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Plant Gene Register

Isolation and Characterization of a cDNA Clone Encoding a Novel Short-Chain Alcohol Dehydrogenase from Norway Spruce (*Picea abies* L. Karst)

Stephan Bauer, Hélène Galliano[1], Friedhelm Pfeiffer, Burkhard Meßner, Heinrich Sandermann, Jr., and Dietrich Ernst*

GSF-Forschungszentrum für Gesundheit und Umwelt GmbH, Institut für Biochemische Pflanzenpathologie (S.B., H.G., H.S., D.E.), Abteilung für Zellchemie (B.M.), D-85758 Oberschleißheim, Germany; and Max-Planck-Institut für Biochemie, Am Klopferspitz 18, D-82152 Martinsried, Germany (F.P.)

The gene family of short-chain ADH is very broad, including members that are only distantly related. The biochemically characterized enzymes show extensive functional differences (Persson et al., 1991) and little is known about the basic functional mechanisms. Enzymes like ADH, Glc dehydrogenase, hydroxysteroid dehydrogenase, acetoacetyl-CoA reductase, fixR protein, and nodG protein belong to the short-chain ADH family (Persson et al., 1991). We report here the isolation of a full-length cDNA clone (pSscADH16), the first report of a short-chain ADH family member cloned from a gymnosperm.

The cDNA insert is 1448 nucleotides in length and contains one open reading frame of 813 nucleotides encoding a protein of 271 amino acids (Table I). The coding sequence seems to be complete because there is an ATG initiation codon. In addition, a G is in position +4 and a C is in position +5. This constellation modulates initiation codon selection in plants (Lütcke et al., 1987). Moreover, the 3′ untranslated region contains putative polyadenylation signals (AATGAA). Sequence comparison of the deduced amino acid sequence with the SwissProt data base revealed highest identity to Glc dehydrogenases (31%). This is similar to residue identities between short-chain ADHs of about 25% (Persson et al., 1991). Moreover, the encoded polypeptide of 271 amino acids fits well with the short-chain ADH subunit size of about 250 amino acids (Persson et al., 1991).

Using the PC/GENE software program, a short-chain ADH signature was found (residues 179–189) (Table I). pSscADH16 protein contains the strictly conserved (Gly^{28}, Gly^{34}, Asp^{85}, Gly^{159}, Tyr^{179}, Lys^{183}) as well as conserved (Gly^{32}, Gly^{45}, Val^{48}, Gly^{104}, Asn^{112}, Ala^{113}, Gly^{114}, Ile^{161}, Ser^{166}, Ala^{181}) residues of short-chain ADHs (Persson et al., 1991).

The pattern of pSscADH16 mRNA expression was examined during elicitation and ozone treatment. In Norway spruce (*Picea abies* L. Karst) cell cultures, as well as in needles of seedlings, the mRNA was abundant. In cell cultures a strong increase was detectable 1 d after elicitor treatment. Treatment of Norway spruce seedlings with ozone (0.6 $\mu L\ L^{-1}$) resulted in a week, although significant, increase of pSscADH16 mRNA. Based on this mRNA expression, the short-chain ADH may protect against biotic as well as abiotic stress, as has been shown in the same tissue for cinnamyl ADH, a member of the Zn-containing ADH gene family (Galliano et al., 1993a, 1993b).

Table I. *Characteristics of a short-chain ADH cDNA from Norway spruce*

Organism:
Norway spruce (*Picea abies* L. Karst); cell culture (Galliano et al., 1993b).
Function:
Short-chain ADH.
Techniques:
Plasmid pSport 1 cDNA library constructed from spruce cell culture poly(A)$^+$ RNA (Galliano et al., 1993a); dideoxy nucleotide chain termination sequencing of both strands (Sanger et al., 1977).
Methods of Identification:
Sequence comparison to SwissProt data base; signature of short-chain ADH (YTASKAAVEMM); strictly conserved amino acids of the short-chain ADH family (Persson et al., 1991).
Features of the cDNA:
Contains 1448 nucleotides consisting of 23 nucleotides in the 5′ untranslated region, 813 nucleotides in an open reading frame, and 612 nucleotides in the 3′ untranslated region; polyadenylated signals were observed.
Structural Features of the Encoded Protein:
Open reading frame of 271 amino acids; M_r 28,724; isoelectric point 5.56.
Subcellular Localization of the Protein:
No transmembrane or membrane-associated structures (Eisenberg et al., 1984; Rao and Argos, 1986).
mRNA Expression:
Abundant in spruce cell cultures and spruce needles; increased levels in ozone-treated spruce seedlings, as well as in elicitor-treated spruce cell cultures.

[1] Present address: Max-Delbrüch-Laboratorium, Carl-von-Linné-Weg 10, D-50829 Köln, Germany.

* Corresponding author; fax 49–89–3187–3383.

Abbreviation: ADH, alcohol dehydrogenase.

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