

COMPUTATIONAL ANALYSIS OF THE SAG 13 GENE ENCODING AN ALCOHOL DEHYDROGENASE

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Abstract

SAG 13 is a senescence associated gene from *Arabidopsis thaliana*, the role of the gene is to encode a protein having alcohol dehydrogenase/ oxidoreductase which is involved in interconversion between alcohol and aldehyde or ketone with the reduction of NAD⁺ to NADH. This present study involves a comprehensive structural analysis of alcohol dehydrogenase and its interactions with specific ligands.

Keywords: *Arabidopsis thaliana*, alcohol dehydrogenase, cinnamyl alcohol

Introduction:

Senescence is the sequence of degradative processes leading to the remobilization of nutrients and eventual death. Using differential screening and subtractive hybridization techniques, researchers have identified genes with increased expression during senescence (15). These genes have been identified in *Arabidopsis*, tomato, barley, potato, cucumber, rice, wheat, and maize. Such genes are often referred to as SAGs or senescence-up-regulated genes. In *Arabidopsis* SAG 13 a type of alcohol dehydrogenase/oxidoreductase is expressed in mature leaves prior to the onset of visible senescence (16). **Alcohol dehydrogenase (ADH)** (EC 1.1.1.1) are a group of seven dehydrogenase enzymes that occur in many organisms and facilitate the interconversion between alcohol and aldehyde or ketone with the reduction of NAD⁺ to NADH (17). The substrate is coordinated to the zinc and this enzyme has two zinc atoms per subunit. One is the active site, which is involved in catalysis. In the active site, the ligands are Cys-46, Cys-174, His-67 and one water molecule. The other subunit is involved with structure. In this mechanism, the hydride from the alcohol goes to NAD⁺. Crystal structures indicate that the His-51 deprotonates the nicotinamide ribose, which deprotonates Ser-48. Finally, Ser-48 deprotonates the alcohol, making it an aldehyde. From a mechanistic perspective, if the enzyme adds hydride to the re face of NAD⁺, the resulting hydrogen is incorporated into the pro-R position. Enzymes that add hydride to the re face are deemed Class A dehydrogenase.

The enzyme is presumably required by plants for NADH metabolism, via reduction of acetaldehyde to ethanol, during periods of anaerobic stress. Crosses between the electrophoretic types and dissociation-reassociation experiments showed that the *Arabidopsis* enzyme behaves as a dimer. The molecular weight of the enzyme has been estimated by gel filtration and by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis to be 87,000. The pH optimum for the oxidation of ethanol is 9.0 and two optima for reduction of acetaldehyde have been obtained, 6.0 and 8.5, respectively. The enzyme exhibits wide substrate specificity for alcohols and is relatively heat resistant.

Materials and Methods:**Detection of Phosphorylation Sites**

The putative phosphorylation sites were analyzed using the Netphos server.

Comparative Homology Modeling

The query protein sequence of Alcohol dehydrogenase was searched to find out related protein structure to be used as template using BLAST (Basic Local Alignment Search Tool) (1) against PDB (Protein Data Bank). The sequence with maximum identity and less e-value was chosen and used as the reference structure for homology modeling. [3GAF, 1AE1 and 1VL8] (10) were used as the template structure for modeling of proteins.

Homology model of alcohol dehydrogenase was constructed using the protein modeling software, MODELLER 9v7. The homology model was generated using the 2.4Å resolution of x-ray crystallographic structure of 3GAF, 1AE1 and 1VL8 receptors as template. A major problem in homology modeling is in having a sequence similarity of less than ~20% between the target sequence and the template sequence. Homology model generated using such low similarity as reported by Wilson et al., 2000 is considered to be unreliable and less

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