Takachi microtitration method, hemagglutination was determined by the photocolorimetric method, of determination of hemagglutination activity, elaborated by us, which allows to monitor the kinetics of hemagglutination process.

As seen from Fig. 3. in the control variant (erythrocytes without lectins) and experimental (erythro-

Table 1. Heamgglutination activity of extracts from all organs of the greater celandine (flower+leaf+root+seed+latex of orange color) to the trypsin-treated rabbit erythrocytes exracted with solutions of different composition

Tissue of *Chelidonium majus* and extracting solution, 1:40 (g/ml)	Protein mg/ml	Hemagglu-tination titre	Hemagglu-tination activity, mkg/ml	Specific activity, ml/mg
PBS (0.9%NaCl + 0.04M KH_2PO_4, pH 7.4)	1.58	4096	0.26	2592.41
PBS (pH 5.0)	1.33	4096	0.22	3079.70
PBS + 0.5mM PMSF (pH 7.4)	1.69	4096	0.28	2423.67
PBS+0.5mM β-mercaptoethanolamine (pH 7.4)	1.36	32768	0.028	24 094.1

$p<0.01$

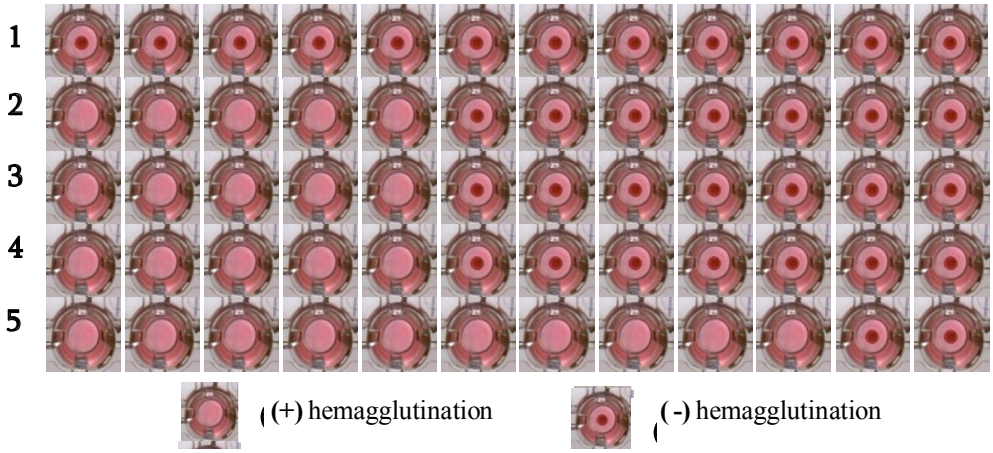


Fig. 2. Visualization of hemagglutination activity of *Chelidonium majus* lectins of immunologic plate using the Takachi method.
1. Control - without lectins
2. In the presence of lectins. Extracting agent: PBS (0.9%NaCl + 0.04M KH_2PO_4, pH 7.4)
3. In the presence of lectins. Extracting agent: PBS (pH 5.0)
4. In the presence of lectins. Extracting agent: PBS + 0.5mM PMSF (pH 7.4)
5. In the presence of lectins. Extracting agent: PBS+0.5mM β-mercaptoethanolamine (pH 7.4)

cytes in the presence of lectins) kinetically the rate of sedimentation of erythrocytes is of reciprocal character. In particular, in the experimental variant the conductivity of light beam increases rapidly because of speedy sedimentation of erythrocytes and reaches maximum (100%) already after 20 minutes, though in the control variant the same index is registered only after 90 minutes. The above mentioned is caused by the agglutination of erythrocytes by the greater celandine lectin (CBL) and quick sedimentation of a big group of erythrocytes caused by this. This, in its turn, causes quick increase in light conductivity of the erythrocyte suspension, which depends on lectin activity.

