Plant Molecular Biology **21**: 1175–1179, 1993.

Update section

Short communication

Kinetic induction of oat shoot pulvinus invertase mRNA by gravistimulation and partial cDNA cloning by the polymerase chain reaction

Liu-Lai Wu [1], Il Song [2], Nadarajah Karuppiah [2] and Peter B. Kaufman [1,*]

[1] *Cellular and Molecular Biology Group, Department of Biology, 4103B Natural Science Building, University of Michigan, Ann Arbor, MI 48109-1048, USA (* author for correspondence);* [2] *Department of Internal Medicine, Medical School, University of Michigan, Ann Arbor, MI 48109, USA*

Received 20 May 1992; accepted 17 December 1992

Key words: cDNA cloning, gravistimulation, invertase mRNA, oat shoot pulvinus, polymerase chain reaction

Abstract

An asymmetric (top vs. bottom halves of pulvini) induction of invertase mRNA by gravistimulation was analyzed in oat shoot pulvini. Total RNA and poly(A)$^+$ RNA, isolated from oat pulvini, and two oligonucleotide primers, corresponding to two conserved amino acid sequences (NDPNG and WECPD) found in invertase from other species, were used for the polymerase chain reaction (PCR). A partial length cDNA (550 bp) was obtained and characterized. A 62% nucleotide sequence homology and 58% deduced amino acid sequence homology, as compared to β-fructosidase of carrot cell wall, was found. Northern blot analysis showed that there was an obviously transient induction of invertase mRNA by gravistimulation in the oat pulvinus system. The mRNA was rapidly induced to a maximum level at 1 h after gravistimulation treatment and gradually decreased afterwards. The mRNA level in the bottom half of the oat pulvinus was significantly higher than that in the top half of the pulvinus tissue. The kinetic induction of invertase mRNA was consistent with the transient accumulation of invertase activity during the graviresponse of the pulvinus. This indicates that the expression of the invertase gene(s) could be regulated by gravistimulation at the transcriptional level. Southern blot analysis showed that there were two to three genomic DNA fragments which hybridized with the partial-length invertase cDNA.

The oat shoot pulvinus, which is the swollen leaf-sheath base, is the graviresponding organ which is responsible for the normal upward growth of oat (*Avena sativa*) shoots [1]. Invertase is responsible for the hydrolysis of sucrose to *D*-glucose and *D*-fructose. These hexoses provide substrate for starch synthesis in the gravisensors (chloroplasts) in the pulvini and for cell wall biosynthesis that occurs in elongating cells of the graviresponding pulvini [1, 4]. It has been demonstrated that the activity of invertase (EC 3.2.1.26) is differentially upregulated after gravistimulation of oat leaf-sheath pulvini [2], where a strong top versus bottom half asymmetry in enzyme activity develops in the graviresponding pulvini. However, information about oat invertase gene(s) ex-

pression at the molecular level is not yet available. In the present study, we demonstrated by northern blot analysis that the invertase mRNA level in oat shoot pulvini is kinetically and differentially upregulated by gravistimulation.

cDNA cloning and characterization of invertase cDNA

Two primers, [TGGAT(C/A/)AA(T)CGA(C)-TCCIAA(C)TGGICCIATG] and [ATCIGGA-CA(C)TTCCCACAT(C/G/T)ACC], were synthesized according to the fully conserved amino acid regions (WINDPNGPM and DPCEWMG) of previously identified β-fructosidase from carrot cell walls, levanase and sucrase from *B. subtilis* and invertase from yeast [5, 7]. cDNAs were obtained by PCR using total RNAs or poly(A)$^+$ RNA as templates. The cDNAs were amplified by PCR using the two primers [6]. The size of the PCR products was determined to be approximately 550 pb by agarose gel electrophoresis. The cDNA was cloned into M13mp18 between the *Bam* HI and *Hin*d III restriction sites. The cDNA was sequenced by means of the dideoxynucleotide sequencing method using Sequenase version 2.0 of United States Biochemical [3]. The cDNA showed a 62% nucleotide sequence homology and a 58% amino acid sequence homology (Fig. 1, uppercase letters) to that of carrot β-fructosidase (Fig. 1, lowercase letters) by a partial comparison. These data indicate that the 550 bp cDNA by PCR is derived from the mRNA encoding oat invertase.

