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Crystallization and preliminary X-ray diffraction analysis of a eumenine mastoparan toxin: a new class of mast-cell degranulating peptide in the wasp venom

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Mastoparans are tetradecapeptides found to be the major component of vespid venoms. A mastoparan toxin isolated from the venom of *Anterhynchium flavomarginatum micado* has been crystallized and X-ray diffraction data collected to 2.7 Å resolution using a synchrotron-radiation source. Crystals were determined to belong to the space group $P6_222$ ($P6_422$). This is the first mastoparan to be crystallized and will provide further insights into the conformational significance of mastoparan toxins with respect to their potency and activity in G-protein regulation.

1. Introduction

Several different bioactive peptides have been isolated from the venoms of wasps and hornets. The mastoparan toxins, the mast-cell degranulating peptides of the vespid venom, are tetradecapeptides and have been reported to be the major peptide in many species of hornet (Nakajima, 1984). Several such peptides have been characterized as causing the liberation of histamine, serotonin and other chemical substances mediating inflammation upon binding to plasma membranes. Some of these peptides also possess a potent haemolytic activity which acts in synergy with the lethal protein of the vespid venom (Ho & Hwang, 1991).

The action of these tetradecapeptides appears to involve multiple signal transduction pathways and may stimulate these activities *via* its effect on G proteins. Previous structural studies using circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy revealed that mastoparans exhibit a random-coil form in aqueous solution, whereas these tetradecapeptides adopt the amphiphilic α-helix conformation in trifluorethanol-containing aqueous solution (Chuang *et al.*, 1996). This conformational behaviour has also been observed for other phospholipid membrane-binding peptides (Higashijima *et al.*, 1983).

Especially interesting is the stimulation of phospholipase A_2 activity by mastoparans. Phospholipase A_2s are perhaps the most ubiquitous enzymes in venoms (Giotto *et al.*, 1998; de Azevedo *et al.*, 1997, 1998, 1999; Ward *et al.*, 1998). They are found in the venoms of snakes, lizards and hornets. Several mastoparans can facilitate phospholipase A_2 activity, a behaviour similar to melittin. Mastoparans cause a 12-, 15- and 50-fold increase in the production of arachidonic acid catalyzed by phospholipase A_2 from bee venom, eastern diamondback rattlesnake and porcine pancreas, respectively (Argiolas & Pisano, 1983).

A mastoparan toxin was isolated from the solitary wasp *A. flavomarginatum micado*, the most abundant eumenine wasp in Japan, and named eumenine mastoparan (Konno *et al.*, 2000). Solitary wasps paralyze insects or spiders with stinging venom and feed the paralyzed victims to their larvae. Accordingly, the venom should contain a variety of constituents acting on the nervous system. However, only a few constituents of the venom have been chemically characterized, despite the thousands of solitary wasp species inhabiting the earth. In the present work, the crystallization and preliminary X-ray analysis of the eumenine mastoparan (Ile-Asn-Leu-Leu-Lys-Ile-Ala-Lys-Gly-Ile-Ile-Lys-Ser-Leu-NH_2), a new class of mast-cell degranulating peptide, is reported in order to provide further insights in the conformational characteristics and the implication for its biological functions.

2. Materials and methods

2.1. Tetradecapeptide synthesis

The peptide was prepared by stepwise solid-phase synthesis using *N*-9-fluorenylmethoxycarbonyl (Fmoc) chemistry with TGS-RAM resin (Shimadzu Corp., Kyoto, Japan) on a Shimadzu PSSM-8 peptide synthesizer. All Fmoc amino acids and the resin were purchased from Shimadzu Corp. Side-chain protective groups included *t*-butoxy for serine and *t*-butoxycarbonyl for lysine. Cleavage of the peptide–resin was performed by treatment with anisole/1,2-ethanedithiol/trifluoroacetic acid solution (5:1:94 by volume), using 10 ml

Table 1
Detailed X-ray diffraction statistics for synchrotron data from a crystal of EMP.

