Vol. 214, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 5, 1995 Pages 163-170

THE COMPLETE AMINO ACID SEQUENCE OF A MANNOSE-BINDING LECTIN FROM " KIDACHI ALOE" (*ALOE ARBORESCENS MILLER VAR. NATALENSIS BERGER*)

Takaaki Koike[1], Koiti Titani[2]*, Masami Suzuki[2], Hidehiko Beppu[1], Hiroshi Kuzuya[3], Kazuhiro Maruta[4], Kan Shimpo[1], and Keisuke Fujita[1]

[1]Institute of Pharmacognosy, Fujita Health University, Hisai, Mie 514-12, Japan

[2]Division of Biomedical Polymer Science, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi 470-11, Japan

[3]Division of Molecular Biology, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi 470-11, Japan

[4]Department of Biochemistry, School of Health Sciences, Fujita Health University, Toyoake, Aichi 470-11, Japan

Received July 29, 1995

Summary : The complete amino acid sequence of a mannose-binding lectin purified from the leaf skin of "Kidachi Aloe" (*Aloe arborescens Miller var. natalensis Berger*) is presented. The 109-residue sequence of the subunit was determined by analysis of peptides of the intact or S-pyridylethylated protein generated by digestion with cyanogen bromide, BNPS-skatole, *Achromobacter* protease I, or trypsin. The subunit contains an intrachain disulfide bridge. The sequence is highly homologous to that of a mannose-binding lectin from snowdrop bulb.

Numerous species of Aloe have been used as a folk medicine for treatment of skin injuries and burns for a long time in Japan [1, 2, 3]. *Aloe arborescens Miller var.natalensis Berger* is called "Kidachi Aloe" in Japanese. Fujita et al. [4] reported that this species of Aloe contains an enzyme hydrolyzing bradykinin, which might explain its anti-inflammatory effects. Thereafter Fujita et al. [5] showed that the enzyme is a carboxypeptidase, capable of

*To whom correspondence should be addressed.

0006-291X/95 $12.00

digesting bradykinin at the C-terminus. We recently isolated a novel lectin with a molecular mass of about 35 kDa, which was estimated by size exclusion chromatography and native-PAGE, composed of three or four identical subunits from the leaf skin of "Kidachi Aloe" (Koike et al., unpublished results). The lectin showed a hemagglutinating activity toward rabbit, but not human and sheep erythrocytes and specifically bound to mannose as well as snowdrop lectin did. The lectin also showed a strong mitogenic activity toward mouse lymphocytes. We report here the complete amino acid sequence, location of an intrachain disulfide bond and *in vivo* proteolysis site of the subunit with the molecular mass of 12,200 Da of this 35 kDa mannose-binding lectin from "Kidachi Aloe".

Materials and Methods

Materials: Fresh "Kidachi Aloe" leaves were harvested at a herb garden of the Institute of Pharmacognosy, Fujita Health University in Hisai, Mie, Japan.

Purification of Aloe Lectin: Fresh leaves of "Kidachi Aloe" were separated into the leaf skin and the leaf pulp (gel portion) with a knife. The leaf skin was used to prepare the 35 kDa-Aloe lectin by a series of procedures including homogenization, precipitation by cold acetone, and chromatography on Sephadex G-25 (Pharmacia), DEAE 52 (Whatman) and Superdex 75 HR 10/30 (Pharmacia) columns (Koike et al., unpublished results).

Reduction and S-Pyridylethylation : The Aloe lectin was reduced and S-pyridylethylated (PE) as described [6]. The S-pyridylethylated (PE) protein was desalted and further separated into two fractions, I and II, by reversed-phase HPLC on a SynChropak RP-8 column (4.6 X 250 mm) (SynChrom) with a linear gradient of acetonitrile into dilute aqueous trifluoroacetic acid (Fig. 1).

Enzymatic Digestion and Chemical Cleavage: The fraction I of the PE-protein was cleaved with cyanogen bromide in 70% formic acid at room temperature overnight as described by Gross [7]. Each fraction of the PE-protein was digested with *Achromobacter* protease I in 50 mM Tris-HCl, pH 9.0, in the presence of 2 M urea at 37℃ overnight; the protein was dissolved in a small volume of 8 M urea and then diluted with 3 volumes of Tris buffer, pH 9.0 [8]. Cleavage with BNPS-skatole followed the procedure of Omenn et al. [9]. The intact protein was cleaved with cyanogen bromide as described above and then with trypsin in 0.1 M ammonium bicarbonate at 37℃ overnight [10]. Peptides were separated by reversed phase HPLC on a SynChropak RP-8 column or an ODS-M column (4.0 × 150 mm) (Shimadzu Techno-Research) with a gradient of acetonitrile into dilute aqueous trifluroacetic acid.

