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Plant Physiology

Determination of Primary Structure of the Mannose-Binding Lectin DB1 from *Dioscorea batatas* Tubers

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ABSTRACT. The amino acid sequence of a mannose-binding lectin DB1 from the yam (*Dioscorea batatas*) tubers was determined. Lectin was combined of two isoforms (Cys86) and (Leu86) with 90% sequence homology between two isolectins. DB1 (Cys86) had two intrachain disulfide bonds located at (Cys29-Cys52) and (Cys54-Cys86), whereas DB1 (Leu86) had one intrachain disulfide bond located at (Cys29-Cys52). DB1 showed a high sequence similarity to snowdrop (*Galanthus nivalis*) bulb lectin with the well documented anti-nutritive effects toward the economically important pests. The results suggest that DB1 may play defensive role in the yam tubers. *© 2008 Bull. Georg. Natl. Acad. Sci.*

***Key words**: Dioscorea batatas, Galanthus nivalis agglutinin, mannose-binding lectin, plant defense.*

Lectins are among wide range of natural defense proteins found in plants [1]. The possible function of serving as a chemical defense against large array of insect pests is well documented [2]. Insecticidal activities were found to be associated mostly with legume lectins [3] and cereals [4]. Recently vast amount of reports were dedicated to insecticidal properties of monocot mannose-binding lectin GNA (*Galanthus nivalis* agglutinin). GNA has been shown to be insecticidal to a range of economically important pests [5]. GNA have been successfully used in the search for alternatives to chemical pesticides in pest control via genetic engineering demonstrating the broad insecticidal activity of this lectin [6]. In this paper we describe the primary structure of new monocot mannose-binding lectin DB1 from *Dioscorea batatas* tubers and demonstrated structural homology to GNA.

DB1 was purified from yam tubers as previously described [7]. DB1 was reduced with 10 mM dithiothreitol in 0.25 M Tris-HCl [pH 8.6] containing 10 mM EDTA and 6 M guanidine hydrochloride at 37°C for 2 h, and reacted with 20 mM iodoacetamide for 20 min at room temperature in the dark. Reduced and carboxamidomethylated [CAM] DB1 was digested with endoproteinase Lys-C [S/E=100:1], endoproteinase Arg-C [S/E=100:1], according to the manufacturers' recommendations, or cyanogen bromide [CNBr] cleavage in 70% formic acid. Each digest was separated by reversed-phase HPLC on a TSKgel ODS 120T column [4.6 x 250 mm] using a linear gradient increase of acetonitrile in 0.1% TFA. The amino acid sequences of isolated peptide fragments were determined by the combined use of a protein sequencer, MALDI-TOF mass spectrometer, and an amino acid analyzer as described [8]. Homologous sequences were searched by the FASTA program accessed by Genome Net WWW.

Oligonucleotide primers [DB1F/DB1R] specific to DB1 were designed based on the amino acid sequence of DB1. cDNA fragments were amplified by means of RT-PCR as follows [F and R indicate sense and antisense primers, respectively]:

DB1F, 5'-TAYGAYAAYGGNAARGCNATHTGGGC-3'; DB1R, 5'-GCNGCNCCRTA -DATNACNACRTT-3'.

Total RNA was extracted using Concert Plant RNA Reagent [Invitrogen, Tokyo, Japan] according to the manufacturer's instructions. Poly $[A]^+$ RNA was purified with a Micro-FastTrack mRNA Isolation Kit [Invitrogen], and reverse transcribed with oligo dT primer using Access Quick RT-PCR System [Promega, Madison, WI, USA]. Amplified DNA fragment [0.6 kbp] generated by PCR with DB1F/DB1R specific primers was subcloned into the pCR-Blunt II TOPO vector [Invitrogen]. DNA was sequenced on an ABI DNA sequencer by cycle sequencing using T7, SP6 and M13 forward [-20] primers and the DYEnamic ET terminator cycle sequencing kit [Amersham Pharmacia Biotech].

The total amino acid sequences of DB1 were determined by both Edman degradation and cDNA sequencing as summarized in Fig. 1. DB1 [Cys86, Leu86] were composed of 108 amino acid residues with a molecular mass calculated to be 11,807 Da and 11,779 Da, which are in good agreement with the values [11,813 Da and 11,785 Da] obtained from MALDI-TOF mass spectrometry, respectively [7]. Two isolectins had 90% sequence homology and were 11 amino acid residues difference from each other. DB1s include four [Cys86] or three [Leu86] half-cysteine residues, respectively. This indicates that extra cysteine residue contribute to disulfide bonds.

