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Plant Gene Register

Nucleotide Sequence of a cDNA Encoding NADP-Sorbitol-6-Phosphate Dehydrogenase from Apple[1]

Yoshinori Kanayama*, Hitoshi Mori, Hidemasa Imaseki, and Shohei Yamaki

Laboratory of Horticultural Science (Y.K., S.Y.) and Research Institute for Biochemical Regulation (H.I.), School of Agricultural Sciences, Nagoya University, Chikusa-ku, Nagoya 464–01, Japan; and Division of Biological Regulation, National Institute for Basic Biology, Myodaijicho, Okazaki 444, Japan (H.M., H.I.)

Sorbitol plays a key role in the translocation of photosynthate in the Rosaceae family, including apple (*Malus domestica*), pear, and stone fruits (4, 7). NADP-S6PDH[2] plays the most important role in biosynthesis of sorbitol in apple because its activity increases before accumulation of sorbitol in seedlings (8), and high activity is maintained in leaves (10). This enzyme reduces G6P to S6P using only NADPH (1, 6). Therefore, S6P dehydrogenase (EC 1.1.1.140), which catalyzes NAD-dependent conversion between fructose 6-phosphate and S6P in microorganisms (3), is different from NADP-S6PDH described in this paper.

We report the isolation of a cDNA clone encoding NADP-S6PDH from apple (Table I). To our knowledge, this is the first report of the complete primary structure of a sorbitol-related enzyme from plants. A cDNA library constructed from apple seedlings was screened with the antibody against NADP-S6PDH purified from loquat leaves (1). The nucleotide and deduced amino acid sequences of the clone cDNA is shown in Figure 1.

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[2] Abbreviations: NADP-S6PDH, NADP-dependent D-sorbitol-6-phosphate dehydrogenase; G6P, D-glucose 6-phosphate; S6P, D-sorbitol 6-phosphate.

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Table I. *Characteristics of a cDNA Encoding Apple NADP-S6PDH*

Organism:
Malus domestica.
Function:
S6P + $NADP^+ \rightleftharpoons$ G6P + NADPH + H^+ (1, 6).
Techniques:
A cDNA library constructed in pBluescript (Stratagene) by the vector-primer method (5) was screened with polyclonal antibodies raised against purified loquat NADP-S6PDH (1). Sequencing of double-stranded DNA was by the dideoxynucleotide method. T7 primer was used for sequencing of deletion mutants, and synthetic oligonucleotide primers were used for the other strand.
Methods of Identification:
Sequence identity with a partial amino acid sequence of a polypeptide obtained by cleavage (2) of purified NADP-S6PDH at Pro^{231} to Ala^{245} of the deduced sequence; detection of NADP-S6PDH activity in an extract of *Escherichia coli* harboring the cDNA; synthesis of an immunoreactive protein similar in size to the purified enzyme in *E. coli.*
Expression Characteristics:
mRNA of approximately 1.4 kb detected by northern blot analysis of polyadenylate-enriched RNA and total RNA.
Regulation:
Unknown.
(G+C) Content:
46.4% in the coding region.
Structural Features of Protein:
Open reading frame of 310 amino acids; M_r 34,900; dimers consisting of two equally sized subunits (1).
Subcellular Location:
Chloroplast and cytosol (9).
DDBJ/EMBL/GenBank Accession No.:
D11080

