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Biochemistry

Isolation, Purification and Biochemical Characterization of N-Acetyl-D-Glucosamine Specific Lectin from the Root of Aloe (*A. aristata*)

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ABSTRACT. N-acetyl-D-glucosamine specific lectin was isolated from the root and bulb of *Aloe aristata*. Its extraction conditions were established. Lectin characteristics were studied on the rabbit's trypsinized as well as non-trypsinized erythrocytes. The effect of temperature on lectin activity of the aloe root was investigated. *© 2011 Bull. Georg. Natl. Acad. Sci.*

Key words: *Aloe aristata, lectin.*

One of the central problems in the functioning of a biological system is the identification of carbohydrates and lectins on the cellular and subcellular levels. The identification is carried out through the specific protein-carbohydrate interaction and accounts for biologically very important processes such as substance metabolism and transport, modulation of enzyme activity, reproduction, cellular communications, cell differentiation, reproduction, growth and development, inflammatory processes, microbes adhesion, synaptic transmission, etc. Information about decoding and identification of carbohydrates is available in their primary structure which is represented on the cell surface as glycoproteins (as well as glycolipids and polysaccharides) and it is adequately identified by lectin specific domains.

The lectins, isolated and biochemically studied to date, appear to be effective tools to study probing of terminal sugar of glycoconjugates on the cell surface.

Lectins have been successfully employed in clinical laboratories for diagnosis and treatment of a variety of pathologies through microbe adhesion.

Proceeding from the foregoing, isolation, identification and study of their biological role of new lectins of plant origin still remains one of the main problems.

We have decided to study the lectins of *Aloe aristata*. Our interest in aloe emerged from the conclusion made on the basis of evidence reported in the litarature focusing on a number of curative properties of aloe. It was likewise most challenging to detect to date the unexplored lectins of aloe and study their physico-chemical properties. Now again the root of *Aloe aristata* was chosen as the object of study.

The aloe root was homogenized and soluble protein was extracted: a saline solution - 0.9% NaCl, 0.4 mM potassium dihydrophosphate, pH 7.4 (PBS). For the purpose of extraction the homogenate was placed on a mechanical mixer for 60 min at room temperature. The obtained homogenate was filtered through a double gauze.

The filtrate was centrifuged (centrifuge TY 5.3 75-4261-76, rotor PY 180) at 8000 g for 20 min. The supernatant obtained was filtered in a cellulose filter and stored at 4ºC. Protein concentration was determined by Lowry et al. [3].

For the purpose of partial purification of the extracted proteins precipitation was made with ammonium sulfate solutions of different saturation (0-20; 20-40; 40-60; 60-80; 80-100%). The obtained sediment was dissolved to a

minimal volume of PBS. Dialysis was made by PBS for 24 h under cold conditions.

To reveal lectin thermostability the extract was incubated in a water thermostat at 20, 40, 60, 80 and 100ºC for 20 min.

Lectin activity of the protein was determined by hemagglutination test with rabbit's trypsinized erythrocytes on immunologic planchettes by Liener's microtitration method [4]. Hemagglutination activity was estimated according to the specific activity of protein extract from aloe root. $SA=T^{-1}C^{-1}$, where T^{-1} is the index of protein dilution in immunological planchette holes, where hemagglutination can be still noticed. C^{-1} is the protein concentration expressed in mg/ml.

To reveal lectin activity protein extracts of aloe root homogenates were prepared and the agglutinative activity was measured on the rabbit's native and trypsinized erythrocytes. The tests were conducted under variable pH (Table 1).

As seen from Table 1, specific activity of lectin is the highest at pH 7.4. Specific activity at pH 7.4 (3413.33), as pH 8.00 (2174.1) and pH 5.00, is 7-fold and twice as high respectively.

In the next series of experiments the extracted proteins were fractionated under different saturation of ammonium sulphate and hemagglutination activity was studied on the rabbit's trypsinized as well as non-trypsinized erythrocytes. As is evident from Table 2, 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 [5].

