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Plant Gene Register

Complete Nucleotide Sequence of Potato Tuber Acid Invertase cDNA

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Acid invertase (EC 3.2.1.26), which hydrolyzes Suc to Glc and Fru, plays an important role in regulating sugar concentrations in many plant storage organs. Its activity is inversely related to Suc concentration but positively related to Glc and Fru concentrations in many plant tissues. Acid invertase activity increases severalfold during storage of potato (*Solanum tuberosum* L.) tubers at low temperatures (Pressey, 1969; Sasaki et al., 1971). Further, the ratio of the increments of Glc to Fru during sweetening of potato tubers at low temperatures is close to 1, indicating that Suc is hydrolyzed via invertase (Zhou, 1994). On the basis of the lack of correlation between levels of sugars and acid invertase activity in potato tubers after 3 months of storage at low temperature, Pressey (1969) suggested that invertase activity was regulated by an invertase-invertase inhibitor system. However, Richardson et al. (1990) demonstrated that during the initial storage of potatoes at low temperature, changes in total acid invertase activity assayed after destroying the endogenous invertase inhibitor reflected the sugar changes more closely than did the basal activity assayed with the inhibitor present. This raises questions concerning both the in vivo role of the invertase inhibitor and the contribution of acid invertase to the accumulation of reducing sugars in potato tubers stored at low temperature.

To study the regulatory mechanism by which acid invertase is regulated, a cDNA library of Uni-Zap XR (Stratagene) made from RNA isolated from cold-stored potato tubers was constructed. Six cDNA clones were isolated by screening the library with radiolabeled mung bean acid invertase cDNA probe (Arai et al., 1992) prepared by PCR. The sizes of these clones are between about 2.0 and 2.4 kb. The longest cDNA, pPAI11, was sequenced. It is 2336 bp long and contains one open reading frame extending from an ATG start codon at position 9 to a TAA stop codon at position 1926, encoding a polypeptide of 639 amino acid residues. The features of this cDNA are summarized in Table I.

Sequence analysis shows that pPAI11 is 60 to 95% identical to carrot, mung bean, and tomato acid invertase (Sturm and Chrispeels, 1990; Arai et al., 1992; Klann et al., 1992; Elliott et al., 1993; Sato et al., 1993). The deduced amino acid sequence is 97.9% similar and 95.6% identical to a tomato fruit vacuolar acid invertase cDNA (Klann et al., 1992; Elliott et al., 1993). Southern blot analysis indicated that the potato acid invertase gene is probably present in one copy per haploid genome. Northern blot analysis showed that the 2.3-kb acid invertase transcript was detected in potato tubers stored at 1°C, suggesting that increases in acid invertase activity in potato stored at low temperatures is regulated at the transcription level.

Table I. *Characteristics of acid invertase cDNA from potatoes*

Organism:	*Solanum tuberosum* L. cv Russet Burbank.
Genome Location:	Nuclear genome.
Gene Copy Number:	Single copy.
Gene Product:	Acid invertase (β-fructosidase, EC 3.2.1.26).
Source:	A cDNA library in Uni-Zap XR constructed with poly(A)$^+$ RNA isolated from potatoes stored at low temperature.
Sequencing Technique:	Dideoxy nucleotide chain-termination method was used to completely sequence both strands.
Method of Identification:	Screening with mung bean acid invertase cDNA. Nucleotide and amino acid sequence comparison with other plant acid invertases.
Expression and Regulation:	Northern blot detected the 2.3-kb acid invertase transcript in potato tubers stored at 1°C, but not in potatoes stored at 10°C.
Features of the Predicted Amino Acid Sequence:	The open reading frame encodes 639 amino acid residues. The deduced amino acid sequence contains a putative signal sequence with a predicted cleavage site at residue 50 (Heijne, 1986; Elliott et al., 1993). The β-fructosidase motif is found in bacterial, yeast, and other plant invertases (Sturm and Chrispeels, 1990). There are six potential N-linked glycosylation sites (Asn-X-Ser/Thr).

NOTE ADDED IN PROOF

After the submission of this paper we became aware of the cloning of an apoplastic invertase from potato (P. Hedley, G.C. Marchray,

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H.V. Davies, L. Burch, and R. Waugh, unpublished results). The amino acid sequence of this invertase is 61.1% similar to the acid invertase reported here.

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