

Metal Species in Some Lectins Detected by Inductively Coupled Plasma Spectrometer

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Abstract—Ten commercial lectins were semi-quantitatively analyzed for their metal species by inductively coupled plasma (ICP) spectrometer. Calcium, silicon, zinc and manganese, which had been found in a lectin purified from a tree trunk, were especially analyzed. Calcium was abundant in the lectins obtained from dicotyledonous source, some of which also contained zinc and/or manganese. Calcium ions in these lectins were discussed in relation to their physiological roles. Silicon was detected in all of the lectins analyzed. Some of the lectins were phosphorylated, suggesting participation of protein kinases for their processing.

1. Introduction

Lectin is defined as a sugar binding (glyco-)protein which agglutinates cells and/or precipitates glycoconjugates^{1~2)}. They are non-immunological products such as sugar binding antibody nor those carbohydrate-binding substances such as carbohydrate-specific enzymes or other proteins which contain only one carbohydrate binding site. The lectins have been mainly studied in leguminous seeds, and recently being found in various tree trunks^{2~3)}. Some of them were so far purified and characterized, i.e. from *Robinia pseudoacacia*^{2~5)}, *Laburnum anagyroides*⁶⁾, *Sambucus nigra* & *S. racemosa*^{7~10)} and *Sophora japonica*^{11,12)}.

The tree lectins were found in protein bodies of the phloem parenchyma cells¹⁰⁾ and showed seasonal fluctuation⁹⁾, and thus the lectin is regarded as a kind of storage protein. Most of them are metalloproteins¹⁵⁾, of which metals are important for their function. The author proposed an idea that the protein in the tree plays a role of calcium pool which is able to regulate the ion concentration, and controls physiological process in the tree trunks¹²⁾. Moreover, the metal is essential for the hemagglutinin activities as demonstrated in Concanavalin A^{13,14)}. However, the metal species present in the proteins have been not systematically studied. This is probably because only trace amounts of metal species are present in the proteins and the conventional methods such as atomic absorption analyses are not able to cover all of the species. Recently, inductively coupled plasma (ICP) spectrometer

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has been developed and provides us quantitative trace analyses for a series of metal species. In *Sophora japonica* trees, silicon, calcium, manganese and zinc were detected in the seed and sap lectins by the ICP analyses¹²⁾. However, no silicon is reported in any protein so far we know, nor some of the metals described above are familiar in the lectin molecules. Therefore, commercial lectins were analyzed by ICP in order to confirm if these metals are detected in a series of the commercial lectins.

2. Experimental

A kit of lectins were obtained from E.Y laboratory Inc., U.S.A. They contained each one mg of wheat germ agglutinin (WGA), Soybean agglutinin (SBA), peanuts agglutinin (PNA), *Maclura pomifera* agglutinin (MPA), *Dolichos biflorus* agglutinin (DBA), *Ulex europaeus* agglutinin (UEA-I), *Griffonia simplicifolia* agglutinin (GS-I; GS-II), Concanavalin A (Con A), and *Bauhinia purpurea* agglutinin (BPA). All of them guaranteed salt free by the company were listed in Table 1 with some of their properties^{15~17)}. Standard silicon, calcium, manganese, zinc, and magnesium solutions of atomic absorption grade (Wako Pure Chemical Industries, Ltd.) were used for their calibration.

Table 1. List of the Lectins Analyzed by ICP

Abbreviation (Plant Source)	MW	Saccharide Specificities
WGA (wheat germ: <i>Triticum vulgaris</i>)	36,000	GlcNAc, sialic acid
SBA (soybean seed: <i>Glycine max</i>)	120,000	A ₁ >A ₂ >O>B: D-GalNAc> α -D-Gal
PNA (peanuts: <i>Arachis hypogaea</i>)	110,000	D-Gal- β -(1 $\rightarrow$ 3)GalNAc, Me- α -Gal
MPA (osage orange: <i>Maclura pomifera</i>)	—	α -D-Gal
DBA (seed: <i>Dolichos biflorus</i>)	104,000	A ₁ >A ₂ ; α -GalNAc
UEA-I (gorse seed: <i>Ulex europaeus</i>)	56,000	O; α -L-Fuc
GS-I (: <i>Griffonia simplicifolia</i>)	114,000	B; melibiose, α -D-Gal
GS-II (seed: <i>Bandeiraea simplicifolia</i>)	120,000	terminal GlcNAc
ConA (jack bean: <i>Canvalia ensiformis</i>)	104,000	α -D-Man, α -D-Glc
BPA (: <i>Bauhinia purpurea</i>)	—	Gal, GalNAc

The properties summarized here were taken from the references 15-17.

