Plant Gene Register

Sequence of a cDNA Encoding Chloroplast Fructose-1,6-Bisphosphatase from Rapeseed

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Chloroplast Fru-1,6-bisphosphatase is a key enzyme of the Benson-Calvin cycle for photosynthetic CO2 assimilation in higher plants. Although the capacity to hydrolyze Fru-1,6-bisP is low in dark-adapted chloroplasts, it increases upon illumination. Numerous authors have identified the Fd-thioredoxin system (Fd, Fd-thioredoxin reductase, thioredoxin) as the mediator in enzyme activation (Wolosiuk et al., 1993). On the other hand, the concerted action of a reductant, a sugar bisphosphate, a bivalent cation, and either co-solvents, chaotropic anions, or high hydrostatic pressure stimulate in vitro the enzyme activity. Thus, the replacement of reduced thioredoxin with nonphysiological modulators disclosed the importance of appropriate conformations not only in the activation process but also for the assembly of the nuclear-encoded Fru-1,6-bisphosphatase in the chloroplast stroma (Lallicora and Wolosiuk, 1990). To analyze these processes, we isolated and sequenced a cDNA coding for the chloroplast Fru-1,6-bisphosphatase from rapeseed (Brassica napus) (Table I).

Polyadenylated mRNA, isolated from 30-d-old green leaves, was used for the synthesis of cDNA, which was subsequently cloned between NotI/SalI sites of pSPORT1 (BRL). The cDNA library was screened for Fru-1,6-bisphosphatase expression with a polyclonal antibody raised in rabbits against spinach chloroplast Fru-1,6-bisphosphatase. Positive clones were confirmed by Southern blot analysis using a DNA probe derived from a cDNA encoding wheat chloroplast Fru-1,6-bisphosphatase.

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Table I. Characteristics of the cDNA encoding the rapeseed chloroplast Fru-1,6-bisphosphatase

| Organism: | Rapeseed (Brassica napus L., cv Global). |
| Function: | Encodes the precursor polypeptide of the monomer of (tetrameric) Fru-1,6-bisphosphatase (EC 3.1.3.11), which catalyzes the hydrolysis of Fru-1,6-bisP to Fru-6-P and Pi in plant chloroplasts. |
| Clone Type, Designation: | cDNA, pBnpFBP1. |
| Source: | cDNA library was prepared in pSPORT1 vector (BRL) using polyadenylated RNA isolated from 30-d-old rapeseed green leaves. |
| Method of Identification: | Library was screened with polyclonal antibodies raised in rabbits against spinach chloroplast Fru-1,6-bisphosphatase. Positive clones were confirmed by Southern blot analysis using a DNA probe derived from a cDNA encoding wheat chloroplast Fru-1,6-bisphosphatase. |
| Sequencing Strategy: | The sequencing of the cDNA insert was carried out on both strands by manual dideoxy sequencing. First, half of the full-length cDNA was determined from fragments generated by restriction endonucleases and subcloned in pSPORT1. Subsequently, the other half was sequenced, using, as primers, synthetic oligonucleotides that partially overlapped with the sequence determined previously. |
| cDNA Characteristics: | The cDNA insert contained 1340 nucleotides [excluding the poly(A) tail] with an open reading frame of 1233 bp; the G/C contents were 49.88 and 48.06%, respectively. |
| Structural Features of Deduced Protein: | The precursor and the putative mature subunit of Fru-1,6-bisphosphatase contained 411 (M, 44,444) and 358 (M, 38,999) amino acid residues, respectively. The putative mature subunit has 93.9, 85.8, and 78.6% amino acid sequence identity with Fru-1,6-bisphosphatase subunits of A. thaliana, potato, and wheat, respectively. The rapeseed enzyme was 85% identical with the primary structure of the spinach counterpart. |

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The nucleotide sequence of the cDNA showed the occurrence of an open reading frame of 1233 bp preceded by a 5'-bp-long noncoding region. The noncoding 3' region (102 bp) was followed by the poly(A) tail. The protein deduced from the open reading frame contains 411 amino acids. Fifty-three amino acid residues in the N-terminal region were significantly similar to chloroplast Fru-1,6-bisphosphatases from *Arabidopsis thaliana*, wheat, and potato (Raines et al., 1988; Horsnell and Raines, 1991; Koßmann et al., 1992). Moreover, the remaining 358 amino acid residues shared extensive identity both with the primary structure deduced in these species and with that found for the spinach counterpart by amino acid sequencing (Table I) (Marcus and Harrsch, 1990). On this basis, it appeared that the 358-amino acid polypeptide of rapeseed constitutes the stromal enzyme monomer.

In all mature chloroplast Fru-1,6-bisphosphatases, the feature relevant to the light-mediated activation was the sequence containing two Cys's separated by only four amino acid residues (-C-I-V-N-V-C-) that was similar to the putative site for the action of thioredoxin described in the spinach enzyme (-C-V-V-N-V-C-) (Marcus et al., 1988). It is worth noting that this unique region was absent in nonphotosynthetic and leaf cytoplasmic Fru-1,6-bisphosphatases (Ladró et al., 1990; Marcus and Harrsch, 1990). Thus, a sequence of highly hydrophobic amino acid residues located between redox-reactive Cys's was evolutionary preserved in light-regulated Fru-1,6-bisphosphatases.

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LITERATURE CITED

Horsnell PR, Raines CA (1991) Nucleotide sequence of a cDNA clone encoding chloroplast fructose-1,6-bisphosphatase from *Arabidopsis thaliana*. *Plant Mol Biol* 17: 185-186