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Short Sequence-Paper

Full-length cDNA sequences for both ferredoxin-thioredoxin reductase subunits from spinach (*Spinacia oleracea* L.)

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Abstract

Full-length cDNA clones for ferredoxin-thioredoxin reductase subunits A and B of *Spinacia oleracea* were obtained and their complete nucleotide sequences were determined. The results are compared with other known FTR sequences.

Key words: Ferredoxin-thioredoxin reductase; Subunit A; Subunit B; cDNA sequence; (*S. oleracea*)

Ferredoxin-thioredoxin reductase (FTR) is an iron-sulfur protein present in organisms performing oxygenic photosynthesis. It is part of the light-dependent ferredoxin-thioredoxin system which regulates the activity of various chloroplast enzymes by covalent redox modification [1]. FTR has been isolated and characterized in several laboratories [2–4]. The enzyme has an estimated molecular mass of 30 kDa and is composed of two nonidentical subunits, one of which is similar in all organisms studied so far (subunit B: M_r 13 000), while the other is variable in size and characteristic of a particular source (subunit A: M_r 7000 to 16 000) [3]. Both nuclear encoded FTR subunits are thought to be synthesized in the cytosol as precursor proteins containing transit-peptides which are post-translationally processed upon import into the chloroplast. To date, the sequence of the gene encoding the variable subunit A from eukaryotic organisms is still unknown. Only the corresponding gene from the cyanobacterium *Anacystis nidulans* has been cloned and sequenced [5]. The DNA sequence for subunit B from corn and spinach has been reported recently [6].

Using a spinach cDNA library we isolated two full-length cDNA clones which are similar and both encode subunit A. Moreover, we also isolated a full-length cDNA clone encoding subunit B which is different from the two recently reported sequences from spinach and from corn [6].

For each subunit two degenerate oligonucleotide primers were synthesized based on known amino acid sequences [7].

Subunit A-specific primers:

PFL014, 32-mer sense primer based on 'KQYVGFW-KGKY' (central part):

5'- AA(A/G) CA(A/G) TA(C/T) GTN GGN TT(C/T) TGG AA(A/G) GGN TA -3'

PFL023, 30-mer anti-sense primer based on 'KEEE-FEIIAE' (C-terminus):

5'- (T/C)TC NGC (T/G/A)AT (T/G/A)AT (C/T) TC (A/G)AA (C/T)TC (C/T)TC (C/T)TC (C/T)TT -3'

Subunit B-specific primers:

PFL015, 32-mer sense primer based on 'KDTYFCVD-KCV' (N-terminus):

5'- AA(A/G) GA(C/T) ACN TA(C/T) TT(C/T) TG (C/T) GTN GA(C/T) AA(A/G) TG(C/T) GT -3'

PFL024, 30-mer anti-sense primer based on 'DE-IREVTSNM' (C-terminus):

5'- CAT (A/G)TT N(G/C)(A/T) NGT NAC (C/T)TC NC(T/G) (A/G/T)AT (T/C)TC (A/G)TC -3'

An aliquot (10 μl) of the spinach cDNA library constructed in λZAP II (Stratagene, Heidelberg, Germany) was used directly in PCR reactions with both sets of specific primers [8]. The amplified products

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The sequence data reported in this paper have been submitted to the EMBL Data Library under the accession numbers X77162 (FTR A1), X77163 (FTR A2, X77164 (FTR B).

SSDI 0005-2728(94)00015-W

were purified, and labeled fragments were produced in PCR reactions using DIG-labeled nucleotides (Boehringer Mannheim, Germany). The cDNA-library was subsequently screened with these probes in parallel for each subunit. In vivo excision of positive λZAP clones was performed as described in the Stratagene protocol. Sequence analysis was done by the dideoxynucleotide chain-termination method using the Sequenase Version II kit (USB Bad Homburg, Germany) with both standard sequencing and FTR-specific primers. In case of the two subunit A clones, the internal *Eco*RI-site 15 bp in front of the stop-codon was used to generate

