Crystallization and Preliminary X-Ray Diffraction Analysis of Three Mastoparans

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Abstract: Mastoparans are tetradecapeptides found to be the major component of wasp venoms. These peptides possess a variety of biological activities. Three related mastoparans, mastoparan from *Polistes jadwagae* (MP-PJ), mastoparanX (MP-X) and its carboxyl-free C-terminal form (MP-X-COO^-), were crystallized. X-ray diffraction data for them were collected at resolutions of 1.2 Å, 2.0 Å and 3.3 Å respectively.

Keywords: Crystallization, preliminary, X-ray diffraction, mastoparan toxins.

INTRODUCTION

Mastoparan toxins are tetradecapeptides reported to be the major peptides in many species of wasps. They have a variety of biological activities. Mastoparans can induce secretion of neurotransmitters [1], hormones [2-4] and cytokines [5] in different types of cells. They can promote histamine release from mast cells [6], and some of these peptides also exhibit potent hemolytic activity [7, 8] and antimicrobial activity [9].

The mechanism underlying the various activities of martoparans is complicated and has not been clarified. It is generally agreed that the activities of mastoparan involve heterotrimeric G-protein by mimicking the intracellular cationic amphiphilic G protein binding domains of GPCRs in a pertussis toxin-sensitive manner [10]. Moreover, the mechanism of independent G protein is also suggested. Mastoparan has been reported to inhibit Ca^{2+}-ATPase [11] and to inhibit K-ATP channels [12] in a G protein-independent manner which can lead to depolarization and Ca^{2+} influx through L-type Ca^{2+} channels. Mastoparan induces Ca^{2+} influx, which is independent of L-type Ca^{2+} channels [13] and which may result from activation of Ca^{2+}-permeant nonselective cation channels [14]. The antimicrobial activity of mastoparan is also reported to be related to membrane permeability [15].

As the function of mastoparan was suggested to be related to its interactions with membrane, G protein or calmodulin, structural studies have been carried out on mastoparans with such substances mainly by nuclear magnetic resonance (NMR) spectroscopy [16-20]. It has been shown that mastoparans exhibit a random coil form in aqueous solution, whereas in trifluoroethanol-containing aqueous solution they will adopt an amphiphilic α-helix conformation [21]. Mastoparan has also received attention because analogues which have a free carboxyl-terminus differ in biological activity from those which have an amidated C-terminus [22-25]. In order to provide further insights into the conformational characteristics of mastoparans, crystallization of mastoparan analogue, eumenine mastoparan, in 50% trifluorethanol-containing aqueous solution was reported [26, 27]. However, the crystal structure of mastoparan itself has not yet been reported.

Mastoparan from *Polistes jadwagae* (MP-PJ, VDWK KIGQHILSVL-NH_2) and mastoparanX (MP-X, INWKGIA AMAKKLL-NH_2) are the major characteristic mastoparans with various types of activity [28-31]. To better understand the conformational differences of mastoparan in different solvent environments and to reveal the functional role of the C-terminus, attempts to crystallize different kinds of mastoparans were carried out in our laboratory. The present work reports the crystallization and preliminary crystallographic study of three mastoparans, MP-PJ, mastoparanX and its carboxyl-free C-terminal form(MP-X-COO^-,) all in aqueous solute without lipid or trifluoroethanol.

MATERIALS AND METHODS

1. Crystallization

The MP-PJ used in the experiments was purchased from Sigma Corp., without further purification and directly dissolved in distilled water at a concentration of 7 mg/ml. An initial screening of crystallization conditions using crystal screens 1 and 2 (Hampton Research) was attempted. Crystals were obtained both with the hanging-drop and sitting-drop vapor-diffusion methods, but were twined most of the time. Single crystals suitable for x-ray analysis were obtained in about 3 days at 18°C containing 1.5 μl of MP-PJ solution (7 mg/ml) mixed with 1 μl reservoir solution and another 1 μl distilled water (Table **1**). The MP-X-NH_2 and MP-X-COO^- were synthesized by AC Scientific Inc.(Xi'an, China) with purity of about 98%. They were also directly dissolved in distilled water at concentrations of 3 mg/ml and 7 mg/ml

