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Crystallization and preliminary diffraction data of neurotoxin Ts-γ from the venom of the scorpion *Tityus serrulatus*

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Abstract

Scorpion neurotoxin Ts-γ was isolated from *Tityus serrulatus* venom and purified to apparent homogeneity by ion-exchange HPLC. Crystals of the toxin were grown using polyethylene glycol 6000 as precipitant and were found to belong to the monoclinic space group $P2_1$ with cell parameters a = 22.20, b = 36.90, c = 31.57 Å, β = 100.85°. The crystals diffract beyond 1.73 Å resolution at a synchrotron beamline, being notably stable during X-ray exposure. The structure has been solved by molecular replacement using the very high resolution structure of Sahara scorpion *Androctonus australis* Hector (PDB code 1AHO) as a search model.

1. Introduction

Scorpion venom contains a set of toxins generally represented by relatively small proteins of two classes: short neurotoxins containing 30–40 residues and long neurotoxins containing 60–70 residues. The long neurotoxins have been classified and are divided into two groups (α and β) with respect to their effects on the Na^+ channels of excitable membranes. The α-neurotoxins have been isolated from the venom of North African and Middle Eastern scorpions, whereas β-toxins were found in the venom of the North and South American scorpions. Both types modify Na^+ channel gating: α-toxins interfere with the ability of channels to inactivate upon prolonged depolarization, and β-toxins shift the threshold of channel activation toward a more negative potential and interfere with channel closing upon repolarization (Kirsh *et al.*, 1989).

At present, the three-dimensional structures of several scorpion neurotoxins have been determined both by X-ray analysis and by NMR. Structures of α and β types include a dense core consisting of a short α-helix, a three-stretch antiparallel β-sheet and a hydrophobic surface region responsible for the toxin affinity. The core is stabilized by four disulfide bridges (Fontecilla-Camps *et al.*, 1980, 1988). The difference in the toxin antigenic polymorphism seems to be due to a difference in the amino-acid composition of the loops protruding from the core structure (Zhao *et al.*, 1992). The complexity of scorpion venoms may be caused by evolutionary pressure resulting in the divergence from a hypothetical ancestral scorpion-toxin molecule (Fontecilla-Camps, 1989).

Brazilian scorpion venom contains both α- and β-type neurotoxins. Some of them have been partially or completely sequenced: TsTx-V (Marangoni *et al.*, 1995), TsIV-5 (Possani *et al.*, 1991; Martin-Euclaire *et al.*, 1994) and Ts-γ (Possani *et al.*, 1985). Interestingly, neurotoxins from the Brazilian scorpion may act directly on tissue, independently of nervous-system effects. For example, toxin TsTx-V causes a 2.0- to 2.4-fold increase in potassium outflow in b-cells from the islets of Langerhan. Toxin TsIV-5 stimulates discharge of zymogen granules from isolated pancreatic exocrine cells (Possani *et al.*, 1991; Marangoni *et al.*, 1995). The precise mechanism of these effects is unknown.

Preliminary modelling studies (Oliveira *et al.*, 1994) revealed a similarity in the three-dimensional structures of the Brazilian scorpion toxins TsTx-V, Ts-γ and TsTx-VI. In contrast, all these toxins showed some structural peculiarities when compared with AaH II from North African scorpions (α-neurotoxin model) and CsE V3 from North American scorpions (β-neurotoxin model). B and J loops, which are present in the models for α and β structures, are absent in the Brazilian scorpion neurotoxins. Presumably, there are other regions in these molecules characterizing them as α or β type. Ts-γ toxin demonstrates different behaviour compared with other β-toxins on the basis of electrophysiological assay (Strichartz *et al.*, 1987) and may belong to an independent group of toxins.

All these data suggest that the Brazilian scorpion neurotoxins form a particular family. The present work was initiated to identify regions responsible for the different biological activity, and to understand the structural basis of such activity and the mechanism of biological action. We report the crystallization of toxin Ts-γ and its preliminary X-ray diffraction study.