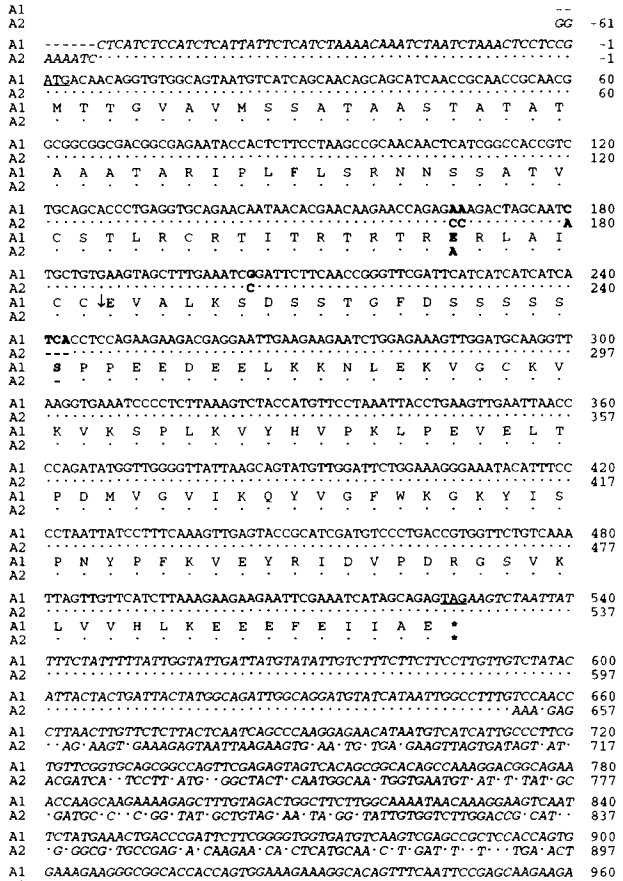

Fig. 1. Nucleotide sequences and deduced amino acid sequences of the two full length cDNA clones coding for subunit A of ferredoxin-thioredoxin reductase from spinach. Dots represent identical positions in sequence A2. Gaps are marked by dashes and significant changes in the coding region are highlighted by bold letters. The start of the mature protein according to published protein sequence information [7,9] (i.e., the putative signal sequence processing site) is indicated by a vertical arrow.

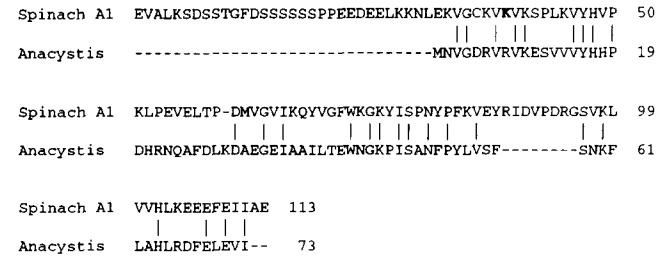

Fig. 2. Comparison of the mature protein sequences of subunit A1 of ferredoxin-thioredoxin reductase from spinach (this paper) and the corresponding subunit from *Anacystis nidulans* [5]. The previously published FTR A protein sequence from spinach [7] differs in one position (39 K → F) marked in bold.

5′-deletions in order to sequence the unusually long 3′-untranslated trailer regions.

The obtained nucleotide sequences of two different cDNA clones of spinach FTR subunit A (A1 and A2) and the corresponding translation of their coding parts are shown in Fig. 1. The A1 gene encodes a protein of 175 amino acid residues, whereas the A2 gene codes for a protein of 174 amino acids. Both include the putative plastidic signal sequence. By comparison with the N-terminal sequence of the mature protein from spinach [7,9] as shown in Fig. 2, subunit A1 would yield a protein of 113 amino acids (M_r 12771), containing one additional amino acid when compared to subunit A2 that consists of 112 amino acids (M_r 12683). Furthermore, one amino acid exchange in the putative signal sequence and two silent nucleotide exchanges are observed between A1 and A2 (Fig. 1). Together with our finding that the sequences differ in their