Kinetic induction of invertase mRNA

Total RNA was isolated from frozen top and bottom halves of gravistimulated oat (*Avena sativa* cv. Victory) pulvini (0.5–1.0 g fresh weight for each sample). Poly(A)$^+$ RNA was purified from total RNA with an oligo(dT)-cellulose chromatography column [11]. Poly(A)$^+$ RNA (5 μg/well) was electrophoresed in a 1% formaldehyde-agarose gel and blotted onto a nylon membrane filter (Hybond-N, Amersham). The filter was hybridized with α-^{32}P-dCTP-labeled partial-length invertase cDNA as a probe and exposed to X-ray films with an intensifying screen at -70 °C. RNA was initially measured with a spectrophotometer at a wavelength of 260 nm. In order to check for equal loading of mRNA in each well, the same filters were stripped of the hybridized invertase cDNA probe and rehybridized to an α-^{32}P-labeled actin gene probe from *Arabidopsis thaliana*. Autoradiographs were scanned with densitometer (LKB 2222-010 UltroScan XL, Bromma).

Our previous studies have shown that there is a time-course change in invertase activity during the gravitropic response of oat shoot leaf-sheath pulvini [2]. Northern blot analysis here showed that a 1.90 kb invertase mRNA was detected from whole pulvini of oat plants (Fig. 2A). The level of this mRNA was very low at time zero, but is was significantly induced 1 h after initiation of gravistimulation. After that time, it decreased. In order to examine the induction pattern of the gravity-induced invertase mRNA in more detail, RNAs were separately isolated from top and bottom halves of oat shoot pulvini after different times of gravistimulation. As shown in Fig. 2B and 2C, there is a clear pattern of kinetic changes in invertase mRNA level during the graviresponse of the respective halves. The 1.90 kb mRNA was detected at a relative low level in the top halves of the pulvini (Fig. 2B), but it occurs in the bottom halves at a very high level (Fig. 2C). The amount of mRNA rapidly increased at 1 h after initiation of gravistimulation, after which time, it gradually decreased. Using a densitometer, the maximum level at 1 h represents a five-fold increase above that of the 0 time control, and a ten-fold higher level than that of top halves of pulvini. These data are typical results of three repeated experiments. Using an actin probe to monitor the amount of mRNA loaded in each well, we observed a weak band in each well (data not shown). It has been demonstrated that a similar asymmetric distribution in the activity of invertase occurs in upper versus lower halves of gravistimulated oat pulvini [2]. Northern blot analysis data here indicate that the kinetic and