The nucleotide sequences were analyzed using a method of rapid amplification of cDNA ends [RACE]. The cDNA of DB1 [Cys86] included 761 nucleotides with an open reading frame of 498 nucleotides encoding for a protein of 147 amino acid residues and a signal sequence of 19 residues. It should be noted that the C-terminal amino acid sequence, Val-Gly-Val-Ser-Gly-Gly-Mey-Phe-Ile-Glu-Ser-Lys-Ala-Thr-Ile-Phe-Gly-Ser-Leu-Phe-Ala-Asn-Glu-Thr-Thr-Ala-Glu-Ala-Lys-Ala-Ala-Arg-Ile-Ser-Met-Val-Val-Asn-Lys which was deduced from the cDNA sequence, could not be detected in any digest prepared with various proteases. A second processing step is probably involved resulting in the removal of a C-terminal extension of 39 amino acid residues [3984 Da] during this post-translational processing of the protein. Furthermore, the hydrophobic character of this C-terminal peptide is consistent with the possibility that it is removed post-translationally.

We reported the presence of mannose-binding lectin DB1 in yam tubers [*Dioscorea batatas*] accounting for 20% of the total tuber protein [7]. Due to exclusive specificity toward mannose and especially toward α[1-3]

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  1    ATGCTCCCGGCCGCCATGGCGGCCGCGGGAATTCGATCTCCTCTTTGCTGCATGGCC  58
-19     M  L  P  A  A  M  A  A  A  G  I  R  S  P  L  C  C  M  A   -1

 58  GATTTCATACTTTACTCTGGGGAATCCCTCAGGTCCGGCCAAGCCTTGTACCGTGGGAGC  118
  1   D  F  I  L  Y  S  G  E  S  L  R  S  G  Q  A  L  Y  R  G  S   20
      ▼1

118  TACACTTTCATTATGCAGAATGACTGCAACCTAGTTTTGTATGATAATGGCAAAGCAATA  178
 21   Y  T  F  I  M  Q  N  D  C  N  L  V  L  Y  D  N  G  K  A  I   40

178  TGGGCTTCCGGCACTAACGGCCGAGGCAGCGGCTGCTACTGCGCTATGCAGAGTGATGGT  238
 41   W  A  S  G  T  N  G  R  G  S  G  C  Y  C  A  M  Q  S  D  G   60

238  AACCTGGTCGTTTATACCAGTAACAACAATGCTGTGTGGGCAAGCAACACCAACGTGGGA  298
 61   N  L  V  V  Y  T  S  N  N  N  A  V  W  A  S  N  T  N  V  G   80

298  CAAGGCCACTACGTCTGCATCCTTCAGAAAGATCGCAACGTCGTCATCTATGGAGGTGCA  358
 81   Q  G  H  Y  V  C  I  L  Q  K  D  R  N  V  V  I  Y  G  G  A  100

358  CGCTGGGCAACCAACACCAACACTGTCGGCGTCTCTGGTGGTATGTTCATCGAAAGTAAG  418
101   R  W  A  T  N  T  N  T  V  G  V  S  G  G  M  F  I  E  S  K  120
                    108▼

418  GCCACCATCTTTGGTTCTTTGCCTGCTAACGAAACTACTGCAGAAGCCAAGGCTGCACGC  478
121   A  T  I  F  G  S  L  P  A  N  E  T  T  A  E  A  K  A  A  R  140

478  ATTTCCATGGTCGTCAACAAGTGATGCTGAAGCTTAGTGAACAATATAATAAGCGCATGC  538
141   I  S  M  V  V  N  K  *
                 147

538  ATCCATCGTGACATCTATGGTTCATGCATGCATGCGAGAGTTATAATAAGTTGCTTCGGC  598

598  CTTGTATTGCATATGTAGCCCGTGTGTGTGAAGTTTCTACTGTTGCTGTTGGTAACGAGA  658

658  AAATAACCTTATTCTTGTGCCAAACATCGATACATGTGGTGAATAAATGTGAATGCATCC  718

718  TATGCCTTTCAGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 761
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Fig. 1. Nucleotide and amino acid sequences of DB1. Nucleotides and amino acid residues are numbered on the side. The putative processing sites for the signal sequence and the C-terminal extension are indicated by arrowheads.

