| | |
|---|---|
| Title | <Original>Metal Species in Some Lectins Detected by Inductively Coupled Plasma Spectrometer |
| Author(s) | KURODA, Hiroyuki |
| Citation | Wood research : bulletin of the Wood Research Institute Kyoto University (1986), 73: 1-7 |
| Issue Date | 1986-12-28 |
| URL | http://hdl.handle.net/2433/53301 |
| Right | |
| Type | Departmental Bulletin Paper |
| Textversion | publisher |

Original

# Metal Species in Some Lectins Detected by Inductively Coupled Plasma Spectrometer

Hiroyuki KURODA*

(Received Sepetember 1, 1986)

**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 pseudoacasia*[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 cells[10] and showed seasonal fluctuation[9], and thus the lectin is regarded as a kind of storage protein. Most of them are metalloproteins[15], 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 trunks[12]. 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

* Research Section of Wood Biology.

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 analyses[12]. 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: *Triticum vulgaris*) | 36,000 | GlcNAc, sialic acid |
| SBA (soybean seed: *Glycine max*) | 120,000 | $A_1 > A_2 > O > B$: D-GalNAc>α-D-Gal |
| PNA (peanuts: *Arachis hypogaea*) | 110,000 | D-Gal-β-(1→3)GalNAc, Me-α-Gal |
| MPA (osage orange: *Maclura pomifera*) | — | α-D-Gal |
| DBA (seed: *Dolichos biflorus*) | 104,000 | $A_1 > A_2$; α-GalNAc |
| UEA-I (gorse seed: *Ulex europaeus*) | 56,000 | O; α-L-Fuc |
| GS-I ( : *Griffonia simplicifolia*) | 114,000 | B; melibiose, α-D-Gal |
| GS-II (seed: *Bandeiraea simplicifolia*) | 120,000 | terminal GlcNAc |
| ConA (jack bean: *Canvalia ensiformis*) | 104,000 | α-D-Man, α-D-Glc |
| BPA ( : *Bauhinia purpurea*) | — | Gal, GalNAc |

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

A DAIICHI 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

| $H_2O$ (Control) | | | | | |
|---|---|---|---|---|---|
| Si | Ca | Zn | Mn | Mg | run |
| 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 |
| Standard Solution (1 ppm) | | | | | |
| Si | Ca | Zn | Mn | Mg | run |
| 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 | | Si | Ca | Zn | Mn | Mg |
|---|---|---|---|---|---|---|
| (nmoles) | | (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 | Mn | Mg |
|---|---|---|---|---|---|---|
| | | | | (micro grams) | | |
| 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 inhomogenious 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 $\alpha$-subunits[18]. 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 $\beta$-subunit in addition to $\alpha$-subunit. $\beta$-Subunit usually consists 20 to 60% of the lectin preparation[19], being considered as a $\alpha$-subunit fragment[19,20]. Unfortunately, it is not characterized so much as $\alpha$-subunit, being unknown how many metal species present in a $\beta$-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 bodies[23]. Calcium ions were known as a regulating factor or second messenger in plants, serving metabolic control such as activation of enzymes via calmodulin[21]. 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 high[12].

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 legumes[22]. 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 dicotyledon[23]. 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 lyophylized 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 process[12]. 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 paper[21] 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.

## Acknowledgment

The author would like to thank Dr. Toru Kuboi of National Institute for Environmental Studies (Tsukuba) for his kind suggestion on this study. The author appreciates Professor Kazuo Sumiya for providing a good experimental circumstance. This work was supported by a grant from the Ministry of Education, Japan.

## References

1) I.J. Goldstein, R.C. Hughes, M. Monsigny, T. Osawa and N. Sharon: Nature, **285**, 66 (1980).
2) Newsletter 1981 of the Nomenclature Committee of IUB (NCIBU) and IBU-IUPAC Joint Commission on Biochemical Nomenclature (JCBN): Eur. J. Biochem., **114**, 1–4.; Arch. Biochem. Biophys., **206**, 458–462; Hoppe-Seyler's Z. Physiol. Chem., **362**, 1–4; J. Biol. Chem., **256**, 12–14.
3) C. Gietl, H. Kauss and H. Ziegler: Planta, **144**, 367–371 (1979).
4) C. Gietl and H. Ziegler: Biochem. Physiol. Pflanzen, **175**, 50–57 (1980).
5) V. Horejsi, C. Haskovec and J. Kocourek: Biochem. Biophys. Acta, **532**, 98–104 (1978).
6) M.D. Lutsik and V.A. Antonyuk: Biochemistry (USSR), **47**, 1448–1453 (1982).
7) W.F. Broelaert, M. Nsimba-lubaki, B. Peeters and W.J. Peumans: Biochem. J., **221**, 163–169 (1984).
8) M. Nsimba-lubaki, W.J. Peumans and A.K. Allen: Planta **168**, 113–118 (1986).
9) M. Nsimba-lubaki and W.J. Peumans: Plant Physiol., **80**, 747–751 (1986).
10) J.S. Greenwood, H.M. Stinissen, W.J. Peumans and M.J. Chrispeels: Planta, **167**, 275–278 (1986).
11) K. Baba and H. Kuroda: under submitting.
12) H. Kuroda and K. Baba: under submitting.
13) A.J. Kalb and A. Levitzki: Biochem. J., **109**, 669–672 (1968).
14) S.H. Koenig, C.F. Brewer, R.D. Brown, III: Biochemistry, **17**, 4251–4260 (1978).
15) I.W. Goldstein: in "The Phytochemistry of Cell Recognition and Cell Surface Interaction (F.A. Loewus and C.A. Ryan eds.) Recent Advances in Phytochemistry Vol. 15", pp.25–35, Plenum Press, New York and London (1981).
16) H. Lis and N. Sharon: Ann. Rev. Biochem., **42**, 541–574 (1973).
17) M.E. Etzler: Ann. Rev. Plant Physiol., **36**, 209–234 (1985).
18) J.W. Becker, G.N. Reeke, Jr., J.L. Wang, B.A. Cunningham and C.M. Edelman: J. Biol. Chem., **250**, 1513–1524 (1975)
19) Y. Abe, M. Iwabuchi, S. Ishii: Biochem. Biophys. Res. Commun., **45**, 1271–1278 (1971).
20) J.L. Wang, B.A. Cunningham, G.M. Edelman: Proc. Natl. Acad. Sci. U.S.A., **68**, 1130–1134 (1971).
21) P.K. Hepler and R.O. Wayne: Ann. Rev. Plant Physiol., **36**, 397–439 (1985).

22) D. WERNER and R. ROTH: in "Inorganic Plant Nutrition (A. LAUCHLI and R.L. BIELESKI eds.) Encyclopedia of Plant Physiol. new ser. Vol. **15B**", 682–694, Springer-Verlag, Berlin, Heiderberg, New York, Tokyo (1983).
23) E. TAKAHASHI: Textbook "Comparative Studies on Plant Nutrition" (in Japanese), p. 106, Yohkendoh Co. Ltd., Tokyo (1974).