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Biochemistry

Allocation of Mannose- and N-Acetyl-D-Glucosamine Specific Lectins in Different Parts of Aloe Plants (*Aloe aristata* Haw.) and their Biochemical Characteristics

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ABSTRACT. Allocation of mannose- and N-acetyl-D-glucosamine specific lectins in different organs of the flower (pistil, filament, anther, petals and peduncle) and root of aloe (*Aloe aristata Haw*) plant was studied. To establish lectin specificity to carbohydrates 0.6 M solutions of simple sugars D-glucose, D-mannose, D-galactose, N-acetyl-D-glucosamine were used. All the sugars were prepared on the basis of PBS buffer. Molecular mass of the protein was determined by gel-filtration on Toyopearl HW 55 column (2.0x38 cm), equilibrated by PBS. It was shown, that aloe flower stalk is distinguished by high content of proteins, but lectin activity practically does not differ from the other parts of the flower stalk. It is noteworthy that when the flower stalk extract was allowed to stay for a certain time with tripsinazed erythrocytes it caused lysis of erythrocytes, which was blocking after adding 0.6 mM mannose to the incubation medium, lysis was developed later, nearly after 20 hours. Chromatography of the total extract of aloe flower yielded four peaks, only the second and third peaks display high lectin activity. In parallel N-acetyl-D-glucosamine specific lectins were separated from the root of aloe. It is important to underline, that after 20 min incubation at 80°C and 100°C the aloe root lectin maintains its hemagglutination ability. The native country for *A. aritsata* is the South Africa with dry and hot climatic conditions. Aloe reflects genetic adaptive possibilities to its traditional ecological environment and it is natural that all its properties are determined by genetic memory. *© 2016 Bull. Georg. Natl. Acad. Sci.*

Key words: *Aloe aristata* Haw., mannose- and N-acetyl-D-glucosamine specific lectin

Lectin-carbohydrate interaction still remains an urgent problem of biology. The majority of lectins are specific proteins, reversibly binding with terminal mono- and oligosaccharides of glycoproteins [1, 2,]. Lectins are actively involved in regulation of life processes and mechanisms of adaptation to the environment [3]. Due to this, localization of lectins and their quantitative distribution in different organs of plant in relation with physiological state of a plant is being intensively studied [4, 5]. In the present work we have investigated distribution of mannose- and N-acetyl-D-glucosamine specific lectins in different parts of aloe flower – stem, pistil, filament, anther, sepals and peduncle, root.

Table 1. Lectin indexes of extract of flower stalk of *Aloe aristata* Haw. Plant

Parts of flower stalk	Titre	Protein concentration mg/ml	Specific hemagglutination activity ml/mg
Lower part	64	1.02±0.04	62.74±5
Middle part	64	0.66±0.05	96.97±3
Terminal part	64	0.71±0.05	90.14±5

Material and Methods

Lectins from different organs of *Aloe aristata* Haw. plant were isolated using saline (0.9% NaCl+1M KH_2PO_4 pH 7.4 (PBS 0.4 mM) at a ratio 10 ml per gram of raw material. Homo-genization of material was made on porcelain bowls. Extraction of lectins from the obtained homogenate was performed on magnetic stirrer at room temperature for 60 minutes. Extract was filtered through the double gauze and the filtrate was centrifuged (centrifuge ТУ 5.3 75-4261-76, rotor РУ 180) at 8000g for 20 minutes. The supernatant was filtered through the cellulose filter and stored at 4°C. Protein concentration was determined according to Lowry [6]. Lectins were concentrated and partially purified using 90% saturated ammonium sulphate solution. Lectin activity of the extract was determined using the hemagglutination test to trypsin-treated rabbit erythrocytes on U-shaped immunologic plates. Hemagglutination degree was evaluated according to the specific activity of the protein extract of aloe plant - SHA=T/C, where T is index of protein dilution, at which hemagglutination is still visible. C is protein concentration in mg/ml.

Specificity of lectins to carbohydrates was studied by the hapten-inhibitory method by Liener [7]. With the aim of establishing lectin specificity to carbohydrates 0.6M solutions of simple sugars D-glucose, D-mannose, D-galactose, N-acetyl-D-glucosamine prepared on the basis of PBS buffer were used. Molecular mass of the protein was determined by gel-filtration on Toyopearl HW 55 column (2.0x38 cm), equilibrated by PBS. Rate of elution was 1 ml/min. Absorption spectra were registered at 280 nm wavelength by the detector of Knauer type. To reveal lectin activity extracts of root was incubated in a water thermostat at 20, 40, 60, 80 and 100°C for 20 min.

