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Plant Gene Register

A Tobacco cDNA Coding for Cell-Wall Invertase[1]

Steffen Greiner, Marion Weil, Silke Krausgrill, and Thomas Rausch*

Botanisches Institut, Ruprecht-Karls-Universität, Im Neuenheimer Feld 360, D-69120 Heidelberg, Germany

The CWI of higher plants is a structurally well-characterized basic protein (pI approximately 9.5) showing a complex glycosylation pattern. CWIs of several species have been characterized at the protein level (Laurière et al., 1988; Weil and Rausch, 1994, and refs. cited therein). Recently, complete cDNA clones have been obtained for CWIs of carrot (Sturm and Chrispeels, 1990), *Arabidopsis thaliana* (Schwebel-Dugué et al., 1994), and potato (Hedley et al., 1993). Sequencing of vacuolar invertase isoform cDNAs of different plant species has revealed high homology to CWIs; however, upon alignment of protein sequences, vacuolar invertase isoforms form a clearly separated group (Unger et al., 1994, and refs. cited therein). In contrast to the detailed knowledge about the structure of CWI, its physiological functions are not entirely clear. CWI has been proposed to play a role in phloem unloading during (a) seed development in maize (Miller and Chourey, 1992), (b) gravistimulation of oat shoot pulvinus (Wu et al., 1993), and (c) assimilate accumulation in tobacco (*Nicotiana tabacum* L.) crown gall tumors (Weil and Rausch, 1990). Apart from its putative role in phloem unloading (Eschrich, 1980) CWI may be involved in osmotic adjustment (regulation of apoplastic water potential) and pathogen defense (Sturm and Chrispeels, 1990). With the aim to characterize further the role of CWI for the growth of transformed suspension-cultured cells and tobacco crown gall tumors (Weil and Rausch, 1990, 1994) we have cloned a cDNA from a tobacco cell culture (Table I).

Table I. *Characteristics of tobacco CWI cDNA*

Organism:	*Nicotiana tabacum* (L.), cv Petit Havana SR1.
Gene Product:	Cell-wall invertase (β-fructosidase).
Function:	Hydrolysis of Suc in the apoplast.
Clone Type:	cDNA clone Ntbfruc1: full length, 1975 bp; pBK CMV.
Sources:	cDNA library in ZAP Express *Eco*RI/*Xho*I, mRNA isolated from suspension-cultured cells.
Techniques:	Homologous screening of a cDNA library with a PCR-amplified probe (784 bp) using consensus primers: sense primer, TGGATIAACGATCCAAATGGACCIATG; antisense primer, TCAAAGAAIGTCTTT/GGAIGCA/GTA. Nonradioactive dideoxy chain termination cycle sequencing of overlapping nested deletion clones (obtained with exonuclease III and mung bean nuclease) in pBK CMV.
Features of cDNA:	5′ End, 83 bp untranslated; 3′ end, 152 bp untranslated; 1740-bp open reading frame (59% AT content).
Characteristics of Deduced Protein:	The open reading frame encodes a polypeptide of 580 amino acids with a calculated M_r of 65,836 (mature polypeptide 61,302) and pI of 9.51. The deduced protein contains a putative signal peptide (Lys^9–Val^{23}) and seven potential glycosylation sites (Asn-X-Ser/Thr) in the mature polypeptide: Asn^{147}, Asn^{159}, Asn^{184}, Asn^{300}, Asn^{337}, Asn^{494}, Asn^{559}.

For the tobacco CWI clone, a hydrophobic stretch of 15 amino acids, located near the N terminus of the open reading frame (position 9–23), may represent part of the putative signal sequence for entry into the ER (Sturm and Chrispeels, 1990). In carrot the mature protein starts with Ser^{49}, i.e. 18 or 10 amino acids downstream of the two putative cleavage sites, suggesting a posttranslational removal of a prosequence (Sturm and Chrispeels, 1990). It is noteworthy that this Ser residue is conserved in all CWIs sequenced thus far. If the corresponding Ser residue 41 in tobacco CWI represents the N-terminal amino acid of the mature protein, the processed polypeptide will have a molecular mass of 61 kD, as has been previously determined for the completely deglycosylated protein by SDS-PAGE, and a pI of 9.51, identical with the value obtained by IEF of native CWI (Weil and Rausch, 1994). Among all higher plant CWI cDNAs, the tobacco clone shows the highest number (seven) of putative glycosylation sites. Sequence alignment of all plant CWIs indicates that three of the putative glycosylation sites (positions 159, 184, and 337 in the tobacco clone) are highly conserved. The previous characterization of the tobacco CWI protein (Weil and Rausch, 1994) has revealed one additional high-Man glycan as compared to the carrot enzyme, which bears two complex glycans and one high-Man glycan (Sturm and Chrispeels, 1990). Thus, in tobacco, only four of seven sites are actually glycosylated. It is interesting that the glycosylation sites that have been shown to bear glycan side chains in the carrot enzyme are conserved in the tobacco sequence.

Clustal analysis of the protein sequences derived from plant CWI cDNAs published thus far reveals high homology of the tobacco enzyme to CWIs of carrot, *A. thaliana*,

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* Corresponding author; e-mail id6@urz-mail.urz.uni-heidelberg.de; fax 49-69-798-4822.

Abbreviation: CWI, cell-wall invertase.

and potato. It is interesting that for potato two putative CWI cDNAs have been identified, one of which (EMBL accession No. Z22645) is more closely related to the tobacco CWI than to the second potato isoform (Hedley et al., 1993). In our cDNA library of tobacco suspension-cultured cells (the total number of screened plaques is 10^6) six CWI cDNA clones were picked, which were all identical except for the total length of the 5′ untranslated region. Furthermore, Southern blot analysis revealed the presence of a single gene. Therefore, at present, we have no indication of an additional CWI isoform in tobacco.

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The EMBL data library accession number for the sequence reported in this article is X81834 (*N. tabacum* mRNA for β-fructosidase).

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