Amino Acid Analysis and Sequence Determination: Peptides were hydrolyzed in 6 N HCl containing 1% phenol at 110℃ for 24 h by the vapor-

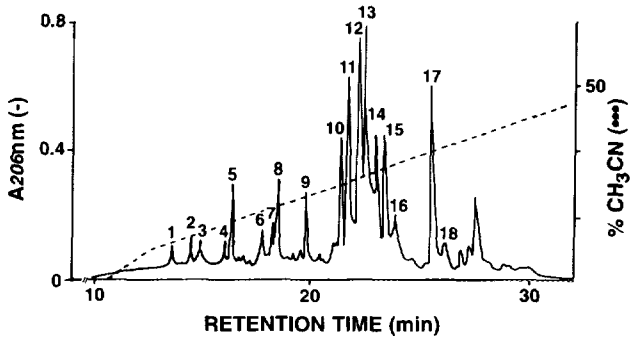

Figure 1. Separation of peptides generated by digestion of the Aloe lectin with cyanogen bromide followed by trypsin. Peptides were separated on a SynChropak RP-8 column with a TFA-acetonitrile system. Purified peptides are identified by prefix MT in Figure 2.

phase method. Amino acid analysis was carried out with a Hitachi model L8500 amino acid analyzer. Sequence determination was carried out with an Applied Biosystems 470A protein sequencer connected to a 120A PTH analyzer.

Mass Spectrometry: Ion-spray mass spectral analysis was performed with a PE-Sciex API-III biomolecular mass analyzer.

Sequence Homology Search: Sequence homology was searched in the protein sequence database (SWISS-PROT) using DNASIS (Hitachi Software Engineering).

Results

Reduction and S-Pyridylethylation of the 35-kDa Aloe Lectin: After reduction and S-pyridylethylation, the product (PE-protein) was separated into two fractions, I and II, by reversed-phase HPLC as described in "Materials and Methods" (data not shown). Fractions I and II were identified to be a mixture of the PE-intact subunit and PE-heavy chain, and PE-light chain, respectively, by SDS-PAGE.

Amino Acid Sequence of the 35-kDa Aloe Lectin: Sequence analysis (Fig. 2) of Fraction I (ca. 400 pmol) and II (ca. 400 pmol) yielded the N-terminal sequence of 39 and 24 residues, respectively, as shown in Fig. 2. The cleavage of the intact lectin (ca. 3.5 nmol) with cyanogen bromide followed by trypsin yielded 18 peptides (MT 1-MT 18) by reversed- phase HPLC as shown in Fig. 1. Sequences of these peptides are shown in Fig. 2.