Results and Discussion

At the first stage of research characteristics of lectins of *Aloe aristata* Haw. flower stalk (peduncle) (length 15 cm) were studied. Parts of stalk, differing with coloration - dark red lower part, light red middle part and very light red terminal part of flower stalk were investigated in experiments (Table 1).

As shown in Table 1, despite the fact that lower part of the aloe flower stalk is distinguished by high content of proteins, it practically does not differ by lectin activity from the other parts of the flower stalk. Protein content of the lower part of stalk is by 35.29% higher, than that of the middle part and it is by 30.39% higher, than protein content of the terminal part of the flower stalk. These results indicate that protein content is higher in lower and middle parts of flower stalks as compared with its terminal part. It is noteworthy that after studying the hemagglutination activity of the flower stalk extract, when an extract was allowed to stay for a certain time, it caused lysis of erythrocytes. This points to the presence in the stalk of a substance with lysoigenic activity, which was not detected in the leaf, flower, root or bulb. After adding 0.6 mM mannose to the incubation medium, lysis developed later, nearly after 20 hours, pointing to the inhibition by mannose of the process of lysogenesis. After treatment of extract with 90% saturated solution of ammonium sulphate, lysis was evidently reduced. Lectin of the flower stalk was found to be mannose-specific and its inhibiting concentration of hemagglutination test to trypsin-treated rabbit erythrocytes being 0.15mM (Table 2).

Table 2. Carbohydrate specificity of lectin of flower stalk of *Aloe aristata* Haw. plant (lectin specificity to carbohydrates is denoted by the symbol "+")

Carbohydrate	Inhibition of hemagglutination activity	Minimal concentration of carbohydrates inhibiting agglutination, mM
D-galactose	-	-
D-mannose	+++	0.15
N-acetyl-D-glucosamine	+	0.3
D-glucose	+	0.3

Table 3. Lectin properties of protein fractions obtained as a result of chromatography of flower stalk extract of the plant *Aloe aristata* Haw.

Peaks of protein fractions	Titre	Protein concentration mg/ml	Specific hemagglutination Activity ml/mg
I	4	0.185±0.04	21.62±5
II	2048	4.05±0.03	505.68±4
III	256	0.315±0.05	812.7±5
Characteristics of the extract before chromatography	1024	1.54±0.03	664.94±5

Table 4. Lectin activity in extracts obtained from different parts of *Aloe aristata* Haw. Flower

Extracts from flower parts	titre	Protein concentration mg/ml	Specific hemagglutination activity ml/mg
Flower peduncle	256	1.284±0.05	199.38±5
Petals	32	1.34±0.05	23.88±5
Pistil	16	2.024±0.05	7.90±5
Filaments	4	0.324±0.05	12.35±5
Anthers	-	1.24±0.05	-

As a result of chromatography of the total extract of flower stalk three protein peaks was revealed, of which peaks II and III high concentration of proteins and lectin activity were registered. Protein fractions of each peak were added by 90% saturated solution of ammonium sulphate, the sediment was dissolved in minimum volume of PBS and protein concentration and specific lectin activity were determined (Table 3).

In the next series of experiments quantitative distribution of lectins in different parts of aloe flower was investigated (Table 4).

As seen from Table 4, flower stalk (peduncle) is distinguished by the highest content of lectins. This fact is may be indicative of a supportive role of lectins. Lectin activity, detected in pollen filaments by the high probability proves the same. It is noteworthy that lectin activity was not detected in anthers. Protein, separated from ammonium sulphate by centrifugation was dissolved in PBS of minimum concentration and lectin activity was not detected in any of fractions. The specific recognition reaction in the self-incompatibility system is thought to be due to the interaction of glycoproteins or proteins of the pollen surface and complementary macromolecules on the cell surfaces of the stigma or style. This interaction leads to either the retardation of growth or penetration of the incompatible pollen tubes or the ovary [8]. Lectins and their receptors were proposed as likely candidates for these recognition macromolecules [9,10].

