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Biochemistry

Partial Purification and Biochemical Characteristics of Lectin CBL-1 Isolated from the Greater Celandine (*Chelidonium majus* L.) Plant

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ABSTRACT. Lectin of hemagglutination activity (CBL-1) has been isolated from the seeds of *Chelidonium majus* L. – medicinal plant distributed in Georgia - using phosphate buffer solution (PBS). CBL-1 belongs to the class of chitin-specific homotypic hololectins. It is shown that CBL-1 content is the highest in protein fractions isolated in conditions of 40-80% saturation with ammonium sulphate. CBL-1 is thermally stabile protein and completely retains its activity after incubation for 30 minutes at 60°C. The highest index of CBL-1 hemagglutination titre is registered within the range pH 7.0-8.0. CBL-1 does not cause agglutination of native or trypsin-treated erythrocytes of either group of human blood. Application of CBL-1 lectin in clinical practice seems to be very prospective. © *2014 Bull. Georg. Natl. Acad. Sci.*

Key words: Chelidonium majus*, lectins.*

Chelidonium majus L. (the greater celandine) plant, which belongs to perennial poisonous herbs and is widely applied in folk medicine in Georgia has been chosen as the object of investigation. The sap obtained from its organs is of orange colour. In conditions of Georgia plant attains 65-80 cm height.

It is known from the practice of folk medicine that the greater celandine is used for the treatment of skin tuberculosis, lupus erythematosus, at low doses - for treatment of liver and gull blade diseases, removal of verruceae. Tincture of the greater celandine is also used against aphids, Cabbageworm or Cabbage White Butterfly (*Pieris rapae*) and other pests. Alcaloids and phenolics of the greater celandine are widely applied as antimicrobial and antitumour medications (Chelidonin, Chelaritrin, Ukraina and others) [1,2].

In the present paper, we aimed to show some biochemical characteristics of the polypeptide CBL-1 which possesses hemmagglitinaton activity.

Materials and Methods

Mature seeds of medicinal plant *Chelidonium majus* L. (family Papaveraceae), distributed in Georgia, were used as the object of investigation.

Soluble protein fraction was isolated using PBS solution+0.5mM β-mercaptoethanol (pH 7.4). Ratio of raw material to extracting solution was 1:40 (g/ml).

Homogenization was performed in a porcelain bowl. Extraction of soluble proteins from the homogenized raw material was carried out on a magnetic shaker during an hour at a room temperature. The mixture was filtered through the double gauze and the filtrate centrifuged at 16 000 g for 15 minutes. The supernatant was filtered first through the filter (Miracloth, Calbiochem, USA), and then gradually through Whatman GF/c and stnpor-0.45μ filters.

For the gradual fractionation by ammonium sulphate at every step of sedimentation solution of ammonium sulphate was added to the extract of soluble proteins in conditions of constant shaking. After the salt has dissolved, stirring was continued for 20 minutes. Protein suspension was left in the fridge at -10°C overnight and then centrifuged at 20 000 g for 20 minutes at 4°C temperature. The volume of the obtained supernatant was measured and the amount of the salt needed for the next step of fractionation was calculated. Excess of inorganic ions was removed by the dialysis on the G-10 Sephadex gel-filtration column (50x2.7 cm). The sediment was dissolved in the minimum volume of PBS and its hemagglutination activity determined.

Lectin activity was determined visually using microtitration method by Takachi [3] on immunologic slides using the hemagglutination test on native and trypsin-treated erythrocytes of A, B, AB and O groups of rabbit and human blood. **Lectin activity** was evaluated by the minimum concentration of protein (mg/ml) which caused agglutination of trypsin-treated rabbit erythrocytes. For the evaluation of lectin activity also **specific activity** (mg/ml) was determined: $SA=T^{-1}\cdot C^{-1}$, where T^{-1} (titre) is the degree of protein dilution in the last well of the titration plate, where hemagglutination still occurs ($T=2^n$, n – is number of agglutination wells, C- protein concentration in mg/ml).

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

For the study of thermal stability of lectins partially purified protein fractions were incubated over the water bath at 20, 40, 60, 80 and 100°C temperature for 10 minutes. The samples were cooled in the icy bath for 15 minutes and centrifuged at 1500 g for 15 minutes for the removal of denaturated protein sediment. Hemagglutination activity in the supernatants was determined at a room temperature.

