Acta Cryst. (1996). D**52**, 201–202

Crystallization and preliminary X-ray diffraction studies of haemorrhagin I from the snake venom of *Agkistrodon acutus*

WEIMIN GONG, ZHONGLIANG ZHU, LIWEN NIU* AND MAIKUN TENG, *at Young Scientist Laboratory of Structure Biology and Department of Biology, University of Science and Technology of China, Hefei, Anhui 230026, People's Republic of China*

(*Received 16 March 1995; accepted 4 July 1995*)

Abstract

Haemorrhagin I from the snake venom of *Agkistrodon acutus* (AaHI) has been crystallized using the hanging-drop vapour diffusion method. The crystals belong to space group $P4_12_12$ or $P4_32_12$ with unit-cell dimensions $a = b = 63.61$ and $c = 95.69$ Å. There is one molecule in the asymmetric unit. Data to 2.35 Å resolution have been collected using a single crystal.

1. Introduction

Many protein components which cause local haemorrhage after injection into experimental animals have been isolated from snake venoms. These active haemorrhagic proteins vary widely in molecular weights from 20 to 100 kDa and p*I*'s from 4.2 to 10.5 (Ownby, 1990). Most of the haemorrhagic proteins from snake venoms are metalloproteinases. The amino-acid sequences of low molecular weight metalloproteinases (20–26 kDa) are highly conserved (Gomis-Rüth *et al.*, 1994). It was assumed that haemorrhagic activity might be related to the ability to attack basement membranes (Bjarnasson & Fox, 1989; Kini & Evens, 1992; Maruyama, Sugiki, Yoshida, Shimaya & Mihara, 1992). This activity might be linked to substrate specificity, which would be determined mainly by subsite architecture in the active-site cleft (Gomis-Rüth *et al.*, 1994). Recently, two crystal structures of snake venom metalloproteinases, adamalysin II from eastern diamondback rattlesnake *Crotalus adamanteus* (Gomis-Rüth, Kress & Bode, 1993; Gomis-Rüth *et al.*, 1994) and atrolysin C from western diamondback rattlesnake *Crotalus atrox*, (Zhang *et al.*, 1994) were solved, respectively. Adamalysin II and atrolysin C have similar tertiary structures although they are quite different in haemorrhagic activities.

Three different haemorrhagic proteins, haemorrhagin I, II and III, have been isolated from snake venom of *Agkistrodon acutus* found in the southern mountains of Anhui Province in China (Xu, Wang, Liu & Lu, 1981). They cause haemorrhagic symptoms after intradermal injection, possess caseinolytic and lethal activities, and have similar molecular weights of about 22 kDa, while the isoelectric points are 4.6, 5.3 and >9.0, respectively. They are all thermolabile and sensitive to decrease of pH and treatment with EDTA. It has been reported that AaHI contains one zinc ion per molecule (Zhang, Chen, He & Xu, 1984). So presumably AaHI is a zinc metalloproteinase. We report here the crystallization and preliminary X-ray diffraction studies of AaHI (*Agkistrodon acutus* haemorrhagin I) crystals that are suitable for high-resolution structure determination.

2. Purification and crystallization of AaHI

The procedures for the purification of AaHI given by Xu *et al.* (1981) were modified as follows. The venom powder was dissolved in Tris–HCl buffer (0.2 *M*, pH 8.0). The venom solution was applied to a DEAE–Sepharose Fast Flow (Pharmacia) column which had been equilibrated with the same buffer, and then eluted with a linear gradient from Tris–HCl buffer, 0.02 *M*, pH 8.0, to Tris–HCl buffer, 0.02 *M*, pH 6.0, containing 0.5 *M* NaCl. The fraction containing AaHI was collected, dialyzed against distilled water, concentrated and further purified by gel filtration on S-200 Sephacryl (Pharmacia) column equilibrated and eluted with 0.15 *M* NaCl. All purification steps were carried out at room temperature (about 288 K). Approximately 20 mg purified AaHI was obtained from 1 g crude venom.