Table 5. Carbohydrate specificity of lectin isolated from *Aloe aristata* Haw. flower (lectin specificity to carbohydrates is denoted by symbol "+")

Carbohydrate	Inhibition of hemagglutination activity	Minimal concentration of carbohydrates inhibiting agglutination, mM
D-galactose	-	-
D-mannose	+++	0.15
N-acetil-D-glucoseamine	+	0.3
D-glucose	+	0.3

Table 6. Carbohydrate specificity of lectin isolated from *Aloe aristata* Haw. stalk. (lectin specificity to carbohydrates is denoted by symbol "+")

Carbohydrate	Inhibition of hemagglutination activity	Minimal concentration of carbohydrates inhibiting agglutination, mM
D-galactose	-	-
D-mannose	+++	0.15
N-acetil-D-glucoseamine	+	0.3
D-glucose	+	0.3

Table 7. Lectin activity of protein peaks obtained as a result of chromatography flower extract of *Aloe aristata* Haw.

Peaks of protein fractions	Titre	Protein concen-entration mg/ml	Specific hemagglutination Activity ml/mg
I	2	0.032±0.05	62.5±5
II	4096	0.4±0.05	10240.00±5
III	64	0.175±0.05	365.7±5
IV	4	0.015±0.05	266.7±5
Characteristics of the extract before chromatography	1024	1.54±0.05	2392.52±5

As lectin activity was detected in anthers of *Aloe aristata* and not in tissues of the pistil, it may be supposed that prior to fertilization the process of pollen-stigma interaction proceeds with participation of carbohydrate receptors of compatible pollen grain and lectins contained in tissues of the stigma. It is know from the literature that anthers contain 34% of carbohydrates, among which the main are glucose, fructose, maltose, saccharose, starch and other derivatives of carbohydrates [11]. It can be supposed that in aloe leaf, flower stalk, petals and anther filaments lectins accomplish supportive function, thus favouring to the formation of firmness of plant organs and their resistance to harmful factors of the environment.

Chromatography of the total extract of aloe flower yielded four peaks, of which only the second and third peaks were distinguished by high lectin activity, similar to the case with flower stalk.

After treating the peaks with ammonium sulphate the protein was dissolved in the minimum volume of PBS and protein concentration and lectin activity were determined (Table 7).

Table 7 demonstrates that as a result of chromatography of aloe flower, like the case with flower stalk, four leaks of protein are revealed. In this case to the second and the third peaks are distinguished with high lectin activity.

In the next series of experiments we extracted lectins from Aloe aristata root homogenate. The ho-

Table 8. Lectin activity of proteins fractionated with ammonium sulphate from aloe roots under different degrees of saturation

Percent of ammonium sulphate saturation	Minimal titre	Protein concentration mg/ml	Specific hemagglutination Activity ml/mg
0-20	512	0.65±0.05	787.±5
20-40	512	0.18±0.02	1280.±5
40-60	512	0.156±0.04	1765.52±4
60-80	128	0.026±0.05	581.82±5
80-100	32	0.012±0.03	457.14±3

Table 9. Carbohydrate specificity of lectin of roots of Aloe aristata Haw. plant (lectin specificity to carbohydrates is denoted by the symbol "+")

Carbohydrate	Inhibition of hemagglutination activity	Minimal concentration of carbohydrates inhibiting agglutination, mM
D-galactose	-	-
D-mannose	+++	0.3
N-acetil-D-glucoseamine	+	0.15

mogenate were fractionated under different saturation of ammonium sulphate and hemagglutination activity was studied. As is evident from Table 8, lectin specific activity is the highest in the fractions obtained under saturation with ammonium sulphate to 40-60%.

The specificity of lectins with respect to carbohydrates was studied by the hapten-inhibitory method [7]. The 0.6M PBS of simple sugars was used for the analysis. The following carbohydrates were used in the experiments: D-galactose, D-mannose, N-acetyl-D-glucosamine (Table 9).

Study of the lectin specificity to carbohydrates revealed that hemagglutination activity is inhibited largely in the presence of 0.15 mM N-acetyl-D-glucosamine, which suggests its specific hapten property (Table 9). It is necessary to underline, that In the next series of experiments we studied the effect of temperature on hemagglutination activity of aloe root lectin. The lectin from the aloe root was shown to be characterized by thermostability and activity is maintained even after incubation at 100°C for 20 min (Table 10).

