X-ray Analysis of An Insect Toxin with Analgesic Effect from Scorpion*Buthus martensii* Karsch

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Introduction

Scorpion venoms contain a number of peptide neurotoxins active specifically on mammals, insects or crustaceans. The scorpion *Buthus martensii* Karsch (BmK) is a widely distributed species in China. Interestingly, its venom is not only much less toxic and never causes death of an envenomed people, but quite contrarily, is used for disease prevention and therapy. Whole scorpion, scorpion tails or their extract are effective in treating some neural diseases such as apoplexy, epilepsy, facial paralysis and hemiplegia, etc, and also used to soothe the nerves and to relieve pains caused by meningitis, cerebral palsy, rheumatism, and so on. In recent experiments we found an active peptide with analgesic effect named dITAP3. Interestingly, this new peptide also shows anti-insect toxicity, but is devoid of any toxicity on mice even at a high dose. In order to understand the pharmaceutical mechanism, it is significant to determine its crystal structure.

Experimental and results

Crystals of dITAP3 were found under several conditions, but the quality was quite disappointing for either being fragile or being heavily twinned. When detergents were introduced into the recipes the situation was greatly improved. Finally the best crystal was grown in the hanging drop.

The single crystal of dITAP3 in size 0.1 mm x 0.1 mm x 0.1 mm was used in the crystallographic analysis and data collection. The preliminary X-ray analysis showed that the number of molecules in the asymmetric unit was 2 and the solvent content estimated according to Matthews equation was 53.4%, corresponding to a V_m of 2.64 $Å^3$/dalton. The crystal of dITAP3 belongs to space group R3 and the unit cell parameters are listed in Table 1.

Diffraction data were collected at room temperature using synchrotron radiation (λ=1.0 Å) on an ADSC Quantum 4 CCD detector at the BL18B experimental station in the Photon Factory (Tsukuba, Japan). The distance from crystal to detector was set 100.0 mm. The exposure time for each image is 70 s, and an oscillation angle of 1.5° in range of total 120° was used. Data processing and analysis were performed using programs *DPS/MOSFLM/CCP4* and *SCALA* [1, 2, 3]. The data statistics are shown in Table 1. The structure determination is currently under way with this data set.

Table 1 Data-collection and process statistics

X-ray source	BL18B, PF
Wavelength (Å)	1.0
Resolution (Å)	2.6
Detector	ADSC Quantum 4 CCD
Crystal-to-detector distance	230.0 mm
2?	20º
Exposure time	50 s
Oscillation angle	1.0º
Total oscillation range	120º
Total frames collected	120
Temperature	room temperature
Space group	R3
Unit-cell parameters (Å)	a=b=73.29, c=68.90
Number of observations	24087
Number of unique reflections	4246
I/Sigma(I)	7.0 (2.0)*
Completeness (%)	99.7 (97.5)
R_{merge} (%)	9.7 (36.2)

* Values in parentheses refer to that in the highest resolution shell 2.74-2.60 Å.

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References

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