Specificity of lectins to carbohydrates was studied using hapten-inhibitory method [4]. 0.6 M solutions of oligosaccharides on the basis of PBS were used for analyses. The 22 carbohydrates were used in experiments: D-galactose, methyl-D-galactose, N-acetyl-D-galactosamine, D-mannose, methyl-D-mannose, D-glucose, L-fucose, D-fructose, L-inositol, L-rhamnose, D-xylose, D-arabinose, L-ribose, D-galacturonic acid, D-lactose, D-maltose, saccharose, D-trehalose, D-cellobiose, D-melibiose and D-raffinose as well as oligomers of N-acetyl glucosamine (partially purified hydrolysate of chitin). Carbohydrate solution was titrated with decreasing concentration from 200 mM, on the immunologic titration slide. In all wells of a slide equal concentration of a lectin solution at a titre 1:4 was introduced. Hapten-specificity was judged by the minimum concentration of a carbohydrate (mM), which caused inhibition of hemaggutinationg 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.

Dependence of hemagglutination activity of lectins on concentration of hydrogen ions H^+ was tested in the PBS buffer of pH 4.0-10.0 range, with 0.5 unit intervals. Solution of purified protein was titrated by the decreasing concentration on the microtitration immunologic slides, in the PBS of the corresponding pH. For the agglutination reaction 2% suspension of trypsin-treated rabbit erythrocytes prepared on the PBS solution of the corresponding pH was used.

Protein concentration was determined by the method of Lowry [5] using the graph calibrated on the basis of bovine serum albuminum (BSA, Sigma).

Table 1. Influence of duration and extraction temperature on the CBL-1 hemagglutination titre, hemagglutination and specific activities

Duration and temperature of extraction	Protein mg/ml	Hemagglutination titre	Hemagglutination activity, mg/ml	Specific hemagglutination activity, ml/mg
15 min; (18^0C)	8.2	1024	0.0040	124.9
30 min; (18^0C)	7.9	1024	0.0038	129.6
1 hr; (18^0C)	8.2	1024	0.0040	129.6
2 hrs; (18^0C)	8.3	1024	0.0041	123.4
24 hrs; (+4^0C)	7.7	1024	0.0037	133.0

$p<0.01$

Results of Investigation and Discussion

In the first series of experiments influence of duration and temperature of the extraction on the hemagglutination titre of CBL-1-1, its hemagglutination and specific activities was studied (Table 1). According to the data presented in scientific literature, time and temperature regime of lectins' extraction are different in some cases. Because of this, while elaboration of a new method of lectins' extraction duration of the extraction procedure and optimum temperature regime are being determined experimentally.

Table 1 shows dependence of the CBL-1 hemagglutination titre, hemagglutination and specific activities on the duration of extraction and temperature regime.

As seen from the Table 1, in our experiments extraction of the homogenate was accomplished during 15 min, 30 min 1 hr, 2 hrs and 24 hrs in conditions of room temperature (18-20°C) and +4°C. The obtained results show that the maximum hemagglutination titre, hemagglutination and specific activities were registered after the minimum time interval (15 min) and change of temperature regime does not affect the mentioned characteristics of CBL-1.

At the second step of experiments partial purification of the CBL-1 was carried out using the method of fractionation by the ammonium sulphate. With this aim gradual fractionation of the extract of soluble proteins of the greater celandine seeds using ammonium sulphate from 0 up to 90% was accomplished in

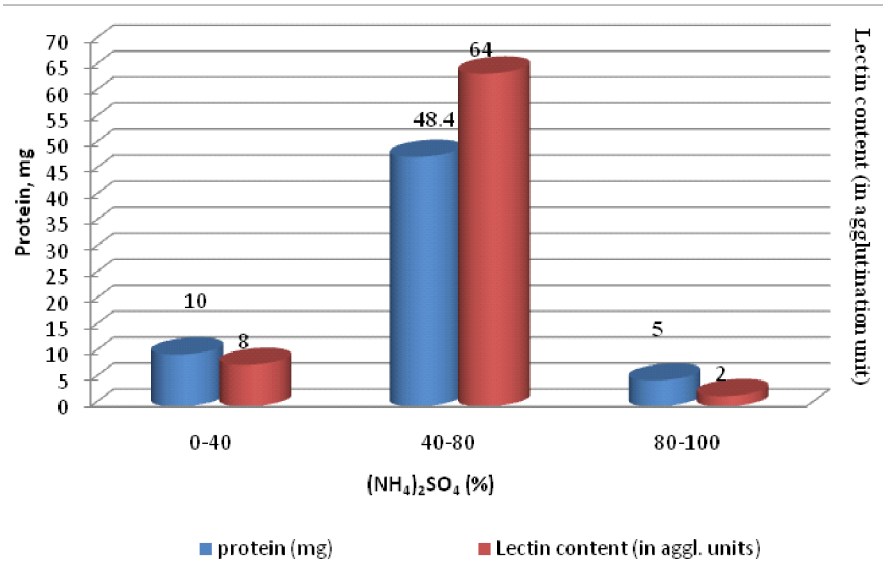

Fig. 1. Fractionation of soluble proteins of the Chelidonum majus seeds with ammonium sulphate