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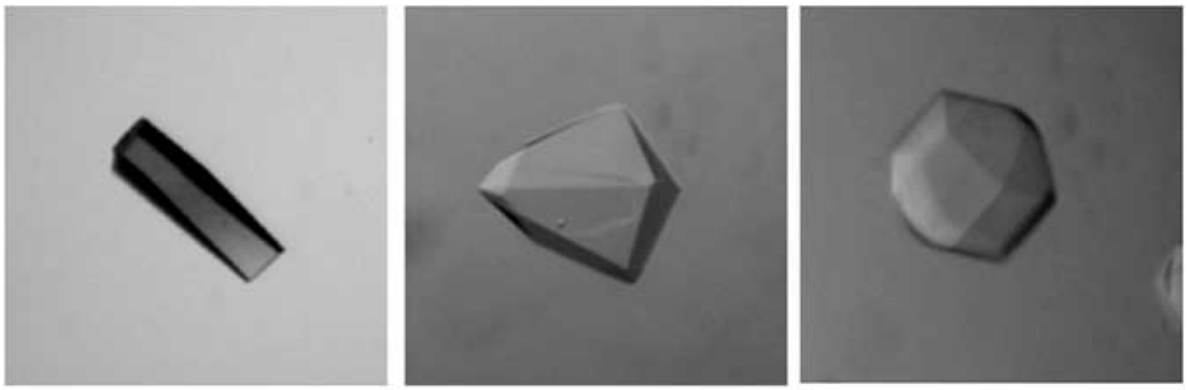

a b c

Figure 1. a) A photomicrograph of a rectangular layer-shaped crystal of MP-PJ suitable for X-ray diffraction experiments, with average dimensions of about 0.05x0.05x0.1mm. **b)** and **c)** are the photomicrographs of the crystals of MP-X-NH_2 and MP-X-COO^- with suitable dimensions for X-ray diffraction experiments.

Table 1. Crystallization Conditions of Mastoparans

	MP-PJ	**MP-X-NH_2**	**MP-X-COO_**
Sample solution(A)	**7mg/ml**	**7mg/ml**	**3mg/ml**
Reservoir solution(B)	0.06 M ammonium sulfate, 0.1M sodium cacodylate pH 6.5, 24% polyethylene glycol (PEG) 1500	0.2M ammonium sulfate 30%PEG MME 2000 0.1M sodium acetate pH 4.6	0.7M ammonium sulfate 0.1M sodium citrate pH 6.0
Drop	1.5μl A+1μl H_2O+1μl B	1μl A+1μl B	2μl A+2μl B
temperature(°C)	18	18	16
growth time	3 days	4 months	4 days
crystal dimensions	0.05x0.05x0.1mm	0.06x0.1x0.1mm	0.1x0.1x0.1mm

respectively. Crystals were obtained with hanging-drop vapor-diffusion. For MP-X-COO^-, single crystals were obtained in about 4 days at 16°C, and for MP-X-NH_2, single crystals were obtained in about 4 months at 18°C (Table **1**).

2. Data Collection and Processing

X-ray diffraction data of MP-PJ were collected at 100K with paratone-N (Hampton Research Corp.) as cryo-protection and at a wavelength of 0.90Å using the Synchrotron Radiation on BSRF (beamline 3W1A of the Beijing Synchrotron Radiation Facility). With an oscillation range of 2.0°, 180 images were collected and the raw x-ray diffraction data were processed to 1.20Å resolution using the program DENZO and scaled with the program SCALEPACK [32]. Detailed data-collection statistics are shown in Table **2**.

RESULTS AND DISCUSSION

The crystals of MP-PJ belong to space group $P2_1$, with unit cell parameters of a=22.13Å, b=59.38Å, c=37.26Å, and β=101.3°. Assuming there are six or eight peptides in an asymmetric unit, with a molecular weight of 1635 Dalton each, the possible calculated solvent contents of the crystals is 49% for 6 monomers and 33% for 8 monomers.