Resolution in range (Å)	No. of independent reflections	$I/\sigma(I)$	Redundancy	Completeness from all data (%)	R_{merge}† in range from all data (%)
20.00–6.58	122	21.43	3.82	97.6	4.8
6.58–5.26	107	20.27	5.49	100.0	7.6
5.26–4.60	100	23.03	5.69	100.0	8.0
4.60–4.19	96	23.24	5.98	100.0	8.0
4.19–3.89	101	23.47	5.71	100.0	8.5
3.89–3.66	91	18.23	6.04	100.0	10.4
3.66–3.48	95	15.26	5.96	100.0	12.6
3.48–3.33	92	16.01	5.85	100.0	13.9
3.33–3.20	93	13.50	6.15	100.0	15.8
3.20–3.09	92	11.22	5.95	100.0	18.6
3.09–2.99	96	11.06	5.77	100.0	16.9
2.99–2.91	87	8.68	5.90	100.0	20.9
2.91–2.83	89	7.40	5.44	98.9	24.6
2.83–2.76	88	8.08	5.43	98.9	22.0
2.76–2.70	87	6.22	4.87	97.8	24.4
All reflections	1436	17.97	5.63	99.5	9.9

† $R_{merge} = 100\sum_{hkl}[\sum_i(|I_{hkl,i} - \langle I_{hkl}\rangle|)]/\sum_{hkl,i}(I_{hkl})$, where $I_{hkl,i}$ is the intensity of an individual measurement of the reflection with indices h, k and l, and $\langle I_{hkl}\rangle$ is the mean intensity of that reflection.

per gram of resin at room temperature for 2 h. After filtering to remove the resin and ether extraction, the crude peptide was purified by semipreparative reverse-phase HPLC (CAPCELL PAK C18, 10 × 250 mm, 40% acetonitrile–water containing 0.1% trifluoroacetic acid). The homogeneity of the synthetic peptide was assessed by analytical HPLC and fast-atom bombardment mass spectral (FAB-MS) analysis.

2.2. Crystallization

The eumenine mastoparan (EMP) used in the crystallization experiments was dissolved in 50% trifluorethanol-containing aqueous solution. Crystals of the EMP have been obtained under several different crystallization conditions using the hanging-drop vapour-diffusion and sparse-matrix methods (Jancarik & Kim, 1991). The best crystals were obtained after 2 d growth from drops in which 2 µl of EMP solution (5 mg ml^{-1}) was mixed with an equal volume of reservoir solution. 0.1 *M* sodium acetate trihydrate pH 4.6 and 2.0 *M* sodium formate was used as the reservoir solution. A crystal was mounted in a borosilicate glass capillary tube for X-ray data collection.

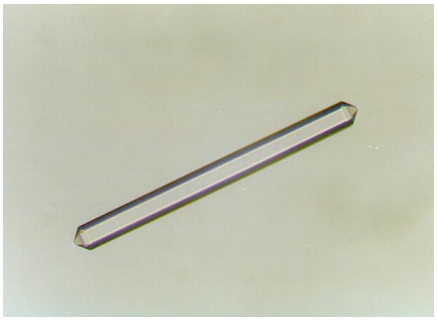

Figure 1
Photomicrograph of the hexagonal-shaped crystal of EMP.

X-ray diffraction data were collected at a wavelength of 1.547 Å using the Synchrotron Radiation Source (Station PCr, Laboratório Nacional de Luz Sincrotron, LNLS, Campinas, Brazil; Polikarpov, Oliva *et al.*, 1998; Polikarpov, Perles *et al.*, 1998) and a 34.5 cm MAR Research imaging-plate detector at a crystal-to-detector distance of 200.0 mm and a temperature of 281 K. Using an oscillation range of 2.0°, 30 images were collected and the raw X-ray diffraction data were processed to 2.7 Å resolution using the program *DENZO* and scaled with the program *SCALEPACK* (Otwinowski, 1993).

Autoindexing procedures combined with analysis of the X-ray diffraction pattern and averaging of equivalent intensities were used in the characterization of the Laue symmetry.