A DAICHI SEIKOSHA ICP2500 spectrometer (PLASMA-THERM INC.) was used for analyzing metals in the lectins. Each sample in a vial was dissolved in one ml of distilled water. Then the samples were respectively analyzed four times by the ICP spectrometer without any pretreatment. The water was counted as a control, and subtracted from the corresponding sample-counts. Then the subtracted counts were converted to the corresponding metal concentration. Abnormal counts in the sample analyzed, if present, were omitted in the calculations. The values for control

Table 2. Examples of Found Values Counted by ICP

Si	Ca	H ₂ O (Control)			run
		Zn	Mn	Mg	
17793	663394	12501	35078	40086	1
17842	657157	12714	34929	39578	2
17942	657395	12596	34741	40110	3
17896	663318	12372	34518	40180	4
17868.3	660316	12545.8	34816.5	39988.5	AV
64.682	3512.77	144.946	242.123	276.579	SD
Si	Ca	Standard Solution (1 ppm)			run
		Zn	Mn	Mg	
97109	2021019	234467	2560289	1455354	1
97432	2029170	234683	2553945	1453961	2
96972	2039359	235550	2562111	1458289	3
96744	2030711	235194	2552541	1450780	4
97064.3	2030065	234974	2557222	1454596	AV
288.694	7517.76	490.673	4678.7	3113.49	SD

Abbreviations: AV and SD show the values for averages and standard deviations of four measurements, respectively.

and standard metals were summarized in Table 2.

3. Results and Discussion

The found values for metal species (Table 3–4) are evaluated under some limitation because of the following reasons. First, one mg of the commercial lectins were contained in the respective vials but the amounts were suspected only one

Table 3. Gram Atom Metals Detected in A Lectin Molecule

Lectins (nmoles)		Si	Ca	Zn	Mn	Mg
		(nano gram atom)				
WGA	27.78	62.2*	3.74	0.79	0.04	1.34
SBA	8.33	27.4*	53.94*	3.74	17.82*	16.12*
PNA	9.09	111.2*	58.03*	4.03	7.28	44.55*
MPA	—	560.3*	23.15*	2.93	0.21	14.85*
DBA	9.62	83.6*	88.32*	10.72*	20.45*	30.81*
UEA-I	17.86	44.9*	38.27*	3.88	10.13*	15.26
GS-I	8.77	24.8*	51.99*	8.74*	1.07	29.78*
GS-II	8.33	25.0*	26.34*	2.09	1.16	19.87*
ConA	9.62	16.2*	29.41*	3.74	3.89	22.29*
BPA	—	56.6*	17.31*	2.41	2.56	12.59

Asterisk (*) shows more than one atom metal per lectin molecule.

Table 4. The Metals in the Commercial Lectins Estimated by ICP

Lectins (nmoles)		Si	Ca	Zn (micro grams)	Mn	Mg
WGA	27.78	1.747	0.150	0.052	0.0026	0.0328
SBA	8.33	0.771	2.162	0.245	0.979	0.392
PNA	9.09	3.124	2.326	0.264	0.400	1.083
MPA	—	15.737	0.928	0.192	0.012	0.361
DBN	9.62	2.349	3.540	0.701	1.124	0.749
UEA-I	17.86	1.262	1.534	0.254	0.557	0.371
GS-I	8.77	0.698	2.084	0.572	0.059	0.724
GS-II	8.33	0.703	1.056	0.137	0.064	0.483
ConA	9.62	0.455	1.179	0.245	0.214	0.542
BPA	—	1.590	0.694	0.158	0.141	0.306

Abbreviations are summarized in Table 1. All of the sample-lectin solutions are 1 mg/ml.

significant figure at mg order. This might result in indefinite weights or moles for the lectins analyzed. Secondary, samples were directly analyzed by ICP without any pre-treatment. This might bring us unusual metal species originated from artifacts when the samples were contaminated in ICP level even if their purity is guaranteed. Finally, the lectin purified by affinity chromatography often shows inhomogenous behavior as shown in following paragraph.

