Molecular Modelling of RNases from Almond Involved in Self-Incompatibility

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Abstract

Gametophytic self-incompatibility (GSI) is a natural mechanism in flowering plants, including almond and other fruit tree species, to prevent inbreeding and promote outcrossing. GSI is typically under the control of a specific locus, known as the *S*-locus, which contains at least two genes. The first gene encodes glycoproteins with ribonuclease (*S*-RNase) activity in the pistils, and the second is a specific F-box gene (SFB) expressed in the pollen. In the Solanaceae, Scrophulariaceae and Rosaceae, active *S*-RNases in the style are essential for rejection of haploid pollen, when the *S*-allele of pollen matches one of two *S*-alleles of the diploid pistil. The *S*-RNase was first identified in *Prunus* more than 20 years ago, whereas SFB was identified only recently. In spite of the knowledge of the genetic structure of the female and male determinants of GSI, the nature of their mutual interactions at genetic and biochemical levels remain unclear. Thus, detailed understanding of the protein structure involved in GSI may help in discovering how proteins involved in GSI function and how they fulfil their biological roles. To this aim, three-dimensional (3D) models of a self-compatible (S_f) and a self-incompatible (S_8) *S*-RNase of almond were constructed, using comparative modelling tools. The molecular models of S_f and S_8 showed that their 3D architectures had the same disposition of the secondary structural elements as typical members of the RNase T_2 family. The modelled structures consisted of mixed α and β folds, with six helices and six beta-strands.

INTRODUCTION

Self-incompatibility (SI) in *Prunus* species shows the gametophytic SI (GSI) system, controlled by a single polymorphic locus containing at least two linked genes, one specifically expressed in the pistil and the other in the pollen (Kao and Tsukamoto, 2004). Pollen tube growth is arrested in the style, whenever the single *S* allele expressed in the haploid pollen matches one of the two *S* haplotypes expressed in the diploid pistil tissue (de Nettancourt, 1977). The pistil component of SI in Rosaceae, Solanaceae and Plantaginaceae has been determined to be an *S*-RNase (McClure et al. 1989). The candidate gene for the pollen component in almond (*Prunus amygdalus* Batsch) has been recently identified to be *SFB* by Ushijima et al. (2003), showing a tight linkage with the *S*-RNase gene. In spite of the knowledge on the genetic structure of the female and male determinants of SI, the nature of their interaction remains unclear. Knowledge of the three-dimensional (3D) structure may help in discovering the recognition mechanism in rosaceous S-RNases at the molecular level and also understand how proteins are involved in the GSI function and fulfil their biological roles.

MATERIAL AND METHODS

S$_f$-RNase (AB467371) and *S*$_8$-RNase (AB481108) sequences were obtained from the almond cultivar 'Blanquerna'.After selecting the 3D structure of the RNase MC1 mutant N71S as the most suitable candidate template, the adjustments between the sequence alignments were performed manually in order to minimize the number of gaps and INDELS. Construction of the 3D model were generated by using MODELLER 9v5. The models with the lowest value of the Modeller objective function were chosen for further refinement. The stereochemical quality and overall G-factors of the protein structures were calculated by using PROCHECK. Additionally, the compatibility of the sequence with the overall fold was estimated using PROSAIIv3. Finally, the molecular graphics were generated with PYMOL.

RESULTS AND DISCUSSION

The 3D structure of the *S*$_f$- and the *S*$_8$-RNases are shown in Fig. 1. The overall dimensions of the molecule were approximately 40 x 50 x 30 Å. The structure belonged to the α and β class, with six helices and six beta-strands. The folding topology of its main chain was very similar to the topologies of the RNase T_2 family enzymes. The *S*$_f$ structure presented a loop region longer than in the *S*$_8$ structure, which connected α helices and β strands. It is well known that extended loops are in many cases susceptible to proteolytic degradation (Branden and Tooze, 1998). Thus, it may be possible that the extended loop present in the *S*$_f$-RNase 3D structure could be prone to degradation and/or inactivation and, as a consequence, allowing its pollen tube growth trough its own pistil. However, further studies are required in order to ascertain the possible role of this loop region involved in the SI mechanism.