The data of MP-X-NH_2 and MP-X-COO^- were obtained using a home diffraction system of FR-E (Biophysics Institute of CAS, Beijing, China) with parantone as cyro-protection and at a temperature of 79 K. Using an oscillation range of 2.0° for both, 70 and 100 images were collected respectively. The data were processed using the program CrystalClear [33] and the results are listed in Table **2**.

The crystals of MP-X-NH_2 and MP-X-COO^- both had hexagonal Laue symmetry with cell parameters of a=b=34.96Å, c=60.52Å, α = γ =90°, β=120° and a=b=82.83Å, c=51.97Å, α = γ=90°, β=120°, respectively. Based on the self-rotation Patterson map, MP-X-NH_2 crystals have an obvious 2-fold symmetric axis in the *ab* directions and therefore belong to the space group P622 with one molecule in an asymmetric unit.

We tried molecular replacement method using the MOLREP of CCP4 [34] program package to solve these structures, however, no solution was apparent by using the NMR structures (PDB code 1A13, 1D7N) of mastoparan as models. Further attempts to solve these structures by the multiple isomorphous anomalous scattering (MAD) methods are in progress.

Table 2. Summary of Data Collection Statistics of Mastoparans

Space group	MP-PJ	MP-X-NH_2	MP-X-COO^-
	$P2_1$	P622	P6
Unit cell dimensions	22.13, 59.38, 37.26,	34.96, 34.96, 60.52,	82.83, 82.83, 51.97,
	90.00, 101.29, 90.00	90.00, 90.00, 120.00	90.00, 90.00, 120.00
Resolution range(Å)	30.0-1.20(1.24-1.20)	27.08-2.10(2.18-2.10)	29.52-3.20(3.31-3.20)
Total number of reflections	180667	36384	22051
Number of unique reflections	27689	1643	3438
Average redundancy	6.52(6.18)	22.14 (22.92)	6.41(6.51)
% completeness	93.7(89.0)	94.9 (93.7)	99.7(100.0)
Rmerge	0.047(0.256)	0.101(0.471)	0.243(0.562)
Output <I/sigI>	46.2(5.7)	17.9 (6.5)	4.2(2.0)