2. Methods and results

Venom was obtained from the telson of *Tityus serrulatus* scorpions by electrostimulation. The scorpion neurotoxin was purified by a method similar to that described by Arantes *et al.* (1989). The venom was applied to a Protein Pack SP 5PW column equilibrated with 0.05 M ammonium bicarbonate buffer pH 8.0, and eluted with a linear gradient (0.05–1.0 M, 60 min) of ammonium bicarbonate pH 8.0 at a flow rate of 1.0 ml min^{-1}. The elution was monitored at 280 nm. The purity of the protein obtained was checked by reverse-phase HPLC. The N-terminal sequencing of Ts-γ with automatic Edman degradation on an Applied Biosystem liquid–gas sequencer showed the presence of only one N-terminal amino-acid residue and confirmed that this protein was Ts-γ (Marangoni *et al.*, 1990). The purified Ts-γ was lyophilized and stored for crystallization.

Crystals of the scorpion neurotoxin were grown by the hanging-drop vapour-diffusion method at room temperature. 10 µl of a 10 mg ml^{-1} solution of the neurotoxin in H_2O was mixed with 5 µl of a 15%(w/v) solution of polyethylene glycol (PEG) 6000 in 50 mM potassium phosphate buffer in the pH

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range 5.2–6.5, and was equilibrated against 15% PEG in the same buffer. Small crystals appeared after a few days at pH 6.0 and 6.2 and were used for seeding. They were crushed with a glass rod and small pieces were placed in a fresh protein–PEG solution equilibrated as above. Crystals were grown for two days at room temperature (298 K) to a maximum size of 0.1 × 0.1 × 0.05 mm. They were found to be suitable for X-ray analysis and were used for synchrotron radiation data collection. X-ray diffraction data were collected in 1.5° oscillation steps on a MAR 345 imaging plate using the protein-crystallography beamline (Polikarpov, Oliva *et al.*, 1998; Polikarpov, Perles *et al.*, 1998) at the Brazilian National Synchrotron Laboratory (Campinas, Brazil) and processed with the *HKL* suite (Otwinowski, 1993). The data were collected in a time mode with 3 min exposure time per frame. Despite its small size (0.1 × 0.1 × 0.05 mm), the crystal diffracted to 1.73 Å and was very stable to X-ray exposure. The synchrotron radiation wavelength was set to 1.38 Å. The crystals were found to be monoclinic and belong to the $P2_1$ space group with cell parameters a = 22.20, b = 36.90, c = 31.57 Å, β = 100.85°. The resulting diffraction data set was 97.1% complete in the 13–1.73 Å resolution range (81.6% complete in the last resolution shell). The resolution cutoff was defined so that 50% of reflections in the highest resolution shell had $I > 3\sigma(I)$. Overall $R_{\rm merge}$ was 6.6%, while $R_{\rm merge}$ in the last resolution shell was 26.0%. 13402 reflections were measured, out of which 5161 reflections were unique.

Assuming a molecular mass of 7000 Da for the neurotoxin and one monomer per asymmetric unit, the V_m value is 1.81 $Å^3$ Da^{-1} and falls in the acceptable range observed for other protein crystals (Matthews, 1968). This parameter indicated a single molecule per asymmetric unit as the most probable choice.

We attempted to solve the Ts-γ neurotoxin structure by a molecular-replacement method using existing crystallographic models of scorpion neurotoxins. There are three crystal structures of scorpion neurotoxins in the Protein Data Base: the Sahara scorpion *Androctonus australis* Hector (Smith *et al.*, 1997; PDB code 1AHO), the scorpion *Centruroides sculpturatus* Ewing (variant 3), found in the south-western region of the United States (Zhao *et al.*, 1992; PDB code 1SN3) and the Chinese scorpion *Buthus martensii* Karsch (Li *et al.*, 1996; PDB code 1SNB). All three structures were used as search models in molecular-replacement procedures performed using *AMoRe* (Navaza, 1994). The best solution was found using a 0.96 Å resolution structure of the neurotoxin from the Sahara scorpion *Androctonus australis* Hector. The rotation function was calculated using diffraction data in the resolution range 10–3.3 Å and a Patterson radius of 13 Å. The rotation peak corresponding to the correct solution was the second highest (correlation coefficient of 17.7%). The highest peak in the rotation-function calculation had a correlation coefficient (CC) of 18.9%, with the third and the fourth solutions having CCs of 17.4 and 17.0%, respectively. The translation search performed with the same program using the Crowther & Blow (1967) translation function resulted in a clear solution with a CC of 20.1% and an R factor of 50.5%. The second translation-function solution had a CC of 14.4% and an R factor of 54.2%. The model was finally subjected to rigid-body refinement in the resolution range 10–3 Å. The resulting R factor was 50.3% and the CC was 28.7%.

