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Crystallization and preliminary X-ray diffraction studies of a novel killer toxin from a halotolerant yeast *Pichia farinosa*

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Abstract

A killer toxin from a halotolerant yeast, *Pichia farinosa* strain KK1, was crystallized at high- and low-salt concentrations. Crystals from the high-salt solution belonged to the tetragonal space group $P4_12_12$ or $P4_32_12$, with unit-cell dimensions of $a = b = 81.10$, $c = 118.46$ Å. The low-salt solution provided crystals that belonged to the same space group, with nearly same cell dimensions. Preliminary diffraction studies showed that the intensity distributions are significantly different between the two crystals. Both types of crystals contained either two or three molecules per asymmetric unit. They diffracted X-rays beyond 2.0 Å resolution and were stable to X-ray irradiation.

1. Introduction

Killer toxin is a proteinous toxin in yeast that kills other sensitive strains. While many distinct killer toxins are known, they have no sequence similarity. One of the well studied killer toxins is the K1-toxin from *Saccharomyces cerevisiae*. It has been assumed that the killing mechanism of the K1-toxin involves damage to the cytoplasmic membrane through the formation of cation channels (Martinac *et al.*, 1990). Recently, the crystal structure of the KP4-toxin from a fungal pathogen of maize *Ustilago maydis*, which has no similarity in chemical properties to the SMK-toxin, was reported. Structural and physiological analyses suggested that the killing mechanism of the KP4-toxin may result from the inhibition of calcium channels (Gu *et al.*, 1995).

A halotolerant yeast, *Pichia farinosa* strain KK1, produces a unique killer toxin termed SMK-toxin (salt-mediated killer toxin), which shows maximum killing activity in the presence of 2 *M* NaCl (Suzuki & Nikkuni, 1989). SMK-toxin consists of two non-covalently associated α (63 residues) and β (77 residues) subunits. The molecular weight of the mature toxin is calculated to be 14 214 from its amino-acid sequence. SMK-toxin is stable only at acidic pH (below 4.5), and the subunits dissociate at a pH above 6.0. Although there is no sequence similarity between the SMK- and K1-toxins, they are somewhat similar in their size, subunit construction, and hydropathy profile. Thus, the SMK-toxin may function in a manner similar to that of the K1-toxin (Suzuki & Nikkuni, 1994), probably through making channels in the cytoplasmic membrane. The reason why the killing activity has the salt dependency is still unclear.

We attempted an X-ray crystal structure analysis of the SMK-toxin to elucidate its salt-mediated killing mechanism. This report describes the crystallization and preliminary X-ray diffraction studies of the SMK-toxin. In order to compare the three-dimensional structures under both high- and low-salt conditions, we crystallized the SMK-toxin under the both conditions.

2. Experimental and results

SMK-toxin was isolated and purified by the procedure described previously (Suzuki & Nikkuni, 1994). The solution of purified SMK-toxin contained 15 mg ml^{-1} protein, 100 m*M* NaCl and 20 m*M* citrate–phosphate buffer, pH 3.5.

We first tried to crystallize the SMK-toxin by salting-out using ammonium sulfate at low pH, because the SMK-toxin is stable only at acidic pH and shows its killer activity only at high salt concentrations. Various conditions were examined by microdialysis method within a matrix of ammonium sulfate concentration (2.3–2.9 *M*) *versus* pH (2.5–4.0). 10 µl of the protein solution (15 mg ml^{-1}) were dialyzed at 293 K against 3 ml of the reservoir solution, which contained ammonium sulfate and an appropriate buffer (20 m*M* tartarate–NaOH for pH 2.5 and 3.0; 25 m*M* formate–NaOH for pH 3.5 and 4.0). After an incubation for about 10 d, tetragonal bipyramidal crystals were obtained at pH 3.5 and 4.0, whereas only an amorphous precipitate was formed at the lower pH. The nucleation of the crystals was apparently too fast at pH 4.0. Crystals and amorphous precipitates were produced simultaneously under a condition of 2.9 *M* ammonium sulfate at pH 3.5. The best result was obtained in the presence of 2.7 *M* ammonium sulfate at the same pH (Fig. 1). Then, we tried to reproduce these crystals, using the hanging-drop vapor-diffusion method. A 10 µl droplet containing protein was equilibrated with 1 ml of reservoir solution at 293 K. Equal volumes of the protein solution (15 mg ml^{-1}) and the reservoir solution

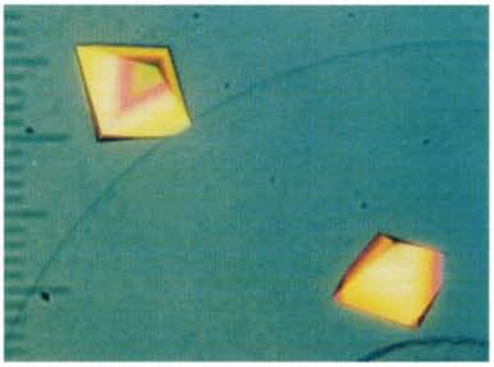

Fig. 1. Tetragonal bipyramidal crystals of the SMK-toxin. The photograph was taken by the use of a polarization microscope. Four divisions of the scale correspond to 0.1 mm.