3. Results and discussion

Fig. 1 illustrates a photomicrograph of a hexagonal-shaped crystal of EMP isolated from the venom of *A. flavomarginatum micado* suitable for X-ray diffraction experiments, with average dimensions of about 0.1 × 0.1 × 0.8 mm. The crystal has the primitive hexagonal Laue lattice, space group $P6_222$ ($P6_422$), with unit-cell parameters a = 43.32 (2), b = 43.32 (2), c = 79.86 (2) Å. Assuming the asymmetric unit content to be two or three peptides of molecular weight of 1.65 kDa each, the possible V_M values are 2.84 and 1.89 Å^3 Da^{-1} (Matthews, 1968), respectively. Assuming a value of 0.74 cm^3 g^{-1} for the protein partial specific volume, the possible calculated solvent content in the crystal are 41% for two peptides in the asymmetric unit and 34.9% for three peptides in the asymmetric unit; the calculated crystal densities are 1.09 and 1.23 g cm^{-3}, respectively. Detailed data-collection statistics for the synchrotron data are given in Table 1.

There are at least two possibilities for the number of molecules in the asymmetric unit, two or three molecules. In order to solve this ambiguity, a self-rotation function was calculated and inspected; however, it was not conclusive. Attempts to solve the structure were performed for the two possibilities and in both cases did not produce a reasonable solution. The two possible space groups were tried in the molecular replacement and refinement. The best solutions were submitted to simulated-annealing refinement as implemented in *X-PLOR* (Brünger, 1992) and did not converge to reasonable values of the R factor and R_{free} (R factor = 39% and R_{free} = 45% for three peptides in the asymmetric unit). The initial model was a mastoparan-X isolated from *Vespa xanthoptera* (Kusunoki *et al.*, 1998; PDB accession code 1a13) solved by nuclear magnetic resonance (NMR) spectroscopy, in which the residues were modified to be in accordance with the EMP sequence. This model is an amphipathic helix in which the residues with hydrophilic side chains are located on one side and the residues with hydrophobic side chains are located on the other side of the molecule. Further attempts to solve the EMP structure will be performed using the multiple isomorphous replacement method.

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References

Argiolas, A. & Pisano, J. J. (1983). *J. Biol. Chem.* **258**, 13697–13702.
Azevedo, W. F. de Jr, Ward, R. J., Canduri, F., Soares, A., Giglio, J. R. & Arni, R. K. (1998). *Toxicon*, **36**, 1395–1406.
Azevedo, W. F. de Jr, Ward, R. J., Gutiérrez, J. M. & Arni, R. K. (1999). *Toxicon*, **37**, 371–384.
Azevedo, W. F. de Jr, Ward, R. J., Lombardi, F. R., Giglio, J. R., Fontes, M. R. M. & Arni, R. K. (1997). *Protein Pept. Lett.* **4**(5), 329–334.
Brünger, A. T. (1992). *X-PLOR Version* 3.1. *A System for Crystallography and NMR*. New Haven: Yale University Press.
Chuang, C., Huang, W., Yu, H., Wang, K. & Wu, S. (1996). *Biochim. Biophys. Acta*, **1292**, 1–8.
Giotto, M. T. S., Garratt, R. C. Oliva, G., Mascarenhas, Y. P., de Azevedo, W. F. Jr, Giglio, J. R., Cintra, A. C. O., Arni, R. K. & Ward, R. J. (1998). *Proteins Struct. Funct. Genet.* **30**, 442–454.
Higashijima, T., Wakamatsu, K., Takemitsu, M., Fujino, M., Nakajima, T. (1983). *FEBS Lett.* **152**, 227–230.

Ho, C. L. & Hwang, L. L. (1991). *Biochem. J.* **274**, 453–456.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Konno, K., Hisada, M., Naoki, H., Itagaki, Y., Kawai, N., Miwa, A., Yasuhara, T., Morimoto, Y. & Nakata, Y. (2000). *Toxicon*, **38**, 1505–1515.
Kusunoki, H., Wakamatsu, K., Sato, K., Miyazawa, T. & Kohno, T. (1998). *Biochemistry*, **37**, 4782–4790.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Nakajima, T. (1984). *Handbook of Natural Toxins*, edited by A. T. Tu, Vol. 2, pp. 109–133. New York: Marcel Dekker.
Otwinowski, Z. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.
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.
Ward, R. J., de Azevedo, W. F. Jr & Arni, R. K. (1998). *Toxicon*, **36**, 1623–1633.