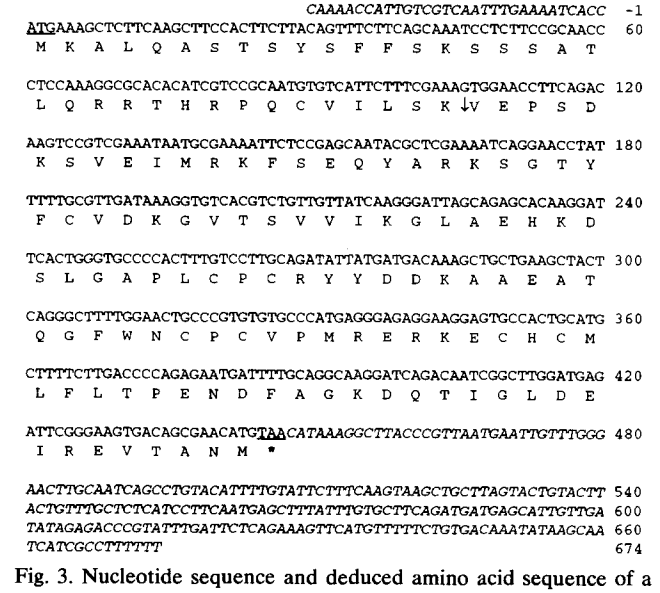

Fig. 3. Nucleotide sequence and deduced amino acid sequence of a cDNA clone coding for subunit B of ferredoxin-thioredoxin reductase from spinach. The presumed start of the mature protein (i.e., the putative signal sequence processing site) is indicated by a vertical arrow [7,9].

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Spinach B1  VEPS-DKSVEIMRKFSEQYARKSGTYFCVDKGVTSVVIKGLAEHKDSLGA  49
Spinach B2  ADPS-DKSMEVMRKFSEQFCRKSDTYFCVDKSVTAVVIKGLADHRDTLGA  49
Corn        ADASNDKSVEVMRKFSEQYARRSNTFFCADKTVTAVVIKGLADHRDTLGA  50

Spinach B1  PLCPCRYYDDKAAEATQGFWNCPCVPMRERKECHCMLFLTPENDFAGKDQ  99
Spinach B2  PLCPCRHYDDKEAEAKQGFWNCPCVPMRERKECHCMLFLTPDNDFAGKEQ  99
Corn        PLCPCRHYDDKAAEVAQGFWNCPCVPMRERKECHCMLFLTPDNDFAGKDQ 100

Spinach B1  TIGLDEIREVTANM  113
Spinach B2  TITLDEIREVTSNM  113
Corn        VISFEEIKEATSKF  114
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Fig. 4. Amino acid sequence comparison of various mature ferredoxin-thioredoxin reductase B subunits showing two different spinach polypeptide sequences B1 (this paper), B2 [6] and one from corn [6]. Cysteine residues are shown in bold letters.

respective untranslated regions, particularly in their unusually long 3′ ends, this suggests that A1 and A2 represent two different genes. The deduced amino acid sequence of subunit A1 is identical to the published spinach protein sequence [7] except for one amino acid exchange at position 39 where Phe is replaced by Lys in the mature protein sequence (Fig. 2). The coding parts of the subunit A clones exhibit only 25% similarity with subunit A from *Anacystis* and are longer by 40 (39) amino acids (Fig. 2). This confirms the earlier finding that the A subunits from different sources differ significantly in size [3].

In Fig. 3 the nucleotide sequence and the deduced amino acid sequence for a clone encoding FTR subunit B from spinach is shown. The entire coding part comprises 148 amino acids including the plastidic signal sequence. By comparison with the N-terminal amino acid sequence of the protein purified from spinach [7,9] as shown in Fig. 4, the deduced amino acid sequence of its cDNA yields a mature protein of 113 residues (M_r 12740). There are differences at various positions compared to the previously published sequences from corn and from spinach [6], however, most changes are conservative (Fig. 4). Seven conserved cysteine residues are present in all three subunit B protein sequences. The additional cysteine residue found in the N-terminal region of the spinach protein [6] is replaced by an alanine residue, as is the case for the corn sequence [6] (Fig. 4).

In contrast to the finding that the FTR subunits A and B differ in size [4] and assuming that the N-termini of both mature subunits [7,9] are complete, our results indicate that both FTR subunits from spinach must be similar in length (113 amino acids). The reason for this discrepancy could be a different processing event in the N-terminus of subunit A in vivo. A further possibility would be an abnormal migration behaviour during SDS-PAGE, as has already been observed for ferredoxin, another small iron-sulfur protein [10].

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