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Table 1. *Comparison between the crystals from two different conditions*

	High salt	Low salt
Crystallization conditions*	2.7 *M* ammonium sulfate, 0.1 *M* glycine–HCl (pH 3.4–4.0)	25–30% PEG 4000–8000, 0.2 *M* ammonium sulfate, 0.1 *M* glycine–HCl (pH 4.0)
Crystal parameters		
Space group	$P4_12_12$ or $P4_32_12$	
Cell dimensions (Å)	$a = b = 81.10$, $c = 118.46$	$a = b = 81.12$, $c = 118.68$
R factors†	DIP data (5.5%‡) ↔ 10.1%§ ↔ DIP data (4.9%‡)	
	4.7%§ ↕	
	PF data (4.6%‡) ↔ 11.0%§ ↔ DIP data (4.9%‡)	

* The hanging-drop method was used. The conditions of reservoir solutions are shown. † The resolution range of used reflections is 50–2.6 Å. DIP data and PF data were collected using a MAC-science DIP100S diffractometer and the screenless Weisenberg camera at the Photon Factory, respectively (see text). ‡$R_{\rm merge} = \sum_h \sum_i |I_{hi} - \langle I_{hi}\rangle| / \sum_h \sum_i |I_{hi}|$, where h specifies unique reflection indices, and i indicates symmetry-equivalent observations of h. §$R_{\rm iso} = \sum_h |F_A - F_B| / \sum_h F_A$, where A and B represent distinct data sets. Replacing from F_A to F_B in the denominator had almost no effect to results.

(2.7 *M* ammonium sulfate, 0.1 *M* glycine–HCl, pH 3.4–4.0) were mixed to make the droplet. The buffer was changed from formate to glycine, which is more suitable for the preparation of heavy-atom derivatives. This procedure allowed the growth of the same type of crystal as that obtained by the microdialysis method.

Crystallization of the SMK-toxin under low-salt conditions was performed by the hanging-drop method using polyethylene glycol as a precipitant. Under a low-salt condition, tetragonal bipyramidal crystals similar to those produced under the high-salt conditions were grown. The crystallization procedure was the same as that in the high-salt case, except for the reservoir solution, which contained 25–30% polyethylene glycol 4000–8000, 0.2 *M* ammonium sulfate, and 0.1 *M* glycine–HCl, pH 4.0.

Preliminary crystal data were obtained with the precession photographs recorded using Cu $K\alpha$ radiation from a Rigaku RU300 rotating-anode generator. Intensity data were recorded using a MAC-Science DIP100S diffractometer with Cu $K\alpha$ radiation from an M18X rotating-anode generator, and also using the screenless Weissenberg camera (Sakabe, 1991) with 1 Å synchrotron radiation at beamline 6A, the Photon Factory, Tsukuba, Japan. The processing of the diffraction data was performed with the *DENZO* and *SCALEPACK* programs (Otwinowski, 1993). The approximate size of the crystals used for the diffraction studies was 0.2 × 0.2 × 0.2 mm. All of the diffraction experiments were performed at room temperature.

Crystals from both high and low salt conditions diffract X-rays beyond 2.0 Å resolution and are stable to X-ray irradiation. The overall merging *R* factors for the native data with $50 \geq d \geq 1.8$ Å and $I \geq \sigma(I)$ were 6–7%. The comparison between the two crystals is summarized in Table 1. The symmetry and systematic absences indicate that both crystals belong to the tetragonal space group $P4_12_12$, or its enantiomorph $P4_32_12$. Assuming that two or three molecules are present in an asymmetric unit, the V_m values are calculated to be 3.4 or 2.3 $Å^3\,Da^{-1}$, respectively, which are within a reasonable range for protein crystals (Matthews, 1968). Cell dimensions of both crystals are practically indistinguishable considering their standard deviations (data not shown). In order to evaluate the difference between the two types of crystals, *R* factors between two distinct data sets ($R_{\rm iso}$) were calculated with various combinations. As shown in Table 1, the values of $R_{\rm iso}$ between two data sets from different types (10–11%) are significantly higher than that from the same type (4.7%), while the merging *R* factors of individual data sets are similar to each other (4.6–5.5%). These results demonstrate that the two types of crystals have different structures. However, the difference is not so large as to be reminiscent of global intra/intermolecular conformational changes. Careful investigation of the crystal structures from the both conditions might elucidate the salt-dependent killing mechanism of the SMK-toxin. We are now preparing heavy-atom derivatives for phasing by the multiple isomorphous replacement method.

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