

Plant Gene Register

Sequence of Mature Phosphoglycerate Kinase from Spinach Chloroplasts¹

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PGKs are present in higher plants in two isoforms, one of which is located in the cytosolic compartment, and the other of which is localized in the stroma of chloroplasts (Anderson and Advani, 1970). The chloroplast isoenzyme catalyzes one step in the photosynthetic carbon reduction cycle, whereas the cytosolic enzyme takes part in glycolysis and gluconeogenesis.

The only cDNA sequences of PGKs from higher plants reported so far are those of isoenzymes from wheat (Longstaff et al., 1989). Here we report the nucleotide sequence for the chloroplast isoform of PGK from spinach (*Spinacia oleracea*). This is the first PGK sequence reported from a dicotyledonous plant.

A partial cDNA for spinach PGK was isolated from a spinach cDNA expression library during a screening with an antibody raised against spinach nucleoside diphosphate kinase. Using this partial clone as a probe for a second screening of the same library, nine clones of cDNAs for PGK were isolated and sequenced. The longest open reading frame found on one of these cDNA clones codes for a protein with 28 additional amino acid residues preceding the putative NH₂ terminus of PGK. Since this open reading frame starts at the 5' end of the cDNA, it is probably incomplete and the 28 NH₂-terminal amino acids represent only part of a chloroplast transit peptide. This notion is supported by the composition of the NH₂-terminal peptide, which conforms with the typical amino acid composition of chloroplast transit peptides (von Heijne et al., 1989). Moreover, this NH₂-terminal peptide shows some sequence homology to the chloroplast transit peptide of the chloroplast isoform of PGK from wheat.

The predicted protein sequence of the spinach PGK shows considerable homology to both PGK isoforms from wheat, pointing toward a high degree of conservation of PGK sequences between monocotyledonous and dicotyledonous plants. At the amino acid level, the identity between the mature spinach protein and the wheat chloroplast enzyme is 87%, whereas it is 85% if compared with the cytosolic isoform from wheat. The close homology between the spinach sequence and the chloroplast PGK from wheat, as well as the

Table 1. Characteristics of PGK from *S. oleracea* L.

Organism:	<i>Spinacia oleracea</i> L.
Function:	Phosphoglycerate kinase, EC 2.7.2.3.
Pathway:	Photosynthetic carbon reduction cycle (Calvin cycle).
Clone Type:	cDNA, partial; full-coding sequence of mature protein plus partial coding sequence of transit peptide plus 3' noncoding region.
Clone Designation:	lsopgk10.
Source:	cDNA-library in λ gt11, mRNA from 16-h light-induced spinach seedlings (Tittgen et al., 1986).
Techniques:	Screening with digoxigenin-labeled DNA, restriction fragment subcloning into Bluescript KS-plasmid vector; nested deletion of restriction fragments >400 bp and subsequent re-ligation and subcloning of shortened plasmid clones; dideoxynucleotide sequencing of double-stranded plasmid DNA.
Method of Identification:	Sequence comparison with GenBank/EMBL data bases.
Features of mRNA Structure:	This clone of 1461 bp contains no 5' noncoding region and a 1299-bp coding region of which the first 84 bp code for a partial transit peptide and the remaining 1215 bp code for the mature protein. A 162-bp 3' noncoding region completes this cDNA clone.
(G + C) Content:	44.7% in total mRNA, 45.9% in coding region, 35.2% in 3' untranslated region.
Structural Features of Protein:	433 amino acids including part of a transit peptide of 28 amino acids at the NH ₂ terminus. The cleavage site of the stromal processing protease is predicted according to the homology with the plastidic wheat-PGK.
Subcellular Location:	Chloroplast stroma, as judged by the existence of a transit peptide and by the closer homology to the plastidic enzyme than to the cytosolic enzyme of wheat.

¹ Supported in part by grants from the Deutsche Forschungsgemeinschaft. U.B. is recipient of a grant from the state of Saarland.

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Abbreviation: PGK, phosphoglycerate kinase.

indications for the existence of a chloroplast transit peptide, indicate that this clone (λ sopgk10) represents the coding sequence for the chloroplast isoform of PGK from spinach.

ACKNOWLEDGMENT

We thank Prof. R. G. Herrmann for kindly providing us with the spinach cDNA library.

Received June 7, 1993; accepted June 15, 1993.

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The GenBank accession number for the sequence reported in this article is X68430.

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