As seen from Table 10, after 20 min. incubation at 80°C and 100°C the aloe root lectin still maintains its hemagglutination ability, whilst the leaf lectin activity from the same variety of aloe is not manifested at the given temperatures.

It is known that the native country for *A. aritsata* is the South Africa with dry and hot climatic conditions. Naturally, it is adapted genetically to high temperature; it is therefore no surprise that aloe root lectin activity is stable even at 80°C-100°C.

Thus, lectin activity of the root and bulb of the variety of *A. aristata*, used as the object of the study, reflects genetic adaptive possibilities to its traditional ecological environment and it is natural that all its properties are determined by genetic memory.

Conclusions

Quantitave distribution of mannose-specific lectins has been separated from deferent organs (pistil, filament, anter, petals, peduncle, root) of Aloe plant (*Aloe aristata* Haw.). It was found that quantitative distribution of lectins is change depending on the length of a plant flower stalk of *Aloe aristata* Haw. The highest concentration of protein was revealed in the lower path of flower stalk of aloe extract. Flower stalk extract of aloe plant causes lysis of trypsin-treated rabbit erythrocytes, while is inhibited by mannose. Ex-

Table 10. Hemagglutination activity of aloe roots lectin after 20 min. incubation at 100°C at room temperature

Temperature, °C	Titre	Protein concentration mg/ml	Specific hemagglutination activity ml/mg
Control room temperature	512	1.15±0.05	445.22±0.03
40	512	0.96±0.02	533.33±5
60	64	1.12±0.05	457.14±0.04
80	2	0.85±0.04	2.35±0.05
100	2	1.15±0.05	1.74±0.03

tracts from other organs of plant do not cause lysis of erythrocytes. Stalk (peduncle) of flower of Aloe is distinguished by high content of lectins. Chromatography of proteins, extracted from the flower stalk and flower plant revealed four peaks of proteins from which only the second and the third protein peaks are characterized with high lectin activity. From the root of Aloe was separated N-acetyl-D-glucosamine-specific lectin. It was shown that after 20 min incubation at 80°C and 100°C the aloe root lectin still maintains its hemagglutination ability, whilst the leaf lectin activity from the same variety of Aloe is not manifested at the given temperatures. The native country for *A. aritsata* is South Africa with dry and hot climate. Naturally, it seems that Aloe is adapted genetically to a high temperature and aloe root lectin is noticeable even at 80°C-100°C, so, it reflects genetic adaptive possibilities to its traditional ecological environment and all its properties are realized by genetic memory.

ბიოქიმია

მცენარე ალოეს (Aloe aristata Haw.) სხვადასხვა ორგანოებში მანოზა- და N-აცეტილ-D-გლუკოზამინ-სპეციფიკური ლექტინის განაწილება და მათი ბიოქიმიური დახასიათება

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მცენარე ალოეს (Aloe aristata Haw.) სხვადასხვა ორგანოდან ყვავილიდან, ყვავილის ღეროდან და ფესვიდან გამოყოფილია მანოზა- და N-აცეტილ-D-გლუკოზამინ-სპეციფიკური ლექტინი. მოცემულია მათი ცილების ქრომატოგრამა და პიკების ლექტინური აქტიურობა. დადგენილია ალოეს ყვავილის ნაწილებში (ბუტკო, მტვრიანას ღერო (ფილამენტი), მტვრიანას პარკი (სამტვრე), გვირგვინის ფურცლები და ყუნწი) ლექტინის რაოდენობრივი განაწილება და ლექტინური აქტიურობა. ღეროში გამოვლენილია ერითროციტების ლიზოგენური ფაქტორი, რომლის მოქმედებას რამდენადმე აკავებს 0,6 mM-ის მანოზა. მცენარე ალოეს ფესვიდან და ბოლქვიდან გამოყოფილია N-აცეტილ-D-გლუკოზამინ-სპეციფიკური ლექტინი. დადგენილია რომ ფესვის ლექტინის ამონიუმის სულფატით სხვადასხვა გაჯერების პირობებში გამომარილებული ცილების ლექტინური აქტიურობის ცვლილება. დადგენილია რომ ალოეს ფესვის ლექტინი მაქსიმალურ აქტიურობას ინარჩუნებს 80-100°C 20 წთ განმავლობაში ინკუბაციის პირობებში, რაც ალოეს აფრიკის მემკვიდრული ეკოლოგიური პირობების გენეტიკური მეხსიერებითაა განპირობებული.

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