The obtained results pointed to the presence of true lectins in the greater celandine, but it remained unclear to which organs was associated their content. Thus in the next series of experiments we studied the allocation of lectins in different organs of the greater celandine plant.

Table 2 shows that flowers, roots, stems, leaves and sap of orange color do not contain protein-lectin possessing hemagglutination activity. Only freshly ripened seeds of *Chelidonium majus* contain lectin and their hemagglutination activity makes 22 ng/ml. Considering the data presented in scientific literature this index points to particularly high hemagglutinating activity and high lectin content in this organ. Of special notice is the fact that hemagglutination titre (T=16 384) of lectin isolated from *Chelidonium majus* using our method 584-1168 times exceeds the hemagglutionation titre of lectin isolated from seed of *Chelidonium majus* by W. J. Peumane and co-authors, which fluctuated within the range of 20-40 [1]. The obtained results allow to sup-

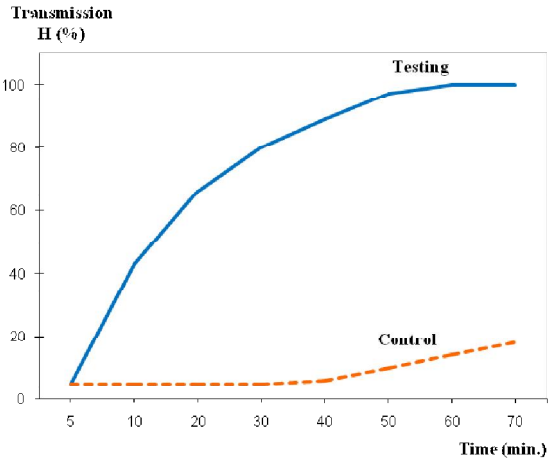


Fig. 3. Kinetics of the process of hemagglutination of red blood cells by *Chelidonium majus* lectin (CBL)
- Control – trypsin-treated erythrocytes without lectins
- Testing - trypsin-treated erythrocytes in the presence of lectins.

Table 2. Distribution of lectins in organs of *Chelidonium majus*

Organ of *Chelidonium majus*	Protein mg/ml	Hemagglutination titre (T)	Hemagglutination activity, mg/ml (HA)	Specific activity, ml/mg (SHA)
Flower	-	-	-	-
Stem	-	-	-	-
Leaf	-	-	-	-
Root	-	-	-	-
Seed	7.21	16 384	0.00022	2272.4
Milky sap	-	-	-	-

Table 3. Specificity of CBL lectins to carbohydrates

Carbohydrate (initial concentration 200 mM)	Inhibition of hemagglutination activity	Minimum carbohydrate inhibiting concentration (mM)
D-galactose	_	
methyl-D-galactose	_	
N-acetyl-D-galactoseamine	_	
D-mannose	_	
methyl-D-mannose	_	
D-glucose	_	
methyl-D-glucose	_	
L-rhamnose	_	
N-acetyl-D-glucosamine	_	
L-fucose	_	
D-galacturonic acid	_	
fructose	_	
oligomers of N-acetyl glucosamine (hydrolysate of partly purified chitin)	+	**1.562**
D-arabinose	_	
L-ribose	_	
D-melibiose	_	
D-lactose	_	
D-maltose	_	
D-trehalose	_	
saccharose		

pose that the lectin (CBL) isolated by us differs from the lectin (CMA) isolated from *Chelidonium majus* by the above mentioned authors.

In special experiments we have investigated the specificity of lectin CBL to carbohydrates (Table 3). Establishing of the specificity of lectins to carbohydrates is necessary for full characterization of lectins. Lectins are known to specifically and reversibly bind the carbohydrates, to cause blocking of active sugar binding centres of lectins and inhibition of agglutination caused by lectins.

20 different carbohydrates at the initial concentration 200 nM have been used for the analyses (Table 3).