Concanavalin A (Con A), of which structure is most extensively studied, was used as an example for evaluating the found values in the present study. It consists four subunits each of which contains one calcium and one manganese if they are α -subunits¹⁸⁾. The present study, however, showed that one Con A molecule contained 1.7 gram atoms of silicon, 0.4 gram atoms of zinc and 2.3 gram atoms of magnesium in addition to 3.1 gram atoms of calcium and 0.4 gram atoms of manganese (Table 3). The inconsistent amounts of the metal species are probably ascribed to the fact that the commercial lectin also contains β -subunit in addition to α -subunit. β -Subunit usually consists 20 to 60% of the lectin preparation¹⁹⁾, being considered as a α -subunit fragment^{19,20)}. Unfortunately, it is not characterized so much as α -subunit, being unknown how many metal species present in a β -subunit. The small calcium content in the protein comparing to the expected one, may be also ascribed to the indefinite weight described above. The other metal in Con A, i.e. manganese was too small in the present study even if it was ascribed to the causes described above. The ion in this preparation was apparently substituted to other divalent ions, e.g. magnesium ion.

Hence, the metal species detected in the commercial lectins, should be semi-quantitatively evaluated. However, significant amounts of calcium, in addition to

silicon, were evidently detected in the nine dicotyledonous lectins but not in monocotyledonous one, i.e. WGA (Table 4). The low calcium in monocotyledon and high in dicotyledon are in accord with natural abundance of both species in the plant bodies²³⁾. Calcium ions were known as a regulating factor or second messenger in plants, serving metabolic control such as activation of enzymes via calmodulin²¹⁾. Lectin degradation may occur in spring because it is regarded as a kind of the storage proteins. Thus, the protein roles as one of the calcium source when the plant initiates growth or cell proliferation. On the other hand, the ion may be stored into the lectin molecule before the plant takes dormant state. Therefore, the lectin is possible to control calcium levels in the plant cells when it is produced or degraded. The protein may also adsorb or release calcium ion in the cells, thus buffering the ion levels. These possibility has been discussed in tree trunks where the lectin level was very high¹²⁾.

Another abundant species detected in the commercial lectins were magnesium and silicon. The former atom is not clear if it is present in the proteins as a substitute for divalent ions. The latter atom is accumulated in Poaceae or Cyperaceae in the stem or leaves, whereas it is a rather low content in legumes²²⁾. The commercial lectins show, however, rather high silicon contents even if it is a legume. Because no silicon was demonstrated as a constituent of any protein so far we know, it is important if silicon atom is present in the protein. In general, the ratio of calcium to silicon in the plant body is less than 0.5 by weight in monocotyledon, while it is more than 2 in dicotyledon²³⁾. This idea is able to adopt directly to the following four lectins, i.e. WGA, SBA, GS-I, Con A and to adopt with small variation to the others except the case of MPA (Table 3). This implicates that silicon present in the protein is quite natural. The author also detected silicon in the seed and sap lectins which were carefully purified and dialyzed, suggesting the presence of silicon in the proteins. The atom might be accumulated on the protein as a silicate bonded to the sugar and/or amino acids. However, the presence of silicon in these commercial lectins is not conclusive because glass vials container for the protein may contaminate them. Silicon might be also concentrated in the lyophilized preparations if the solvent (water) contained small amounts of silicate. Further studies are needed if silicon is presented in the proteins or how it present.

Some of the lectins surveyed here also contain zinc, which is present in enzymes such as alcohol dehydrogenase and carbonic anhydrase. In order to find physiological functions, it is interesting to know whether the lectin preparation accompanies their activities. Qualitative survey for various metal species suggests that some of the lectins seem to be phosphorylated (data not presented). This find-

ing reminds us the participation of protein kinases which are regulated by calcium dependent process¹²⁾. Another interesting species were also detected in these preparation, but no conclusive discussion was possible at this point. At any rate, the metal species detected in the previous paper²¹⁾ were also found in these commercial lectins. ICP analyses of lectins provide us useful and new probes for studying the function and structure of lectin molecules.

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