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Literature Cited

Branden, C. and Tooze, J. 1998. Introduction to Protein Structure, 2nd Edition, Garland Publishing, New York, 410 p.

de Nettancourt, D. 1977. Incompatibility in Angiosperms. Springer-Verlag, Berlin-Heidelberg-New York.

Kao, T. and Tsukamoto, T. 2004. The molecular and genetic bases of *S*-RNase based self-incompatibility. Plant Cell 16:S72-S83.

McClure, B.A., Haring, V., Ebert, P.R., Anderson, M.A., Simpson, R.J., Sakiyama, F. and Clarke, A.E. 1989. Style self incompatibility gene products of *Nicotiana alata* are ribonucleases. Nature 342:955-957.

Ushijima, K., Sassa, H., Dandekar, M.A., Gradziel, T.M., Tao, R. and Hirano, H. 2003. Structural and transcriptional analysis of the self-incompatibility locus of almond: identification of a pollen-expressed F-box gene with haplotype-specific polymorphism. Plant Cell 15:771-781.

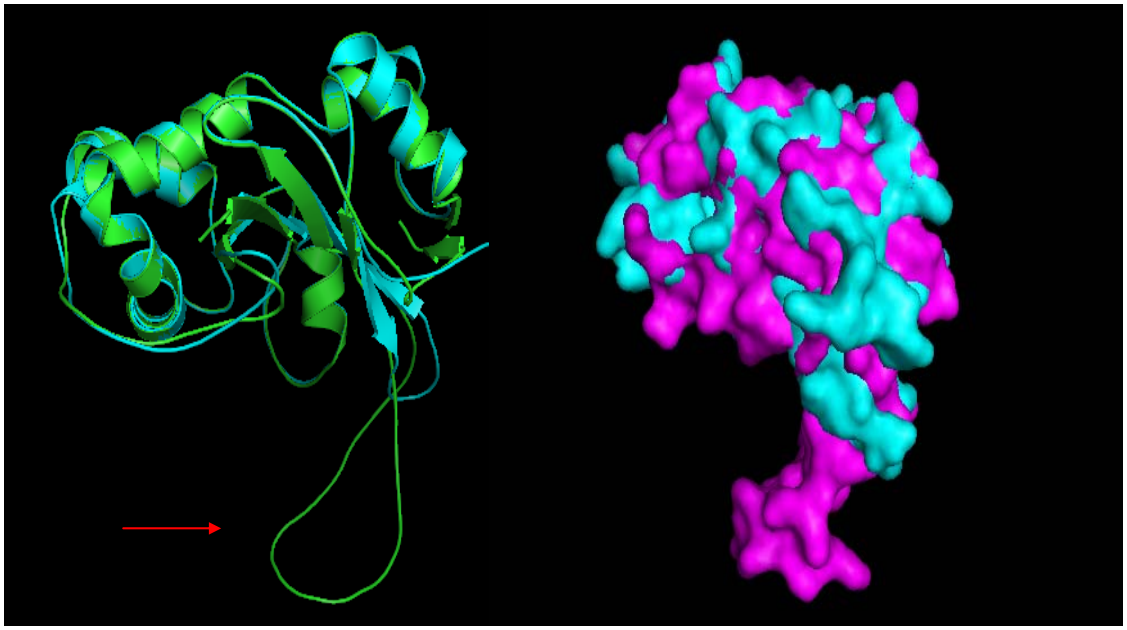

Fig. 1: Cartoon diagram and surface representations of the modelled structure of almond S_f-RNase (cyan cartoon and surface) and S_8-RNase (green cartoon and pink surface), showing secondary structural elements and surface morphologies.