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   1 CCGCTCTAGAACTAGTGGACGAGAGAAAAACAGAAACAGGCTGCAGCAGTCGCTGAGAGA
  61 GTTTGGAGAGTGAGAAAACATGTCCACCGTCACCCTGAGCAGTGGCTACGAGATGCCGGT
                     M  S  T  V  T  L  S  S  G  Y  E  M  P  V  14
 121 CATCGGTCTCGGCCTTTGGCGTCTGGAGAAGGACGAGCTTAAAGAAGTCATCTTAAATGC
       I  G  L  G  L  W  R  L  E  K  D  E  L  K  E  V  I  L  N  A  34
 181 TATTAAGATTGGCTATCGCCATTTTGACTGTGCTGCTCATTACAAGAGTGAAGCAGACGT
       I  K  I  G  Y  R  H  F  D  C  A  A  H  Y  K  S  E  A  D  V  54
 241 TGGAGAAGCACTTGCAGAAGCATTTAAGACTGGACTTGTTAAGAGGGAAGAACTTTTCAT
       G  E  A  L  A  E  A  F  K  T  G  L  V  K  R  E  E  L  F  I  74
 301 TACCACCAAGATTTGGAATTCAGACCATGGGCATGTGGTGGAGGCCTGTAAGAACAGCCT
       T  T  K  I  W  N  S  D  H  G  H  V  V  E  A  C  K  N  S  L  94
 361 CGAGAAGCTTCAGATAGATTATCTGGATCTCTACCTGGTTCACTACCCAATGCCCACAAA
       E  K  L  Q  I  D  Y  L  D  L  Y  L  V  H  Y  P  M  P  T  K  114
 421 GCACAATGCAATTGGTAAAACTGCCAGTCTTTTGGGCGAGGATAAGGTGTTGGACATCGA
       H  N  A  I  G  K  T  A  S  L  L  G  E  D  K  V  L  D  I  D  134
 481 TGTAACAATTTCCCTTCAACAAACCTGGGAGGGCATGGAAAAGACCGTCTCTTTGGGCTT
       V  T  I  S  L  Q  Q  T  W  E  G  M  E  K  T  V  S  L  G  L  154
 541 AGTTCGCAGCATTGGTCTCAGCAACTATGAGCTCTTTCTAACTAGAGATTGCTTGGCTTA
       V  R  S  I  G  L  S  N  Y  E  L  F  L  T  R  D  C  L  A  Y  174
 601 CTCCAAAATAAAGCCTGCTGTGAGCCAATTTGAAACCCACCCCTATTTCCAGCGCGACTC
       S  K  I  K  P  A  V  S  Q  F  E  T  H  P  Y  F  Q  R  D  S  194
 661 TCTCGTCAAATTCTGTATGAAACACGGCGTTCTTCCCACAGCTCACACCCCTCTCGGAGG
       L  V  K  F  C  M  K  H  G  V  L  P  T  A  H  T  P  L  G  G  214
 721 TGCTGCTGCCAACAAGGATATGTTTGGTTCTGTTTCACCTTTGGATGATCCAGTTCTCAA
       A  A  A  N  K  D  M  F  G  S  V  S  P  L  D  D  P  V  L  N  234
 781 TGATGTGGCTAAGAAATACGGAAAGAGCGTGGCACAAATCTGTCTGAGGTGGGGAATTCA
       D  V  A  K  K  Y  G  K  S  V  A  Q  I  C  L  R  W  G  I  Q  254
 841 GAGGAAAACAGCAGTGATTCCAAAATCATCGAAAATTCAGCGATTGAAAGAGAATTTGGA
       R  K  T  A  V  I  P  K  S  S  K  I  Q  R  L  K  E  N  L  E  274
 901 GGTTCTTGAATTCCAGCTGAGCGATGAAGACATGCAGCTCATCTACAGTATCGACAGGAA
       V  L  E  F  Q  L  S  D  E  D  M  Q  L  I  Y  S  I  D  R  K  294
 961 GTATCGTACCAGTCTACCTTCCAAGACTTGGGGCTTAGACGTGTATGCATAAGCGTGCCA
       Y  R  T  S  L  P  S  K  T  W  G  L  D  V  Y  A                310
1021 TTCAAAAACCTTCGAATTGCTGCCTCCGCAACTTCTTCCAAGGCTGTTCAACGGAAGCGA
1081 AATGGAAACTATCGTGAATCTTACTTACAATAAACTGAGCTTCATATAATTTTCCAGAAG
1141 CTCATCTATCTGCTAGTTTGAAAACTTCATTATTCGCCCTTTGCATTAGGCCTTGCAAAG
1201 GAAAATATAATAAACGGCCCTTGTATTTTTTTTGGTACTTAATAAATGAGTTATTAAAGA
1261 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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Figure 1. Nucleotide sequence and deduced amino acid sequence of a cDNA encoding apple NADP-S6PDH. The partial amino acid sequence determined by Edman degradation of a peptide fragment obtained from NADP-S6PDH is underlined.