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Crystallization and preliminary crystallographic study of rBmKαIT1, a recombinant α-insect toxin from the scorpion *Buthus martensii* Karsch

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α-Insect scorpion toxins are a distinct group of scorpion neurotoxins for which no crystal structures are yet available. A novel α-insect toxin named BmKαIT1 from the scorpion *Buthus martensii* Karsch (BmK) has been expressed as an inclusion body in *Escherichia coli* and purified by chromatography after renaturation. Recombinant BmKαIT1 (rBmKaIT1) was crystallized using the vapour-diffusion technique in hanging drops at 296 K. The crystals, which were grown in sodium phosphate, belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 30.24 (1), b = 36.51 (3), c = 57.08 (2) Å. Diffraction data were collected to 2.1 Å resolution using synchrotron radiation. There appears to be one rBmKαIT1 molecule in the asymmetric unit.

1. Introduction

Long-chain scorpion toxins are known to specifically interact with the voltage-gated sodium channel. They belong to a well defined family of homologous proteins that contains 60–70 amino-acid residues cross-linked by four disulfide bonds. These toxins are highly specific to mammals, insects or crustaceans (Possani, 1984; Gordon *et al.*, 1998). They can be divided into two major classes: α-toxins and β-toxins (Gordon *et al.*, 1998; Gurevitz *et al.*, 1998; Goudet *et al.*, 2002). Scorpion α-toxins bind to site 3 on sodium channels in a voltage-dependent manner. They slow or inhibit the sodium-current inactivation and thus induce prolongation of action potentials. The α-toxins can be further divided into three groups: (i) the classic α-toxins, which interact highly specifically with mammals, (ii) the insect α-toxins, which are highly active towards insects, and (iii) the α-like scorpion toxins, which are active towards both mammals and insects. Scorpion α-toxins are studied as a pharmacological probe for analyzing the channel kinetics, gating mechanisms and structural properties as well as the pathophysiology of sodium-channel-related diseases.

The three-dimensional structures of some scorpion toxins have already been characterized by NMR or X-ray diffraction (Possani *et al.*, 1999; He *et al.*, 1999). It seems that all the scorpion toxins have a similar structural fold, characterized by the presence of a common core consisting of an α-helix packed against a three-stranded antiparallel β-sheet. Although the structures are homologous, they showed differences in some regions which are proposed to be responsible for their differential binding to the voltage-gated sodium channel. Therefore, it is important to elucidate the structures of different types of scorpion toxins, including α-insect toxins. The solution structure of an α-insect toxin, LqhαIT, has been solved (Tugarinov *et al.*, 1997); however, no crystal structure of an α-insect toxin is available.

BmKαIT1, a toxin from *B. martensii* Karsch venom, was first isolated and characterized by Wu and coworkers and shows a high specificity towards insects (Wu *et al.*, 1999). It shows a high sequence similarity to the previously described α-insect toxin LqhαIT. The encoding amino-acid sequence BmαTX12 from the cDNA (Genbank accession No. AF151796) is nearly the same as BmKαIT1, except for an amino-acid difference at residue 29 (Zhu *et al.*, 2000). In BmKαIT1 this residue is aspartic acid, while in BmαTX12 it is asparagine. Our previous work has shown that only the D29N mutant of the recombinant BmKαIT1 can be obtained from renaturation of the inclusion body and regain full activity as the natural toxin, while the recombinant toxin of the original sequence could not be refolded properly under the same conditions (unpublished work). Based on our results, we assumed that the amino-acid sequences of BmKαIT1 and BmαTX12 are the same; in other words, residue 29 in BmKαIT1 should be asparagine. Another possibility is that a deamidation reaction occurs after the protein is correctly folded *in vivo* (Wright, 1991).

Preparation of a sufficient amount of highly pure and properly refolded recombinant toxin is difficult, probably owing to the multiple cross-linking possibilities of the four intramolecular disulfide bonds. In this paper, we

Figure 1
Crystals of rBmKαIT1.

describe the first crystallization and preliminary X-ray diffraction analysis of the α-insect toxin rBmKαIT1.