Refinement was undertaken using the program *REFMAC* from the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994). Forty cycles of positional and B-factor refinement of the molecular-replacement model against all the data between 10 and 1.73 Å resolution gave a model with the R factor = 37.0% and $R_{\rm free}$ = 43.56%. Further steps of model rebuilding and refinement are underway.

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References

Arantes, E. C., Prado, W. A., Sampaio, S. V. & Giglio, J. R. (1989). *Toxicon*, **8**, 907–916.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Crowther, R. A. & Blow, D. M. (1967). *Acta Cryst.* **23**, 544–549.

Fontecilla-Camps, J. C. (1989). *J. Mol. Evol.* **29**, 63–67.

Fontecilla-Camps, J. C., Almassy, R. J., Suddath, F. L., Ealick, S. E., Watt, D. D. & Bugg, C. E. (1980). *Proc. Natl Acad. Sci. USA*, **77**, 6496–6500.

Fontecilla-Camps, J. C., Habersetzer-Rochat, C. & Rochat, H. (1988). *Proc. Natl Acad. Sci. USA*, **85**, 7443–7447.

Kirsh, G. E., Skattebol, A., Possani L. D. & Brown, A. M. (1989). *J. Gen. Physiol.* **93**, 67–83.

Li, H.-M., Wang, D.-C., Zeng, Z.-H., Jin, L. & Hu, R.-Q. (1996). *J. Mol. Biol.* **261**, 415–431.

Marangoni, S., Ghiso, J., Sampaio, S. V., Arantes, E. C., Giglio, J. R., Oliveira, B. & Frangione, B. (1990). *J. Protein Chem.* **9**, 595–601.

Marangoni, S., Toyama, M. H., Arantes, E. C., Giglio, J. R., da Silva, C. A., Carneiro, E. M., Gonçalves, A. A. & Oliveira, B. (1995). *Biochem. Biophys. Acta*, **1243**, 309–314.

Martin-Euclaire, M. F., Ceard, B., Ribeiro, A. M., Diniz, C. R., Rochat, H. & Bougis, P. E. (1994). *Fed. Eur. Biochem. Soc. Lett.* **342**, 181–184.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.

Otwinowski, Z. (1993). *Data Collection and Processing. Proceedings of the CCP4 Study Weekend*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 55–62. Warrington: Daresbury Laboratory.

Oliveira, P. S. L., Horjales, E. & Caracelli, I. (1994). *XXIII Annual Meeting of Brazilian Biochemistry and Molecular Biology Society (SBBQ)*, p. 134.

Polikarpov, I., Oliva, G., Castellano, E. E., Garratt, R., Arruda, P., Leite, A. & Craievich, A. (1998). *Nucl. Instrum. Methods A*, **405**, 159–164.

Polikarpov, I., Perles, L. A., de Oliveira, R. T., Oliva, G., Castellano, E. E., Garratt, R. & Craievich, A. (1998). *J. Synchrotron Rad.* **5**, 72–76.

Possani, L. D., Martins, B. M., Fletcher, M. D. & Fletcher, P. I. Jr (1991). *J. Protein. Chem.* **296**, 3178–3186.

Possani, L. D., Martins, B. M., Svendsen, I., Rode, G. S. & Erickson, B. W. (1985). *Biochem. J.* **229**, 739–750.

Smith, G. D., Blessing, R. H., Ealick, S. E., Fontecilla-Camps, J. C., Hauptman, H. A., Housset, D., Langs, D. A. & Miller, R. (1997). *Acta Cryst.* D**53**, 551–557.

Strichartz, G., Rando, T. & Wang, G. K. (1987). *Annu. Rev. Neurosci.* **10**, 237–267.

Zhao, B., Carson, M., Ealick, S. E. & Bugg, C. E. (1992). *J. Mol. Biol.* **227**, 239–252.