Data presented in the Table indicate that of the saccharides used only oligomers of N-acetylglucoseamine (hydrolysate of partly purified chitin) inhibited the hemagglutination activity of lectins.The obtained data point that lectin isolated by us from seeds of the greater celandine belongs to the class of chitin specific, homotypic (inhibited by only one type of oligosaccharide) and hololectins having two or more carbohydrate-binding centers. When binding with membrane receptors containing

oligomers of N-acetyl glucosamine, CBL as hololectin forms bonds with cells of neighboring erythrocytes and causes their sticking of agglutination.

Thus protein (CBL) possessing hemagglutination activity is isolated from seeds of the greater celandine - *Chelidonium majus*, medicinal plant distributed in Georgia using the salt solution (PBS). Minimum hemagglutination activity of lectin to trypsin-treated rabbit erythrocytes makes 0.0022 mg/ml. Lectin CBL belongs to the class of chitin specific, homotypic hololectins. It has been demonstrated that as a result of using (PBS) as extracting agent lectin activity was manifested only in seeds of the greater celandine and none of other organs of an adult plant (flowers, leaves, stems, roots and latex of orange color). The results of experiments allow to suppose that using the method elaborated by us the chitin specific lectin of a new type is isolated from *Chelidonium majus* which differs from lectins from *Chelidonium majus* isolated by other authors.

ბიოქიმია

მცენარე ქრისტესისხლადან (Chelidonium majus L.) ახალი ლექტინის გამოყოფა, მისი ზოგიერთი თვისების და მცენარეში განაწილების შესწავლა

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საქართველოს სამკურნალო მცენარე ქრისტესისხლას (Chelidonium majus L.) თესლებიდან მარილწყალხსნარით (PBS) გამოყოფილია ჰემაგლუტინაციური აქტივობის მქონე ლექტინი (CBL-1). ლექტინის მინიმალური ჰემაგლუტინაციური აქტივობა ბოცვრის ტრიფსინიზირებული ერითროციტების მიმართ 0.00022 მგ/მლ–ია. ლექტინი CBL-1 მიეკუთვნება ქიტინ სპეციფიკური, ჰომოტიპური ჰოლოლექტინების კლასს. ჰემაგლუტინაციური პროცესის კინეტიკიდან ნაჩვენებია, რომ საკონტროლოში (ერითროციტები ლექტინების გარეშე) და საცდელში (ერითროციტები ლექტინების თანაობისას) ერითროციტების დალექვის სიჩქარე კინეტიკურად რეციპროკული ხასიათისაა. კერძოდ, საცდელში სინათლის სხივის გამტარებლობა სწრაფად იზრდება ერითროციტების სწრაფი დალექვის გამო და აღწევს მაქსიმუმს (100%) უკვე 20 წუთის შემდეგ, საკონტროლოში იგივე მაჩვენებელი კი მხოლოდ 90 წუთის შემდეგ ფიქსირდება ნაჩვენებია, რომ PBS-ით გამოყოფილი ლექტინი აღმოჩენილი იქნა მხოლოდ ქრისტესისხლას თესლებში და არა ზრდასრული მცენარის სხვა ნაწილებში (ყვავილები, ფოთლები, ღეროები, ფესვები და ნარინჯისფერი წვენი). განსაკუთრებით აღსანიშნავია, რომ ქრისტესისხლას თესლიდან ჩვენი მეთოდით ექსტრაგირებული ლექტინის ჰემაგლუტინაციური ტიტრი, რომელიც შეადგენს 16 384, 584-1168-ჯერ აღემატება პეუმანისა და მისი თანაავტორების მიერ გამოყენებული მეთოდით გამოყოფილი ქრისტესისხლას თესლის ლექტინის ჰემაგლუტინაციურ ტიტრს, რომელიც მერყეობდა 20-40-ის

ფარგლებში. დადგენილია, რომ ჩვენს მიერ გამოყენებული მეთოდებით მიღებული ლექტინი CBL-1 განსხვავდება სხვა ავტორების მიერ ქრისტესისხლადან გამოყოფილი ლექტინებისაგან.

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