Table 1. Hemagglutination activity of aloe root extracts with different pH buffers (PBS)

Extract solution	Minimal titre	Protein concentration (mg/ml) 50 □l of root extracts	Minimal activity of lectin (mg/ml)	Content of lectin	Specific activity of lectin
0.9% NaCl, 04 mM potassium dihydrophosphate, pH 5 (PBS)	2^9	0.882	0.000861	1024	580.5
0.9% NaCl, 04 mM potassium dihydrophosphate, pH 7.4 (PBS)	2^{12}	1.2	0.000146	8192	3413.33
0.9% NaCl, 04 mM potassium dihydrophosphate, pH 8 (PBS)	2^{11}	0.942	0.0002295	4096	2174.1

Table 2. Lectin activity of proteins extracted with ammonium sulphate from aloe roots under different degrees of saturation

Percent of ammonium sulfate saturation		Minimal titre	Protein concentration, (mg/ml), 50 l of root extracts	Minimal activity of lectin (mg/ml)	Content of lectin	Specific activity of lectin
0-20	Trypsinized erythrocytes	2^9	0.65	0.00063476562	1024	787.69
	Non-trypsinized erythrocytes	2^4		0.0203125	32	24.61
20-40	Trypsinized erythrocytes	2^9	0.4	0.000390625	1024	1280
	Non-trypsinized erythrocytes	2^3		0.025	16	20
40-60	Trypsinized erythrocytes	2^9	0.29	0.00028320312	1024	1765.52
	Non-trypsinized erythrocytes	2^4				
60-80	Trypsinized erythrocytes	2^8	0.22	0.0004296875	512	1163.63
	Non-trypsinized erythrocytes	2^4		0.006875	32	72.72
80-100	Trypsinized erythrocytes	2^6	0.0725	0.00056640625	128	882.76
	Non-trypsinized erythrocytes	2^2		0.0090625	8	55.17

The 0.6 M PBS solution of simple sugars was used for the analysis. The following carbohydrates were used in the experiments: D-glucose, D-mannose, N-acetyl-D-glucosamine and L-fucose.

Study of the lectin specificity to carbohydrates revealed that hemagglutination activity is inhibited largely in the presence of only 75 mM N-acetyl-D-glucosamine, which suggests its specific hapten property (Table 3).

In the next series of experiments we studied the effect of temperature on hemagglutination activity of aloe lectin. The lectin from the aloe root was shown to be characterized by thermostability and activity is maintained even at 100ºC for 20 min after incubation (Table 4).

As seen from Table [4], after 20 min incubation at 80ºC and 100ºC 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 [6].

The native country for *A. aritsata* that was the object of our study is South Africa where it is in dry and hot climatic conditions. Naturally, it is adapted genetically to a high temperature; it is therefore no surprise that aloe root lectin activity is noticeable even at 80ºC-100ºC.

In special experiments we have studied the aloe bulb lectin and its biochemical properties. To reveal the probable lectin the bulb homogenate was extracted with variable pH solutions of PBS (Table 5).

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

Table 3. Specificity of aloe root lectin to carbohydrates

Initial concentration of carbohydrates – 200 mM	Inhibition of hemagglutination activity	Minimal inhibitory concentration (mM) of carbohydrates
D – galactose	–	–
D – mannose	–	–
N-acetyl-D-glucosamine	+	75
L-fucose	–	–

– no inhibition of hemagglutination activity; + hemagglutination

Table 4. Hemagglutination activity of aloe root lectin after 20 min incubation at 100ºC at room temperature

Temperature, ºC	Minimal titre	Protein concentration (mg/ml) 50 l of root extract	Minimal activity of lectins (mg/ml)	Content of lectin	Specific activity of lectin
Control – room temperature	2^{11}	1.15	0.0002805	4096	1780.86
40	2^{11}	0.96	0.000234	4096	2133.33
60	2^{11}	1.12	0.000273	4096	1828.57
80	2^{1}	0.85	0.2125	4	2.35
100	2^{2}	1.15	0.14375	8	3.48

Table 5. Lectin activity of aloe bulb lectin samples extracted by variable pH PBS

Extraction solutions	Minimal titre	Protein concentration (mg/ml), 50 l of root extracts	Minimal activity of lectins (mg/ml)	Content of lectin	Specific activity of lectin
0.9% NaCl, 0.4 mM potassium dihydrophosphate, pH 5 (PBS)	2^{4}	0.85	0.0265625	32	18.82
0.9% NaCl, 0.4 mM potassium dihydrophosphate, pH 7.4 (PBS)	2^{6}	0.84	0.0065625	128	76.19
0.9% NaCl, 0.4 mM potassium dihydrophosphate, pH 8 (PBS)	2^{5}	0.84	0.013125	64	38.09

ბიოქიმია

ალოე *A. aristata*-ს ფესვიდან N-აცეტილ-D-გლუკოზამინსპეციფიკური ლექტინის გამოყოფა, გასუფთავება და ბიოქიმიური დახასიათება

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