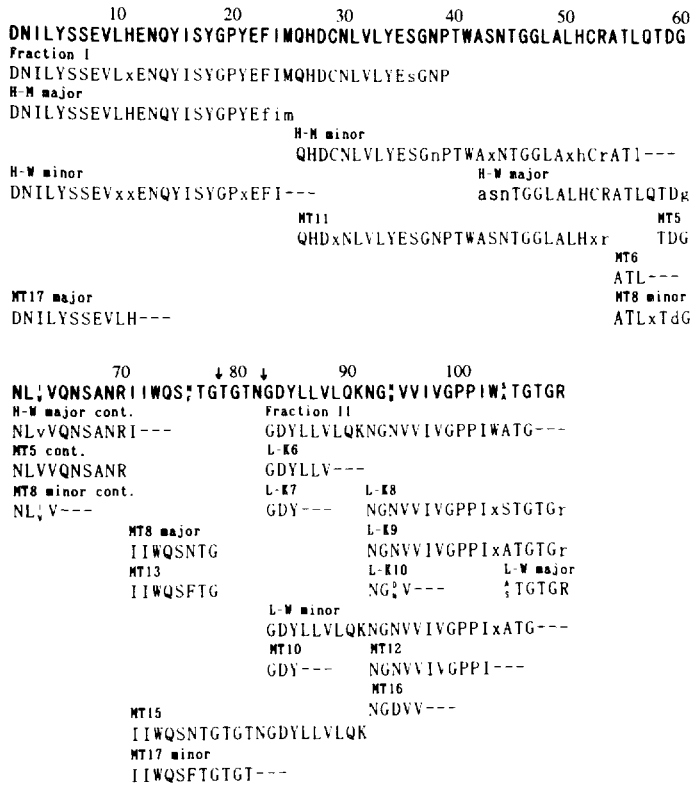

Figure 2. Summary of the sequence proof of the Aloe lectin. The proven sequencesof specific peptides are given in one-letter code below the summary sequence (bold type). Prefixes H-M and H-W denote peptides generated by cleavage of the PE-heavy chain with cyanogen bromide and BNPS-skatole, respectively. Prefixes L-W and L-K denote peptides generated by cleavage of the PE-light chain with BNPS-skatole and *Achromobacter* protease I, respectively. Prefix MT denotes peptides generated by cleavage of the intact protein with cyanogen bromide followed by trypsin. Sequences written in upper case letters were proven by Edman degradation; those in lower case letters indicate tentative identifications. Unidentified residues are shown by x. Dashes indicate that the peptide was no longer analyzed.

Analysis of Fraction I (ca. 125 pmol) after cyanogen bromide cleavage, but without separation by HPLC, yielded two sequences, H-M major and H-M minor. We assumed that the major sequence is derived from residues 1-25, already determined by analysis of intact Fraction I, and the minor sequence corresponds to that from Gln-26. Similarly, analysis of Fraction I (ca. 300 pmol) after BNPS-skatole cleavage (without separation) yielded two sequences, H-W minor and H-W major. Digestion of Fraction II (PE-light

chain) (ca. 270 pmol) with *Achromobacter* protease I yielded five peptides (L-K 6-L-K 10) by reversed phase HPLC (data not shown). BNPS-skatole cleavage of Fraction II (ca. 300 pmol) (without separation) yielded two sequences (L-W minor and L-W major). These results taken together provided the complete amino acid sequence of 109 residues of the Aloe lectin as summarized in Fig. 2.

Sequence Homology: Internal homology of the 35 kDa-Aloe lectin was observed. A portion of amino acid sequence of Met 25-Leu 48 shows a significant homology to those of Leu 56-Gly 80 and Leu 89-Gly 108 (Fig. 3). Such an internal homology has been also reported for the snowdrop lectin (*Galanthus nivalis L.*) [11]. The amino acid sequence of the Aloe lectin is highly homologous to the snowdrop lectin (Fig. 4); identity between the two lectins was calculated to be about 60%.

Discussion

We purified a novel 35 kDa mannose-binding lectin with strong hemagglutinating and mitogenic activities from the leaf skin of "Kidachi Aloe" and determined the complete amino acid sequence of the subunit of 109 residues as shown in Fig. 2. It is likely that the lectin essentially consists of identical subunits. At moment, however, it is unclear whether it consists three or four subunits, because the molecular mass of the intact lectin was estimated to be roughly 35 kDa by native PAGE and size exclusion chromatography, and that of the subunit was estimated to be about 9 kDa by SDS-PAGE, but calculated to be 12,200 Da from the sequence obtained in this

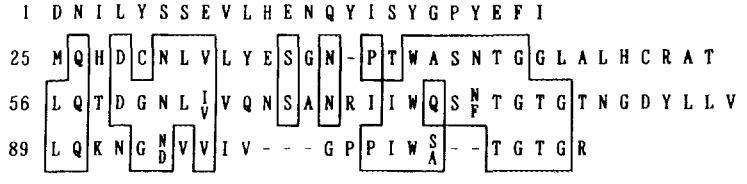

Figure 3. Internal homology of the Aloe lectin. Residues 1-24, 25-55, 56-88, and 89-109 of the Aloe lectin sequence are aligned to give the maximum homology.

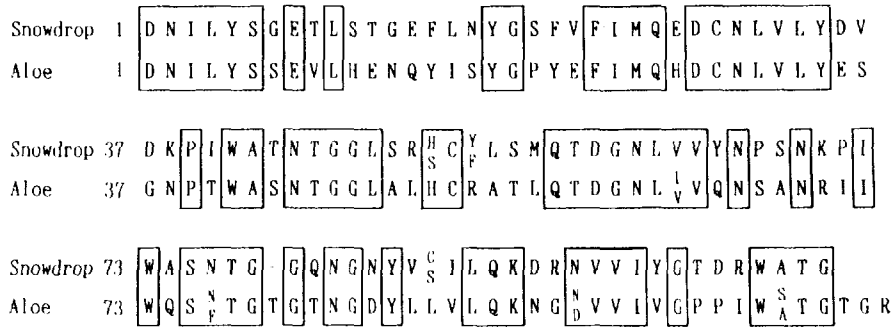

Figure 4. Amino acid sequence homology between the Aloe lectin and the snowdrop lectin. Residue numbers indicate positions of amino acid residues from the N-terminus of the mature proteins. Residues identical to those of the Aloe lectin are boxed.

study. Lectins from monocotyledous species have been reported in the families Liliaceae [12, 13], Amaryllidaceae [14], Alliaceae [15] of the class Liliatae. Recently Van Damme et al. isolated a leek (*A. porrum*) lectin and reported that this lectin is a trimer rather than tetramer of polypeptides with a molecular mass 12.5-13 kDa [15].

