X-ray crtystal structure analysis of Japanese Pear S_3-RNase

Takanori MATSUURA[1], Hiroaki SAKAI[1], Masaki UNNO[1], Shigemi NORIOKA[*1]
[1]Institute for Protein Research, Osaka Univ., 3-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Introduction

Self-incompatibility is a mechanism that prevents self-fertilization in flowering plants. Rosaceous species such as Japanese pear and apple have gametophytic self-incompatibility controlled by a single, multi-allelic locus, the *S*-locus.

When a pollen grain lands on a stigma, a discrimination process takes place as to whether the *S*-allele of the pollen matches one of the two *S*-alleles of the pistil. The pollen grain germinates on the stigma and grows toward the embryo; but, if its *S*-allele matches one of the *S*-alleles of the pistil, pollen tube growth is arrested in the style, and no fertilization take places. A pistil-specific protein encoded by the *S*-locus has been shown to be a ribonuclease (S-RNase) that recognizes the pollen *S*-allele. Two models for the *S*-allele-specific inhibition of pollen tube growth involving S-RNase, the *S*-allele-specific uptake and RNase inhibitor models, have been proposed by researchers working on self-incompatibility, but at present there is not enough evidence to support either model. How S-RNase discriminates between self- and nonself-pollen, what the counterpart molecule interacting with S-RNase is, and how S-RNase interacts with that molecule have yet to be clarified.

In order to elucidate the mechanism of the S-RNase-based gametophytic self-incompatibility at molecular level, we have been studying the structure and function of Japanese pear S-RNases and searching unknown pollen *S*-product(s).

Results

The crystals of Japanese pear S_3-RNase belong to the space group of $P2_1$ with unit cell dimensions of a=45.65, b=52.60, c=47.57 Å, and β=106.5-[1]. The native diffraction data were collected up to a 1.5 Å resolution. Since the Japanese pear S_3-RNase has sequence homology of 28% with RNase MC1 from bitter gourd, we had tried to solve the crystal structure by the molecular replacement method using the model coordinates of RNase MC1. However, in spite of the extensive search, any promising MR solution did not resulted in interpretable electron density map. Thus, the multiple isomorphous replacement method was employed to solve the crystal structure of S_3-RNase. Two kinds of ethylmercurithiosalicylic acid derivatives, one mercury(II) ammonium thiocyanate derivative and one lead(II) acetate derivative were prepared by soaking the crystals in heavy atom reagents. All diffraction data of derivative crystals were collected using the CCD detector Quantum 4R (ADSC) at the beam line of BL6A at Photon Factory and they were processed by the programs MOSFLM and SCALA.

In order to locate the heavy atom coordinates for each derivative, the Patterson maps were automatically interpreted by the program SOLVE. Initial phase calculation was done by the program SHARP and the phases were improved by the program DM. Subsequently, the main chain trace was done by wARP. Based on the automatically traced main chain coordinates, a total of 200 amino acid residues were successfully built by the program O. X-ray crystallographic refinement was carried out by the program CNS. The solvent water molecules were picked up from the difference Fourier map using the automated scripts implemented in CNS. The lower cut off level to pick up solvent water molecules in the residual electron densities, was set to 3σ.

The Japanese pear S_3-RNase has two sugar chain binding sites, one is Asn18 and the other is Asn116. Part of the additional sugar chain identified as two N-Acetyl-Glucosamine (NAG) residues was clearly visible at Asn116 in the difference Fourier map and modeled to fit the densities. On the other hand, no clear electron densities corresponding to sugar moiety were observed at Asn18, suggesting the highly disordered conformation. The crystal structure was refined to and R- and free R-factor of 18.4%, 20.9%, respectively in the resolution range of 500-1.5 Å.

The paper will be published in the near future.

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References

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*norioka@protein.osaka-u.ac.jp