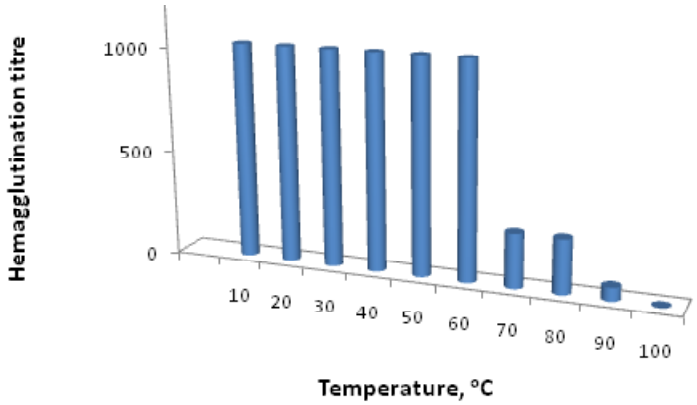

Fig. 2. Influence of temperature on hemagglutination activity of greater chelandine seed lectin (CBL) activity.

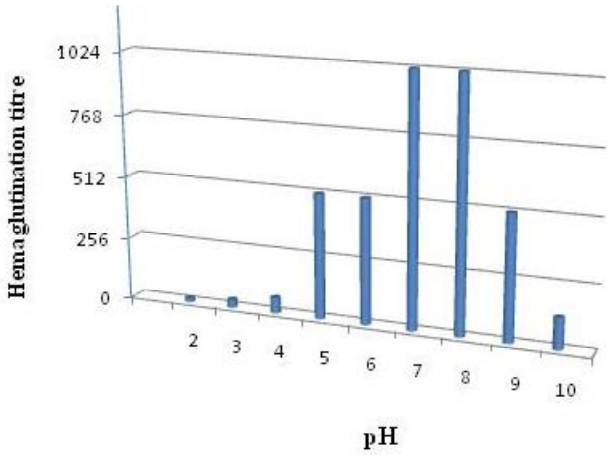

Fig. 3. Influence of concentration of H+ions on hemagglutination activity of the greater chelandine seed lectin (CBL).

conditions of 20% intermediate saturation. Fig. 1 demonstrates the data of protein amount and lectin content obtained at each step of fractionation.

As seen from Fig. 1, the amount of precipitated protein and also content of CBL-1 is highest in protein fractions obtained in coditions of 40-80% saturation with ammonnum sulphate. Considering this the fraction partially purified by this method, in which the content of CBL-1 was the highest, was used in next experiments.

Based on literature the majority of lectins is known to be thermally stable. In the next series of experiments influence of temperature on hemagglutination activuty of CBL-1 was studied. As seen from Fig. 2 CBL-1 is characterized by thermal stability and retains activity after incubation during 30 minutes at 60°C. As a result of futher increase of temperature gradual inactivation of lectin takes place and at 100°C complete inhibition of hemaglutination actvity takes place (Fig. 2).

In the next series of experimens influence of concentration of H^+ ions on hemaggltinaton titre of CBL-1 was investigated. It is known from scientific literature that usually plant lectins express hemagglutination activity in a wide range of pH with maxumum of activty in a neutral medium. Though there are lectins which express maxumum activity at certain values of H^+ ions concentration. Considering the above said in special experiments we studied the effect of H^+ ions on hemagglutinaton activity of the greater celandine seed lectin within the range of pH 2.0-10.0

Table 2. Hemagglutination activity of CBL-1 against native and trypsin-treated erythrocytes of A, B, AB and O groups of human blood

Human blood groups	Erythrocytes	Hemagglutination activity of CBL-1, mg/ml
I (0)	native	_
	trypsin-treated	_
II (A)	native	_
	trypsin-treated	_
III (B) BB	native	_
	trypsin-treated	_
IV(AB)	native	_
	trypsin-treated	_

Results of investigation presented in Fig. 3 show that the highest index of CBL-1 hemagglutination titre is registered within the range pH 7.0-8.0, which points to the fact that CBL-1 manifests maximum hemagglutination activity in the medium with neutral pH.

