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Crystallization and preliminary X-ray crystallographic analysis of *S*-allelic glycoprotein S_{F11}-RNase from *Nicotiana alata*

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Nicotiana alata S_{F11}-RNase is an *S*-glycoprotein associated with gametophytic self-incompatibility. Crystals of S_{F11}-RNase have been grown at room temperature using polyethylene glycol as a precipitant. A crystal diffracted to better than 1.4 Å resolution at 100 K at the SPring-8 synchrotron-radiation source, indicating that it is very suitable for high-resolution structure analysis. The crystal belongs to the space group $P2_1$, with unit-cell parameters a = 65.86 (11), b = 44.73 (5), c = 64.36 (7) Å, β = 90.27 (9)°. The asymmetric unit contains two monomers, giving a crystal volume per protein mass (V_M) of 2.05 Å^3 Da^{-1} and a solvent content of 39.6% by volume. A full set of X-ray diffraction data was collected to 1.55 Å resolution with a completeness of 97.4%. A heavy-atom derivative has been successfully prepared with ethylmercury thiosalicylate (EMTS) and structure analysis is in progress.

1. Introduction

Self-incompatibility in hermaphrodite flowering plants is a mechanism for avoiding self-fertilization through rejection of self and related pollen by the pistil. Self-incompatibility is controlled by a single *S*-locus with multiple alleles. When the haploid *S*-allele of pollen matches one of the diploid *S*-alleles of the pistil, pollen-tube growth is arrested in the pistil and no fertilization takes place. In gametophytically self-incompatible plants from the Solanaceae, Rosaceae and Scrophulariaceae, glycoproteins with ribonuclease activity encoded by *S*-locus (*S*-RNases) and specifically expressed in the style have been identified (McClure *et al.*, 1989; Xue *et al.*, 1996; Ishimizu *et al.*, 1998) as members of the RNase T_2 family (Kawata *et al.*, 1990; for reviews, see Dodds *et al.*, 1996; Kao & McCubbin, 1996). Functional analyses using transgenic plants have shown that *S*-RNase is responsible for the self-incompatibility response that discriminates between pollen bearing different *S*-alleles (Lee *et al.*, 1994; Murfett *et al.*, 1994) and that active ribonucleases are essential for rejection of self pollen by the pistil (Huang *et al.*, 1994). However, the mechanism of *S*-allele discrimination between pollen and *S*-RNase remains obscure, as the pollen *S*-gene product that interacts with the *S*-RNase is not yet known.

The primary structures of multiallelic *S*-RNases share conserved and hypervariable (HV) regions in the Solanaceae (Ioerger *et al.*, 1990) and the Rosaceae (Ishimizu *et al.*, 1998). It is likely that amino-acid residues in the HV regions are responsible for the discrimination of *S*-alleles in the self-incompatibility response. In fact, four amino-acid substitutions in the HV region of the *Solanum chacoense* S_{11}- and S_{13}-RNase have been reported to be sufficient to discriminate between two different *S*-alleles *in vivo* (Matton *et al.*, 1997). Furthermore, *Pyrus pyrifolia* (Rosaceae) S_3- and S_5-RNases differ in only two amino-acid positions in the HV region (Ishimizu *et al.*, 1998). These facts support the idea that the higher order structure of the HV region is probably involved in the discrimination of self pollen from non-self pollen by the pistil.

To elucidate the precise molecular mechanism of *S*-allele discrimination between the pollen and the pistil, we initiated a three-dimensional structure analysis of *N. alata* S_{F11}-RNase. This protein was chosen because it has a single N-glycosylation site occupied by a previously characterized glycan chain (Oxley & Bacic, 1995). As the first step of structure analysis, we report here preliminary X-ray crystallographic data as well as the crystallization conditions of S_{F11}-RNase from *N. alata*.

