Poster C-15

Molecular evolution and structural aspects of plant alcohol dehydrogenase

Authors:
Claudia Elizabeth Thompson (*Universidade Federal do Rio Grande do Sul*)
Loreta Brandão de Freitas (*Universidade Federal do Rio Grande do Sul*)
Francisco Mauro Salzano (*Universidade Federal do Rio Grande do Sul*)
Osmar Norberto de Souza (*Pontifícia Universidade Católica do Rio Grande do Sul*)

Short Abstract: The alcohol dehydrogenase genes encode glycolytic enzymes. Phylogenetic analysis indicate duplication events within angiosperms. Coefficients of functional divergence indicate site-specific shift of evolutionary rate in the Adh1 and Adh2 gene groups and different families. Amino acids residues important for functional divergence were identified in the ADH tridimensional structure.

Long Abstract:

INTRODUCTION

The glycolytic proteins in plants are coded by small multigene families, which provide an interesting contrast to the high copy number gene families studied to date. The alcohol dehydrogenase (Adh) genes encode glycolytic enzymes that have been characterized in some plant families. Although the amino acid sequences of zinc-containing long-chain (LC) ADHs are highly conserved, the metabolic function of this enzyme is variable. Besides this, they have different patterns of expression and are submitted to differences in nonsynonymous substitution rates between gene copies. It is possible that the Adh copies have been retained as a consequence of adaptative amino acid replacement which has conferred a subtle change in function.

In plants, this gene family has been most intensively studied in the Poaceae botanical family. In Zea mays, for example, the two Adh genes differ in their pattern of tissue-specific expression. Adh1 gene is expressed in dry seed and pollen tissues, while both Adh1 and Adh2 are expressed in roots tissues under anoxic conditions. Similar variation in the expression of Adh genes is found in a wide variety of plant species, including other grasses, sunflower, eucalyptus, and pine. Besides this, functional assays reveal important differences among three alleles from the Adh1 locus in maize. Firstly, the protein products encoded by these alleles differ in their specific activity. Secondly, the alleles vary in their abilities to recombine intragenically. Allelic differences in protein function, different patterns of expression, gene conversion, and recombination make the Adh locus interesting from the evolutionary viewpoint.

Despite the large number of studies involving the Adh gene family, there does not exist a wide ranging study correlating molecular evolution and structural biology of the plant alcohol dehydrogenases. Moreover, this is the first study where a plant ADH tridimensional structure is proposed. Here, we extend previous studies of this gene family, with the goal of using molecular evolutionary and molecular modeling tools to understand the process of diversification involved in this multigene family.

MATERIAL AND METHODS

The amino acid and DNA sequences from gymnosperms and angiosperms were obtained from the National Center of Biotechnology Information (NCBI). Alignments were performed with the ClustalW program and were inspected and manual changes made when necessary, using GeneDoc 2.6. The phylogenies were estimated by neighbor-joining (NJ), available in the MEGA program version 3.1 (Molecular Evolutionary Genetics Analysis) and by maximum likelihood (ML) methods using PhyML and TreeFinder programs. ADH sequences from Pinus banksiana were used as the outgroup. The bootstrap analyses were conducted using the previously cited programs. Afterwards, with the objective of evaluating the presence of positive selection, analyses were performed using the maximum likelihood models recommended by Yang. Sites which yielded posterior probabilities higher than 95% were considered significantly affected by selection. A statistical framework modeling the functional divergence was implemented by the Diverge program to estimate the coefficient of functional divergence (θ).

We obtained results for the tridimensional structure of the plant alcohol dehydrogenases using Equus caballus liver alcohol dehydrogenase (PDB code 1N8K). Its structure has been solved to a 1.13Å resolution. The program MODELLER8 was used to build protein models according to the comparative protein modeling methodology. Finally, the best model was evaluated and selected on the basis of the results obtained by PROCHECK and VERIFY-3D.

RESULTS AND DISCUSSION

The phylogenetic analysis indicate that there have been a number of separate duplication events within angiosperms, that is to say genes labeled Adh1, Adh2 and Adh3 in different groups may not be homologous. No indication of positive selection was encountered for the plant Adh gene family. The phylogenetic tree clearly shows three primary lineages corresponding to monocot, dicot, and pine genes. Gene duplications have taken place independently in each of these lineages. No indication of positive selection was encountered for the plant Adh gene family. However, the coefficients of functional divergence (θ) estimated between the Adh1 and Adh2 gene groups indicate statistically significant site-specific shift of evolutionary rate between them. θ varied markedly from 0.541 up to 0.729. Moreover, the θ of the Adh genes between different botanical families are significantly greater than zero, suggesting that altered functional constraints may take place at some amino acid residues after speciation. The variation of θ is from 0.436 up to 0.666.

Theoretical tridimensional models of seventeen plant alcohol dehydrogenase were constructed and verified to be stereochemically valid. The amino acids residues important for the functional divergence of this enzyme were identified in the ADH tridimensional structure.