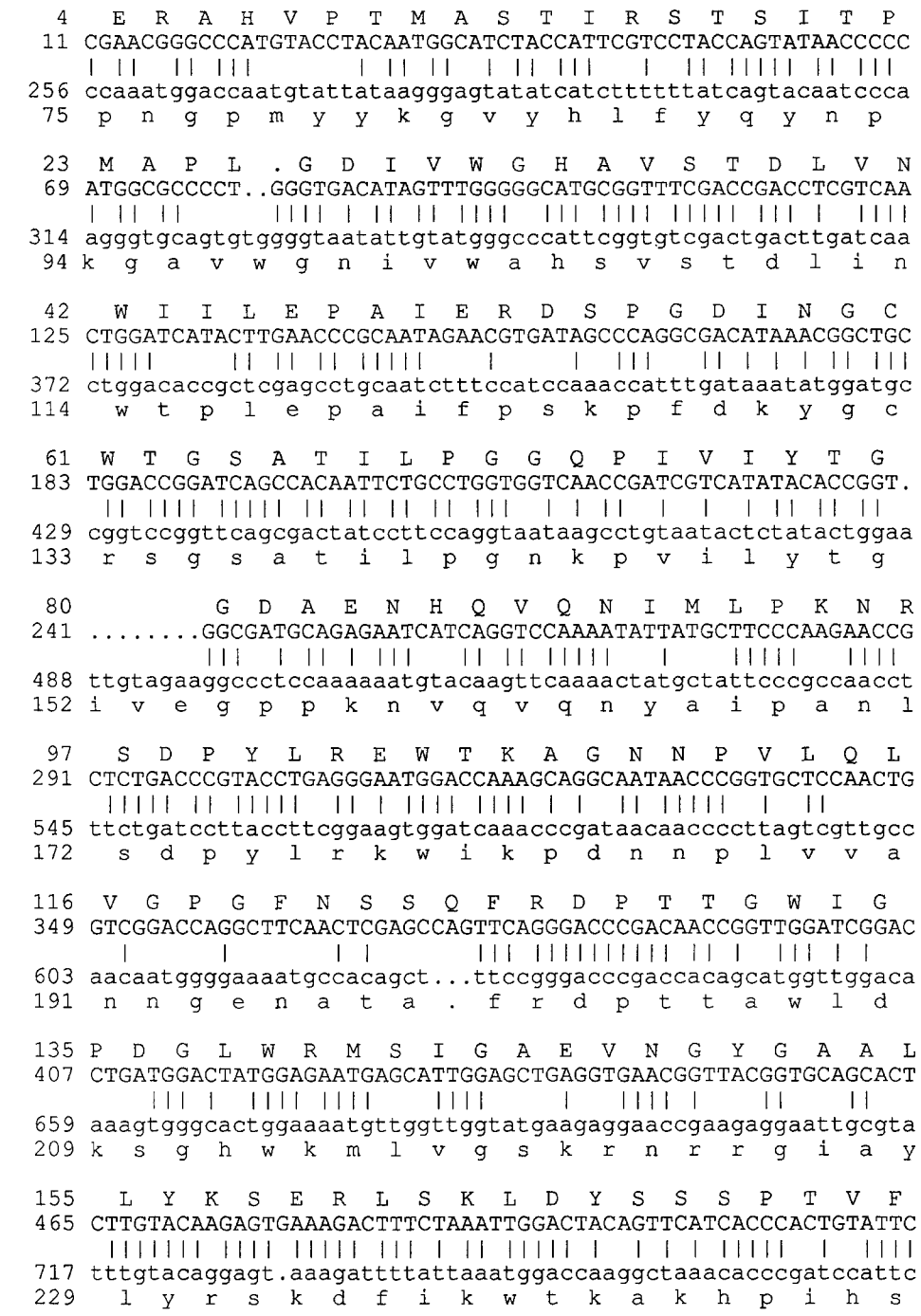

Fig. 1. Comparison of oat invertase partial-length cDNA nucleotide sequence and its deduced amino acid sequence (uppercase letters) with those of carrot β-fructosidase (lowercase letters).

asymmetric induction of invertase mRNA by gravistimulation was consistent with the time-course changes in invertase activities in the same tissue halves. A marked, transient accumulation of invertase transcripts was followed by a transient increase in the enzyme activity. This allows

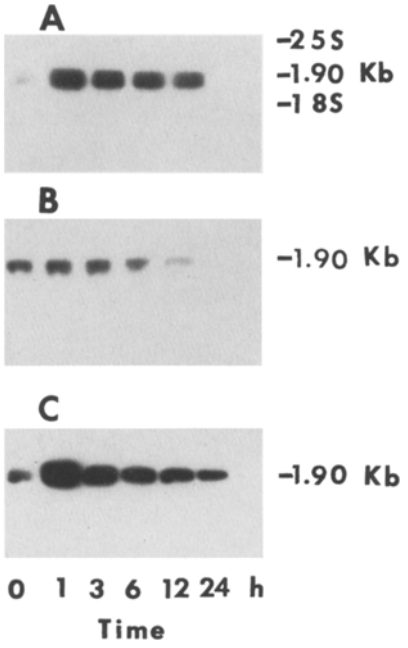

Fig. 2. Northern blot analyses of oat shoot invertase mRNA isolated from gravistimulated oat shoot pulvini. Poly(A)$^+$ RNA (5 μg/well) was electrophoresed in 1% agarose gel, blotted onto a nylon membrane and hybridized with α-^{32}P-dCTP-labeled invertase cDNA (PCR product) as a probe. A. mRNA isolated from intact gravistimulated oat shoot pulvini. B. mRNA isolated from the top halves of the pulvini. C. mRNA isolated from the bottom halves of the pulvini. Lanes 1–6 represent mRNA isolated from oat pulvini during different times of gravistimulation. Lane 1: 0 h is the vertical control. Lane 2: gravistimulated for 1 h. Lane 3: 3 h. Lane 4: 6 h. Lane 5: 12 h. Lane 6: 24 h (B and C) of gravistimulation, respectively.

one to speculate that the induction of invertase mRNA level may account for the changes of invertase activity that occur during the graviresponse. It also suggests that the expression of invertase gene(s) in oat pulvini might be regulated by gravistimulation at transcriptional or post-transcriptional levels.