2. Experimental

2.1. Purification and crystallization

S_{F11}-RNase from homozygous *N. alata* was prepared by the method of McClure *et al.* (1989) with slight modifications. Briefly, hydroxyapatite chromatography was carried out as the final purification step. The S_{F11}-RNase fraction eluted as a single peak was collected, dialyzed against 100 mM cacodylate buffer pH 6.5 and concentrated to 20 mg ml^{-1} in a Centricon-10 concentrator (Amicon) at

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Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (1.61–1.55 Å).

Resolution (Å)	65.94–1.55
No. of observations	237209
Unique reflections	53199
Data completeness (%)	97.4 (95.9)
Average $I/\sigma(I)$	14.4
$R_{\rm merge}$† (%)	6.1 (30.7)

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h, $\sum_h$ is the sum over all measured reflections and $\sum_i$ is the sum over i measurements of a reflection.

277 K. Protein concentration was estimated based on the absorbance at 280 nm by assuming a 10 mg ml^{-1} concentration to correspond to an A_{280} of 16.7 for a 1 cm light path. Crystallization was performed with the hanging-drop vapour-diffusion method using 24-well tissue-culture plates (Hampton Research) at 293 K. Each hanging drop, prepared by mixing 2 µl each of the protein solution and the reservoir solution, was placed over a 0.4 ml reservoir solution.

2.2. Collection of X-ray diffraction data

The diffraction data were collected at 100 K on a Rigaku R-AXIS IV image-plate detector coupled to synchrotron radiation at beamline BL45-XU of the SPring-8 (Yamamoto *et al.*, 1998). The synchrotron X-ray wavelengths were 1.0086 Å for the form I crystal and 1.0200 Å for the form II crystal. The raw data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). Systematic extinctions in the intensity data were checked using the program *HKLVIEW* (Collaborative Computational Project, Number 4, 1994).

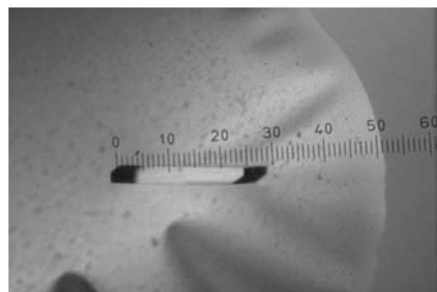

Figure 1
A monoclinic form II crystal of *N. alata* S_{F11}-RNase. Approximate dimensions of the crystal are 0.3 × 0.1 × 0.05 mm.

3. Results

Two crystal forms (form I and II) grown under similar conditions at pH 6.5 were obtained at room temperature after 1–7 d in 100 m*M* cacodylate buffer pH 6.5 containing 28–34%(*w*/*w*) PEG 6000 and 0.8 *M* NaCl. They are needle-like crystals with almost identical dimensions (Fig. 1). Both belong to the monoclinic system, with space group $P2_1$. The form I crystal diffracts to 2.7 Å resolution and has unit-cell parameters a = 75.48 (7), b = 79.38 (4), c = 67.25 (1) Å, β = 90.15 (2)°. The presence of four monomers with a relative molecular mass of 23 005 (excluding carbohydrate) in the asymmetric unit gives a crystal volume per protein mass ($V_{\rm M}$) of 2.18 Å^3 Da^{-1} and a solvent content of 43.1%. These values lie within the range usually found for protein crystals (Matthews, 1968).

The unit-cell parameters of the form II crystal were determined to be a = 65.86 (11), b = 44.73 (5), c = 64.36 (7) Å, β = 90.27 (9)°. The presence of two monomers in the asymmetric unit gives a crystal volume per protein mass ($V_{\rm M}$) of 2.05 Å^3 Da^{-1} and a solvent content of 39.6%, which lie within the ranges usually found for protein crystals (Matthews, 1968). The form II crystal was suitable for high-resolution crystal structure analysis, as it gives sharp diffraction beyond 1.4 Å resolution. Table 1 summarizes the statistics for data collection from the form II crystal.

Attempts to prepare heavy-atom derivatives for phase determination have been performed. A heavy-atom derivative has been successfully prepared with the heavy-atom reagent ethylmercury thiosalicylate (EMTS). Structure determination by the multiple-wavelength anomalous diffraction (MAD) method is in progress.

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