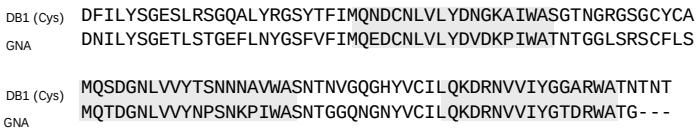

Fig. 2. Aligned amino acid sequences of DB1 and snowdrop lectin GNA. Carbohydrate recognition domain [CRD] are in the boxes.

linkage DB1 was classified into monocot mannose-binding lectin family. At the monosaccharide level, the lectins confined into this family bind mannose, but in contrast to Man-binding legume lectins such as ConA do not accommodate glucose into its carbohydrate-binding site. Snowdrop (*Galanthus nivalis*) bulb lectin GNA is the first lectin reported from this family having well defined insecticidal properties to a range of economically important pests [5, 9]. DB1 had 64% sequence homology to GNA, especially to its carbohydrate-binding site [Gln26, Asp28, Asn30, Val32, Tyr34, Asp37, Lys38] [10, 11]. Moreover, these amino acid residues were highly conserved in DB1 [Fig. 2]. Positioning of disulfide bridges is crucial for ligand contact for some lectins. DB1 contained four or three cysteine residues [Cys86 and Leu86, respectively] at positions 29, 52, 54 and 86. Consequently, either one or two disulfide bridges lending stability to the molecule hold polypeptide. Possibly, an extra cyctein residue [Cys54] forms interchain disulfide bond. GNA contains three cysteins at the positions 29, 52 and 86 per subunit and has one intrachain disulfide bond located at Cys29-Cys52. Cys86 is free cystein [12]. Apparently, homology between CB moieties and defined structural similarities determines the sugar target selection and might be an argument of functional resemblance between DB1 and GNA. GNA binding specificity is limited to mannose sugars in α-1,3- and α-1,6- linkages; it binds to comparatively few glycoproteins on the gut epithelium of insects and higher animals. DB1 has shown similar antinutritive effects towards *Helicoverpa armigera* and *Helicoverpa assulta* (Lepidoptera) larvae at different stages of development. The rate of adults successfully emerging from pupae fed on DB1 was 33% when incorporated into artificial diet at a level of 0.01% (w/w) [13].

Yam tubers of *D. batatas* are stored for a year after harvesting and are particularly vulnerable, since they are more attractive to potential parasites. In addition, as the resting storage organs yam tubers may lack an active defense system to resist various pests. DB1 existed in yam tubers at significant amounts (20% of total protein content), where it may function as storage protein. Preferential association of GNA-like protein with those parts of plant that are most susceptible to attack by pests and predators might be an additional argument for the proposed protective role of DB1.

მცენარეთა ფიზიოლოგია

Dioscorea batatas ტუბერის მანოზა-სპეციფიკური DB1 ლექტინის პირველადი სტრუქტურის დადგენა

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ნაშრომში დადგენილია *Dioscorea batatas* ტუბერის მანოზა-დამაკავშირებელი ცილა ლექტინის (DB1) ამინომჟავური თანამიმდევრობა. ლექტინი წარმოდგენილია ორი იზოფორმით: DB1(Cys86) და DB1(Leu86),

რომლებშიც ამინომჟავური თანამიმდევრობა თანმხვედრია. DB1(Cys86)-ში არის ორი შიდა დისულფიდური ბმა, განლაგებული Cys29-Cys52 და Cys54-Cys86 მდგომარეობაში. DB1(Leu86)-ში გამოვლენილია ერთი შიდა დისულფიდური ბმა Cys29-Cys52 პოზიციაში. DB1-ში ამინომჟავური თანამიმდევრობა თანმხვედრი აღმოჩნდა თეთრყვავილას (*Galanthus nivalis*) ბოლქვის ლექტინის პირველად სტრუქტურასთან. როგორც ცნობილია თეთრყვავილას ბოლქვის ლექტინი გამოირჩევა ტოქსიკური მოქმედებით მავნებლების მიმართ. აღნიშნულიდან გამომდინარე, გამოთქმულია ვარაუდი მცენარეში DB1 ლექტინის დამცველობითი როლის შესახებ.

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