Southern blot analysis

In order to estimate the size of invertase gene(s), genomic DNA was isolated from etiolated oat seedlings, and 25 μg of DNA was digested with

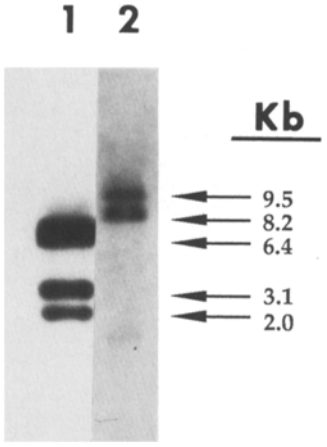

Fig. 3. Southern blot analysis of oat genomic DNA. DNA (25 μg/well) was digested with restriction enzymes and electrophoresed on a 0.6% agarose gel. The gel was denatured, neutralized and the DNA was transferred onto a nylon membrane filter. After baking at 80 °C under vacuum for 2 h, the filter was hybridized to digoxgenin-dUTP-labeled partial-length invertase cDNA used as a probe. The bands were visualized by chemiluminescent detection with Lumi-Phos 530 (Boehringer Mannheim Biochemicals) and exposed to X-ray film for 30 min at room temperature with an intensifying screen. Two restriction enzymes were used: *Eco* RI (Lane 1) and *Bam* HI (Lane 2).

different restriction enzymes at 37 °C for 2 h. The digested DNA was electrophoresed in a 0.6% agarose gel and blotted onto a nylon membrane filter and dried at 80 °C under vacuum [6]. The filter was then hybridized with digoxigenin-dUTP-labeled (a non-isotope labeling method, Boehringer Mannheim Biochemicals) invertase cDNA used as a probe. As shown in Fig. 3, two or three genomic DNA fragments with different sizes were hybridized to the partial-length invertase cDNA probe. These data indicate that more than a single invertase gene may exist for different isoforms of invertase in oat plants.

Acknowledgements

This work was supported by National Aeronautics and Space Administration Grants NAGW-1600 and NAG-10-0069.

References

1. Brock TG, Kaufman PB: Growth regulators: an account of hormones and growth regulation. In: Bidwell PGS (ed) Growth and Development Plant Physiology, vol. 10, pp. 277–326. Academic Press, New York (1991).
2. Gilbeaut DM, Karuppiah N, Chang S-R, Brock TG, Vadlamudi B, Kim D, Ghosheh NS, Rayle DL, Carpita NC, Kaufman PB: Cell wall and enzyme changes during the graviresponse of leaf-sheath pulvinus of oat (*Avena sativa*). Plant Physiol 94: 411–416 (1990).
3. Innis MA, Myambo KB, Gelfand DH, Brow MAD: DNA sequencing with *Thermus aquaticus* DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA. Proc Natl Acad Sci USA 85: 9436–9440 (1988).
4. Karuppiah N, Vadlamudi B, Kaufman PB: Purification and characterization of soluble (cytosolic) and bound (cell wall) isoforms of invertase in barley (*Hordeum vulgare*) elongating stem tissue. Plant Physiol 91: 993–998 (1989).
5. Kim WT, Silverstone A, Yip WK, Dong JG, Yang SF: Induction of 1-amino-cyclopropane-1-carboxylate synthase mRNA by auxin in mung bean hypocotyls and cultured apple shoots. Plant Physiol 98: 465–471 (1992).
6. Maniatis T, Fritsch EF, Sambrook J: Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1990).
7. Sturm A, Chrispeels MJ: cDNA cloning of carrot extracellular β-fructosidase and its expression in response to wounding and bacterial infection. Plant Cell 2: 1107–1119 (1990).