Note: Values in brackets are for the last resolution shell

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REFERENCES

[1] Ohara-Imaizumi, M., Nakamichi, Y.S., Ozawa, Katsuta, H., Ishida, H. and Nagamatsu, S. (**2001**) *Biochem. Biophys. Res. Commun. 289*, 1025-1030.
[2] Murayama, T., Oda, H. and Nomura, Y. (**1996**) *J. Cell Physiol. 169*, 448-454.
[3] Straub, S.G., James, R.F., Dunne, M.J. and Sharp, G.W. (**1998**) *Diabetes 47*, 1053-1057.
[4] Yajima, Y., Uchino, K., Ito, H. and Kawashima, S. (**1997**). *Endocrinology 138*, 1949-1958.
[5] Mizuno, K., Nakahata, N. and Ohizumi, Y. (**1998**) *Toxicon 36*, 447-456.
[6] Wu, T.M., Chou, T.C., Ding, Y.A. and Li, M.L. (**1999**) *Immunol. Cell. Biol. 77*, 476-482.
[7] Mendes, M.A., Monson de Souza, B., Delazari dos Santos, L. and Palma, M.S. (**2004**) *Rapid. Commun. Mass Spectrom. 18*, 636-642.
[8] Mendes, M.A., Monson de Souza, B. and Palma, M.S. (**2005**) *Toxicon 45*, 101-106.
[9] Park, N.G., Yamato, Y., Lee, S. and Sugihara, G. (**1995**) *Biopolymers 36*, 793-801.
[10] Higashijima, T., Uzu, S., Nakajima, T. and Ross, E.M. (**1988**) *J.. Biol.. Chem. 236*, 6491–6494.
[11] Longland, C.L., Mezna, M. and Michelangeli, F. (**1999**) *J.. Biol.. Chem. 274*, 14799–14805.
[12] Eddlestone, G.T., Komatsu, M., Shen, L. and Sharp, G.W. (**1995**) *Mol.. Pharmacol. 47*, 787–797.
[13] Komatsu, M., Mc Dermott, A.M., Gillison, S.L. and Sharp, G.W. (**1993**) *J.. Biol.. Chem. 268*, 23297–23306.
[14] Nie, L., Kanzaki, M., Shibata, H. and Kojima, I. (**1998**) *Endocrinology 139*, 179–188.
[15] Whiles, J.A., Brasseur, R., Glover, K.J., Melacini, G., Komives, E.A. and Vold, R.R. (**2001**) *Biophys. J. 80*, 280-93.
[16] Ohki, S.Y., Yazawa, M., Yagi, K. and Hikichi, K. (**1991**) *J. Biochem. (Tokyo), 110*, 737-742.
[17] Hori, Y., Demura, M., Iwadate, M., Ulrich, A.S., Niidome, T., Aoyagi, H., Asakura, T. (**2001**) *Eur. J. Biochem. 268*, 302-309.
[18] Yu, K., Kang, S., Kim, S.D., Ryu, P.D. and Kim, Y. (**2001**) *J. Biomol. Struct. Dyn. 18*, 595-606.
[19] Sukumar, M., Ross, E.M. and Higashijima, T. (**1997**) *Biochemistry 36*, 3632-3639.
[20] Kusunoki, H., Wakamatsu, K., Sato, K., Miyazawa, T. and Kohno, T. (**1998**) *Biochemistry 37*, 4782-4790.
[21] Chuang, C.C., Huang, W.C., Yu, H.M., Wang, K.T. and Wu, S.H. (**1996**) *Biochim. Biophys. Acta 1292*, 1.
[22] Sandvik, A. K. and Dockray, G. J. (**1999**) *Eur. J. Pharmacol. 364*, 199-203.
[23] Katayama, H., Ohira, T., Aida, K. and Nagasawa, H. (**2002**) *Peptides 23*, 1537-1546.
[24] Ali, M.F., Soto, A.M., Knoop, F.C. and Conlon, J.M. (**2001**) *Biochim. Biophys. Acta. 1550*, 81-89.
[25] Sforca, M.L., Oyama, S. Jr., Canduri, F., Lorenzi, C.C., Pertinhez, T.A., Konno, K., Souza B.M., Palma, M.S., Ruggiero Neto, J., Azeuedo, N.F. Jr., Spisni, A. (**2004**) *Biochemistry 43*, 5608-5617.
[26] Canduri, F., Delatorre, P., Fadel, V., Lorenzi, C.C., Pereira, J.H. Olivieri, J.R., Ruggiero Neto, J., Konno, K., Palma, M.S., Yamane, T., de Azevedo, W.F. Jr. (**2000**) *Acta Crystallogr. D. Biol. Crystallogr. 56*, 1434-1436.
[27] Delatorre, P., Olivieri, J.R., Ruggiero Neto, J., Lorenzi, C.C., Canduri, F., Fadel, V., Konno, K., Palma, M.S., Yamane, T., de Azevedo, W.F. Jr. (**2001**) *Biochim. Biophys. Acta 1545*, 372-376.
[28] Murase, T. and Iio, T. (**2002**) *Biochemistry 41*, 1618-1629.
[29] Wakamatsu, K., Okada, A., Miyazawa, T., Ohya, M. and Higashijima, T. (**1992**) *Biochemistry 31*, 5654-5660.
[30] Nakajima, T., Yasuhara, T., Uzu, S., Wakamatsu, K., Miyazawa, T. Fukuda, K., Tsukamoto, Y. (**1985**) *Peptides 6*, 425-430.
[31] Sukumar, M. and Higashijima, T.G. (**1992**) *J.. Biol.. Chem. 267*, 21421-21424.
[32] Otwinowski, Z., Sawyer, L., Isaacs, N. and Bailey, S. (**1999**) Proceedings of the CCP4 Study Weedend, Daresbury Laboratory, Warrington. pp. 56-62.
[33] Pflugrath, J.W. (**1999**) The finer things in X-ray diffraction data collection. *Acta Crystallogr. D. Biol. Crystallogr. 55*, 1718-1725.
[34] Collaborative Computational Project, Number 4. (**1994**) The CCP4 suite: programs for protein crystallography, *Acta Crystallogr. D. Biol. Crystallogr. 50*, 760-763.

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