Molecular modeling of S-RNases involved in almond self-incompatibility

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INTRODUCTION

Most almond (Prunus amygdalus Batsch) cultivars are self-incompatible (SI, Social i Company, 1999). SI in the Prunus species shows the gametophytic self-incompatibility (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 haplo-types expressed in the diploid pistil tissue. The pistil component of SI in Rosaceae, Solanaceae, and Plantaginaceae has been determined to be an S-RNase (McCure et al., 1989). The Prunus S-RNase is of the T1 type (Igic and Kohn, 2001), with five conserved domains (Cl, C2, C3, RC4, and C5) and one hyper-variable region (Sassa et al., 1997). The candidate gene for the pollen component in almond has been identified to be an SFB by Ushijima et al. (2003), showing a tight association with the S-RNase gene (Ikeda et al., 2005).

Gametophytic self-incompatibility (GSI) is a mechanism in flowering plants, to prevent inbreeding and promote outcrossing. GSI is under the control of a specific locus, known as the S-locus, which contains at least two genes, the RNase and the SFB. Active S-RNases in the style are essential for rejection of haploid pollen, when the pollen S-allele matches one of two S-alleles of the diploid pistil. However, 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 the proteins involved in GSI may function and how they fulfill their biological roles. To this end, 3D models of the SC (S) and two SI (S1 and S2) S-RNases of almond were constructed, using comparative modeling tools. The modeled structures consisted of mixed α and β folds, with six helices and six β-strands. However, the self-compatible (S) RNase contained an additional extended loop between the conserved domains RC4 and C5, which may be involved in the manifestation of self-compatibility in almond.

Keywords: almond, self-(in)compatibility, 3D modeling, RNase T2

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Consequently, our objective was to identify the 3D structures of the almond S-RNases and SFBs through molecular modeling tools and to investigate a link between their 3D structures and the SI mechanism.

MATERIALS AND METHODS
Three different S-RNases from two almond cultivars were modeled because their sequences and physiological activity were available (Fernández i Martí et al., 2009). The S-RNase sequences have been deposited in the EMBL/DEJ/GenBank under AB467371 (S$_5$-RNase from “Blanquerna”), AB481108 (S$_8$-RNase from “Blanquerna”), and AB488496 (S$_{23}$-RNase from “Vivot”).

The modeling procedure started with the alignment of the sequence to be modeled (target) with related known 3D structures (template) derived from the Protein Data Bank (PDB) using FASTA and BLAST (EMBL nucleotide database). In this procedure, the template to be selected among all possibilities must show the highest identity with the target, at least higher than 35%. The coordinates of this template protein were used as a template for further modeling.

Once the best candidate template was selected, the sequence adjustment between the S-RNase sequences and the template was performed manually to minimize the number of gaps and insertions/deletions. The frame of the 3D model was constructed by MODELLER 9v5. A total of 40 models were constructed for each S-RNase. The four models with the lowest value of the Modeller objective function were chosen for further refinement. Energy function was evaluated through PROSAII (Sippl, 1993). This program detects errors in protein structures and thus serves to indicate their quality.

On the other hand, stereo-chemical quality and the overall G-factors of the protein models were calculated using PROCHECK (Laskowski et al., 1993). This software compares the residue-by-residue geometry of a set of closely related structures. The models with lower number of amino acid residues in disallowed regions were selected as the most suitable models. A Ramachandran plot (also known as a Ramachandran map or a Ramachandran diagram) outputted by PROCHECK visualizes dihedral angles ψ against ϕ of amino acid residues in the protein structure, thus showing the possible conformation of ψ and ϕ angles for a polypeptide (Ramachandran et al., 1963).

During a further modeling, the loop refinement protocol was used to generate 40 new models from the previously best model. The same steps as described above were followed, selecting the best four models according to their lowest values of the Modeller objective function, and then selecting the...
“best of the best” from the results obtained by PROSAIIv3 and PROCHECK. Finally, the molecular graphics were generated with PYMOL, which visualizes protein structures (http://www.pymol.org).

RESULTS AND DISCUSSION

The 3D models of the Sf, S8, and S23 RNases were compared with the related known 3D structures derived from PDB. The best candidate template selected was the RNase MC1 mutant with accession 1J1G (Numata et al., 2003), because the identity between this template and the target sequences was 42%. On the other hand, the SFBf, SFB8, and SFB23 models could not be generated because sequence identity higher than 30% was not found in PDB.

Protein structures represent combinations of secondary structural elements, α-helices and β-strands that are inter-connected by loops. These structural elements form the core regions (the inside of the molecule) and are connected by loop regions on the protein surface with surface-exposed α-helices and β-strands. The structure of the S-RNases belonged to the α and β class, with six α-helices and six β-strands connected by loops. The folding topologies of its main chains were very similar to the topologies of the RNase T2 family enzymes. Their overall dimensions were approximately 40 Å × 50 Å × 30 Å.

Ramachandran plot statistics for the S-RNases showed than 97% amino acid residues were positioned in the “allowed” regions. In fact, when structures place 95–97% or more of the amino acid residues in the “allowed” positions, they are considered to be reliable in modeling experiments, and this indicates how well the structures fits with the expected main chain length and torsion angle distributions (Laskowski et al., 1993; Kleywegt and Jones, 1996). The best four models of the Sf, S8, and S23 RNases were selected for further modeling. As shown in Figure 1, in the model sfi1_BL00040001 (Figure 1A), all residues were positioned in the allowed region (red arrow), whereas in the model sfi1_BL00010001 (Figure 1B), 1.6% of the residues were in a disallowed region (green arrow). Thus, the model BL00040001 was selected as the best model to be analyzed. Higher numbers of residues in the disallowed region reflect a distorted geometry in the models, because there are higher proportions of residues falling outside the limits of main chain bond length and torsion angles derived from the small molecule library (Engh and Huber, 1991). These results indicate that our models were optimal.

When the three S-RNases were superpositioned, the Sf-RNase structure contained an additional extended loop, which was not present in the S8 or S23 models. This loop, shown in Figures 2 and 3 contained the amino acid residues CKG NPQ RQA KSQ...
PKN RGK SQP SQQ ATT QFL, which were placed between the conserved domains RC4 and C5. Through the software Pymol, it has been possible to visualize which amino acid residues comprised α-helices, β-strands, and loops. It has been suggested that loops in 3D structures serve to interconnect α-helices and β-strands, and also that longer surface-exposed loops could be susceptible to proteolytic degradation (Branden and Tooze, 1998). As the main structural difference found between the Sf-, Sr-, and Sd-RNases resides in the presence of this “extended looping region,” this long loop could be prone to degradation and, as a consequence, this S-RNase could be less stable. As a result of this possible degradation, the pollen tube could grow through its own pistil giving rise to SC.

Additionally, the 3D models of the Sf-, Sr-, and Sd-RNases were compared with that of another Rosaceous species, the Pyrus pyrifolia Sf-RNase (Matsuura et al., 2001). The structure of the pear Sr-RNase was consistent with the models of the almond Sd and Sf RNase. The fact that both the pear and almond S-RNases confer SI, and that their models did not contain this extended loop, the main structural differences between the SI and the SC RNases could reside in the presence of the loop. Therefore, the amino acid residues that form the extended loop positioned at the surface of the Sf-RNase (Figure 2), between the conserved domains RC4 and C5, could be responsible for the differences in function of RNAs.

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