It is known from the literature that lectins' capacity for cell agglutination is not restricted by the ability for agglutination of rabbit erythrocytes only. Lectins possess potential ability to evoke agglutination of all types of cells of an organism despite their origin (all types of human or animal cells, also bacteria, fungi and viruses). On the one hand, the necessary condition for the occurrence of processes of this type is presence of lectins able to specifically and reversibly bind with carbohydrates being in the composition of glycoconjugates screened on surfaces of a certain type cells or microorganisms and, on the other hand, polyvalency of a lectin, or presence of two or more sugar-binding centres on a molecule. In above described conditions lectin specifically binds with glycoconjugates located on two or more neighbouring cells and causes their agglutination or sticking by means of forming the bonds between them.

In special experiments we have investigated specificity of lectin CBL to carbohydrates (Table 3). Establishing of the specificity of lectins to carbohydrates is necessary for the 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.

As seen from Table 3, 18 different carbohydrates at the initial concentration 200 nM were used for the analyses: (D-galactose, methyl-galactose, N-acetyl-D-glucosamine, N-acetyl-D-galactoseamine, D-mannose, lactose, D-glucose, fructose, inositol, rhamnose, arabinose, L-ribose, chitin, galacturonic acid, saccharose, celobiose, melibiose, raffinose).

Data presented in the Table indicate that of used saccharides only oligomers of N-acetylglucseamine (hydrolysate of partly purified chitin) inhibited hemagglutiniation activity of lectins.The obtained data point that lection 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 centres. 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.

The difference in factors satisfying the above conditions are responsible for the existence of qualitatively different types of lectins and correspondingly selective agglutination of cells and microbes.

Considering the above said we carried out special series of experiments in order to study the agglutination ability of native and trypsin–treated erythrocytes of different groups of human blood with lectin CBL-1 isolated from seeds of the greater celandine.

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		

+ hemagglutination activity of lectin is inhibited
- hemagglutination activity of lectin is not inhibited

As seen from Table 2, CBL-1 does not cause agglutination of erythrocytes of any of groups of human blood, notwithstanding the fact whether native or trypsin-treated erythrocytes were used in experiments.

The obtained results show that CBL-1 qualitatively differs from lectins isolated from the greater celandine by other authors. As it is known, lectins isolated by other scientists in contrast to CBL-1 cause agglutination of native as well as trypsin-treated erythrocytes of all groups of human blood [14].

The obtained results have deepened an interest to the further investigation of a new type of lectin isolated from seeds of the greater celandine. Its localization only in a single organ, seeds, caused great interest to further research aimed at clarification of their physiological role in this particular organ. After establishing the fact that CBL-1 does not cause agglutination of erythrocytes in any of groups of human blood, and thus its toxicity to an organism is excluded, study of its biological activities with the aim of application in practical medicine became very interesting.

ბიოქიმია

მცენარე ქრისტესისხლას (*Chelidonium majus* L.) ლექტინის CBL-1-ის ნაწილობრივ გასუფთავება და ზოგიერთი ბიოქიმიური მახასიათებლები

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***აკადემიის წევრი: საქართველოს საპატრიარქოს წმიდა ანდრია პირველწოდებულის სახელობის ქართული უნივერსიტეტი, თბილისი*

საქართველოს სამკურნალო მცენარე ქრისტესისხლას (*Chelidonium majus L.*) თესლებიდან მარილწყალხსნარით (PBS) გამოყოფილია ჰემაგლუტინაციური აქტივობის მქონე ლექტინი (CBL-1). CBL-1 მიეკუთვნება ქიტინ სპეციფიკური, ჰომოტიპური ჰოლოლექტინების კლასს. CBL-1-ის შემცველობა ყველაზე მაღალია ამონიუმის სულფატით 40-დან 80%-მდე გაჯერების პირობებში მიღებულ ცილოვან ფრაქციებში. დადგენილია, რომ CBL-1 ხასიათდება თერმოსტაბილურობით და აქტივობას სრულად ინარჩუნებს 60^0C-ზე 30 წთ-ის განმავლობაში ინკუბაციის შემდეგ. CBL-1 ჰემაგლუტინაციის ტიტრის ყველაზე მაღალი მაჩვენებელი ფიქსირდება pH 7,0 – 8,0 ფარგლებში. CBL-1 არ იწვევს ადამიანის სისხლის არც ერთი ჯგუფის ერითროციტების აგლუტინაციას, მიუხედავად იმისა ნატიური თუ ტრიპსინიზირებული ერითროციტები იყო გამოყენებული ექსპერიმენტებში.

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