The subunit appears to be partially cleaved *in vivo* at residues 78 and 82 by unknown endopeptidase (and exopeptidase), producing in some extent the heavy (residues 1-78) and light (residues 82-109) chains. The subunit sequence shows some microheterogeneities at least at residues 63, 76, 94 and 104, as often observed in plant proteins. It also shows an internal homology. The sequence of Gln 26-Gly 46 is highly homologous to those of Leu 56-Gly 80 and Leu 89-Gly 108, suggesting that the lectin gene has evolved from an ancestor gene by gene duplication.

The subunit contains two half-cystine (Cys/2) residues at residues 30 and 52, which are S-pyridylethylated only in the presence of tri-n-butylphosphine (Koike et al., unpublished results) and therefore participates in disulfide bond(s). A molecular mass of peptide MT 11, which contained both Cys/2 residues, was estimated to be 1018 by Ion-spray mass spectral analysis, indicating the two Cys/2 residues are forming an intrachain disulfide bond. The sequence shows a high homology with that of the snowdrop lectin (Fig. 4).

Since both "Kidachi Aloe" (*Aloe arborescens Miller var. natalensis Berger*), a member of the family Liliaceae and snowdrop (*Galanthus nivalis L.*), a member of the family Amaryllidaceae, belong to the class Liliatae, a high homology between the two lectins is naturally understandable. They are similar to each other in their properties such as binding specificity for mannose and hemagglutinating activity toward rabbit but not human and sheep erythrocytes (Koike et al., unpublished results) [16]. Since the snowdrop lectin is synthesized as a secretory protein with signal peptide [11], the Aloe lectin should also be a secretory protein.

Acknowledgments

We would like to dedicate this paper to the late Dr Keisuke Fujita (the Founding President and Professor of the Fujita Health University) who died on June 11, 1995. This work was supported by Grants-in-Aid for Scientific Research from the Fujita Health University.

References

1. Fujita, K. (Ed.) (1993) Aloe arborescens Miller var. natalensis Berger. The Pharmacological and Therapeutic Effects. I, pp. 1-139 Fujita Health University Press, Toyoake Japan.
2. Reynolds, T. (1985) Botanical J. Linnean Society **90,** 157-177.
3. Grindlay, D., and Reynolds, T. (1986) J. Ethnopharmacol. **16,** 117- 151.
4. Fujita, K., Teradaira, R., and Nagatsu ,T. (1976) Biochem. Pharmacol. **25,** 205-206.
5. Fujita, K., Ito, S., Teradaira, R., and Beppu, H. (1979) Biochem. Pharmacol. **28,** 1261-1262.
6. Usami, Y., Fujimura, Y., Suzuki, M., Ozeki, Y., Nishio, K., Fukui, H., and Titani, K. (1993) Proc. Natl. Acad. Sci. USA **90,** 928-932.
7. Gross, E. (1967) Methods Enzymol. **11,** 238- 255.
8. Masaki, T., Tanabe, M., Nakamura, K., and Soejima, M. (1981) Biochim. Biophys. Acta **660,** 44-50.
9. Omenn, G.S., Fontana, A., and Anfinsen, C. B. (1970) J. Biol. Chem. **245,** 1895-1902.
10. William, E. B, and Wold, F. (1973) Biochemistry **12,** 828-834.
11. Van Damme, E. J .M., Kaku, H., Perini, F., Goldstein, I. J., Peeters, B., Yagi, F., Decock, B., and Peumans, W. J. (1991) Eur. J. Biochem. **202,** 23-30.
12. Oda, Y., and Minami, K. (1986) Eur. J. Biochem. **159,** 239-245.
13. Peumans, W. J., Allen, A. K., and Cammue B. P. A. (1986) Plant Physiol. **82,** 1036-1039.

14. Van Damme, E. J. M., Allen, A. K., and Peumans, W. J. (1988) Physiol. Plant. **73,** 52-57.
15. Van Damme, E. J. M., Smeets, K., Engelborghs, I., Aelbers, H., Balzarini, J., Pusztai, A., Van Leuven, F., Goldstein I. J., and Peumans, W. J. (1993) Plant Mol. Biol. **23,** 365-376.
16. Van Damme, E. J. M., Allen, A. K., and Peumans, W. J. (1987) FEBS Lett. **215,** 140-144.