2. Expression and purification

The recombinant toxin rBmKαIT1 with an additional Met at its N-terminus was expressed as an inclusion body in *Escherichia coli* strain BL21(DE3). After cell lysis, the inclusion body was collected and washed with 1% Triton X-100, 50 m*M* Tris–HCl pH 7.5, 2.5 m*M* EDTA and 20 m*M* β-mercaptoethanol and then dissolved in 6 *M* guanidine hydrochloride, 50 m*M* Tris–HCl pH 7.5, 2.5 m*M* EDTA and 0.2 *M* DTT; this was followed by dialysis against 4 *M* guanidine hydrochloride pH 2.0. Renaturation occurred when the denatured solution was rapidly diluted to 50 m*M* Tris–HCl pH 7.5 and 50 m*M* KCl. The oxidation of the disulfide bonds was traced by the Ellman reaction. The renatured protein was concentrated and purified by gradient elution with acetonitrile on a RP-HPLC column. The purity of the recombinant toxin was characterized by both SDS–PAGE and ESI–MS. The molecular weight was determined to be 7310.63 $\pm$ 1.07 Da (calculated value of 7310.5 Da; data not shown), indicating the presence of the additional methionine at the N-terminus.

3. Crystallization of rBmKαIT1

The lyophilized rBmKαIT1 sample was dissolved to a final concentration of 10 mg ml^{-1} in 10 m*M* acetic acid containing 0.02%(*w*/*v*) NaN_3 pH 4.2. Crystallization was carried out using the hanging-drop vapour-diffusion method. 4 µl of protein solution was mixed with an equal volume of reservoir solution and was equilibrated against 1.0 ml reservoir solution consisting of 1.0 *M* Na_2HPO_4, 0.02%(*w*/*v*) NaN_3 and 5.0%(*v*/*v*) dioxane adjusted to pH 5.9 using HCl. Colorless needle-shaped crystals were obtained after 3 d at 296 K (Fig. 1), with maximum dimensions of 0.02 $\times$ 0.04 $\times$ 0.40 mm.

Table 1
Diffraction data statistics.

Standard deviations are given in parentheses. Values in square brackets refer to the highest resolution shell (2.24–2.11 Å).

X-ray source	Photon Factory, BL-18B
Detector	ADSC Quantum 4R CCD
X-ray wavelengh (Å)	1.000
Temperature (K)	293
Space group	$P2_12_12_1$
Unit-cell parameters	
a (Å)	30.24 (1)
b (Å)	36.51 (3)
c (Å)	57.08 (2)
Resolution limit (Å)	2.11
Total reflections	26193 [4044]
Unique reflections	3971 [622]
Observed reflections $[I/\sigma(I) > 2]$	3495 [514]
$R_{\rm merge}$† (%)	10.4 [19.9]
Completeness (%)	99.9 [99.9]
Completeness $[I/\sigma(I) > 2]$ (%)	88.1 [82.6]
Multiplicity	6.5 [6.5]
$\langle I/\sigma(I)\rangle$	5.0 [3.5]

† $R_{\rm merge} = \sum_{hkl}\sum_i |I(hkl)_i - \langle I(hkl)\rangle| / \sum_{hkl} I(hkl)$, where $I(hkl)_i$ is the *i*th measurement of the intensity of reflection *hkl* and $\langle I(hkl)\rangle$ is the mean intensity of reflection *hkl*.

4. Data collection and processing

A crystal of rBmKαIT1 was sealed in a quartz capillary (Hilgenberg). Data collection was performed on beamline BL18B of the Photon Factory at the High Energy Acceleration Research Organization, Tsukuba, Japan using an ADSC Quantum 4R CCD camera (Watanabe *et al.*, 1995) at 293 K. Image data were processed with *DPS* (Rossmann & van Beek, 1999) and data sets were scaled and merged using *SCALA* (Collaborative Computational Project, Number 4, 1994). Diffraction data statistics are summarized in Table 1. The space group was determined to be orthorhombic, space group $P2_12_12_1$, with unit-cell parameters (standard deviations in parentheses) a = 30.24 (1), b = 36.51 (3), c = 57.08 (2) Å. Assuming one molecule of rBmKαIT1 in the asymmetric unit, the value of the Matthews coefficient $V_{\rm M}$ is 2.15 Å^3 Da^{-1}, corresponding to a solvent content of 46.5%, both of which are within the normal values for protein crystals (Matthews, 1968). Data statistics are listed in Table 1. The structure of rBmKαIT1 was solved using the molecular-replacement method, with the structure of BmKM1 (PDB code 1sn1; He *et al.*, 1999) as the search model. The model was refined and coordinates have been deposited in the PDB (code 1omy). Analysis of the structure will be published elsewhere.

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