AaHI was crystallized using the hanging-drop vapor-diffusion method (McPherson, 1982) at room temperature. 4 µl of the precipitating solution containing 51%(*v*/*v*) 2-methyl-2,4-pentanediol (MPD) was mixed with 4 µl of the 20 mg ml^{-1} AaHI solution and then equilibrated against 0.5 ml of precipitating solution. Both the precipitating and protein solutions were buffered with 0.02 *M* Tris–HCl at pH 7.5. After 3 d the tetragonal bipyramid-shaped crystals appeared and the single crystals of maximum dimensions 0.7 × 0.3 × 0.3 mm could be harvested one week later.

3. Characterization of AaHI crystals by X-ray diffraction

One crystal was used and examined for diffraction characterization using a Siemens X200B area detector mounted on Rigaku rotating-anode X-ray generator at National Laboratory of Biomacromolecules, Peking. Cu $K\alpha$ radiation was generated at 10 kW. A total of 540 ω-scanning oscillation exposures in step of 0.25° were recorded at 150 mm crystal-to-detector distance. The detector 2θ angle was set at 20° and the exposure time for each frame was 200 s.

Data reduction was accomplished using the *XENGEN* package (Howard *et al.*, 1987) adapted to run on VAX/VMS computers. The crystals are tetragonal with unit-cell dimensions $a = b = 63.61$ and $c = 95.69$ Å. Processing in space group $P4$ showed that there were systematic absences for $00l$ reflections with $l \neq 4n$ and $h00$ reflections with $h \neq 2n$. The crystals thus belong to one of the space groups $P4_12_12$ or $P4_32_12$. A total of 8039 unique reflections were collected with completeness of 91.9% at 2.35 Å resolution. 93.2% of the reflections had the intensities with $I/\sigma > 2.0$. The $R_{\rm merge}$ [$R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$] of the data was 0.0849.

On the basis of molecular weight and cell dimensions, one molecule could be present in the asymmetric unit, corresponding to a V_M value of 2.19 Å^3 Da^{-1} and a solvent content of about 44% (Matthews, 1968). The crystal structure determination is being continued.

Support for this project to LN has been provided by research grants from Chinese Academy of Sciences, National Laboratory of Biomacromolecules and National Education Committee of China.

References

Bjarnasson, J. B. & Fox, J. W. (1989). *J. Toxicol. Toxin Rev.* **7**, 121–209.

Gomis-Rüth, F. X., Kress, L. F. & Bode, W. (1993). *EMBO J.* **12**, 4151–4157.

Gomis-Rüth, F. X., Kress, L. F., Kellermann, J., Mayr, I., Lee, X., Huber, R. & Bode, W. (1994). *J. Mol. Biol.* **239**, 513–544.

Howard, A. J., Gilliland, G. L., Finzel, B. C., Poulos, T. L., Ohlendorf, D. H. & Salemme, F. R. (1987). *J. Appl. Cryst.* **20**, 383–387.

Kini, R. M. & Evens, H. J. (1992). *Toxicon*, **30**, 265–293.

McPherson, A. (1982). *Preparation and Analysis of Protein Crystals*. New York: John Wiley.

Maruyama, M., Sugiki, M., Yoshida, E., Shimaya, K. & Mihara, H. (1992). *Toxicon*, **30**, 1387–1397.

Matthews, B. W. (1968). *J. Mol. Biol.* **38**, 491–497.

Ownby, C. L. (1990). *Handbook of Toxinology*, edited by W. T. Shell & D. Mebs, pp. 601–654. New York: Marcell Dekker.

Xu, X., Wang, C., Liu, J. & Lu, Z. (1981). *Toxicon*, **19**(1), 633–644.

Zhang, S., Botos, I., Gomis-Rüth, F. X., Doll, R., Blood, C., Fox, J. W., Bode, W. & Meyer, E. (1994). *Proc. Natl Acad. Sci. USA*, **91**, 8447–8451.

Zhang, J., Chen, Z., He, Y. & Xu, X. (1984). *Toxicon*, **22**(6), 931–935.