Crystallization and preliminary X-ray diffraction studies of La1 from *Liocheles australasiae*

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A novel scorpion venom peptide, La1 from *Liocheles australasiae*, with a molecular weight of 7.8 kDa, is presumed to possess a single von Willebrand factor type C (VWC) domain, a common protein module, based on the position of eight Cys residues in its sequence. The biological function of La1 is still unknown. Deciphering its three-dimensional structure will be helpful in understanding its biological function. La1 was crystallized by the sitting-drop vapour-diffusion method using magnesium sulfate as a precipitant. The crystals belonged to the monoclinic space group *C2*, with unit-cell parameters $a = 63.0$, $b = 30.2$, $c = 32.3$ Å, $\beta = 108.5^\circ$, and diffracted to 1.9 Å resolution. The calculated V_M based on one molecule per asymmetric unit was $1.87 \text{ Å}^3 \text{ Da}^{-1}$. The solvent content was 34.1%.

1. Introduction

Scorpion venoms are composed of a number of peptides, many of which are reported to be neurotoxic to various organisms such as mammals, insects and crustaceans by their ability to specifically modulate ion channels in excitable membranes (Bergeron & Bingham, 2012; Rodriguez de la Vega & Possani, 2005; Zlotkin, 2005). Scorpions use these neurotoxic components to defend themselves against enemies and prey insects. Antimicrobial peptides have also been isolated from scorpion venoms, which are assumed to be necessary to protect the species from microbial infections *via* their venom glands (Zeng *et al.*, 2005). Scorpion venom peptides are classified into two main types: disulfide-bridged peptides and non-disulfide-bridged peptides (Zeng *et al.*, 2005). The disulfide-bridged peptides are further classified into two groups based on their activity and molecular size. The Na^+ -channel-specific toxins are composed of 60–76 amino-acid residues that are cross-linked by four disulfide bridges, whereas those of the K^+ -channels are composed of 31–39 amino-acid residues cross-linked by three or four disulfide bridges (Possani *et al.*, 1999).

In addition to these biologically active peptides, components of unknown function have also been identified in the venom (de la Vega *et al.*, 2013). We isolated a peptide, La1, from the venom of the scorpion *Liocheles australasiae* (Miyashita *et al.*, 2007). Although La1 is the most abundant component in the venom, its biological function is still unknown. Recently, many scorpion peptides with similar sequences to La1 have been reported and are termed ‘La1-like peptides’ (Abdel-Rahman *et al.*, 2013). Intriguingly, peptides homologous to La1 were found not only in scorpions, but also in ticks (Ribeiro *et al.*, 2011), insects (Sheldon *et al.*, 2007) and shrimps (Chen *et al.*, 2011), suggesting that La1-like peptides have an important biological role. La1 consists of 73 amino-acid residues cross-linked by four disulfide bonds. Although the disulfide-bridge patterns of these peptides, including La1, have not yet been determined, the peptides are presumed to possess a single von Willebrand factor type C (VWC) domain, a common protein module, based on the position of eight Cys residues in the sequence (Sheldon *et al.*, 2007).

The solution structure of the chordin-like cysteine-rich repeat, referred to as the VWC module, from collagen IIA (O’Leary *et al.*,

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2004) and the crystal structure of the N-terminal VWC domain of crossveinless-2 (CV-2) from zebrafish (Zhang *et al.*, 2008) have been reported. Both three-dimensional structures consist of two sub-domains tethered by a disulfide bridge. The N-terminal region, whose folding is stabilized by two disulfide bonds, consists of a two-stranded antiparallel β -sheet, while the C-terminal region is more irregular, with its folding constrained by two disulfide bonds. Knowledge of the three-dimensional structure of La1 will be helpful in understanding its biological function. Here, we report the crystallization and preliminary X-ray analysis of La1 from the scorpion *L. australasiae*.

2. Materials and methods

2.1. Sample preparation

Full-length La1, which consists of 73 amino-acid residues (7.8 kDa; FGESCIAGRIVPLGQQVTDQRDCALYKCVNYNKKFAL-ETKRCATVNLKSGCKTVPGGAGAAFPSCCPMVTCK) cross-linked by four disulfide bonds, was synthesized using the Fmoc solid-phase method using a sequential native chemical ligation with three segment peptides. Disulfide formation was carried out by air oxidation through dialysis against a series of buffers with a pH gradient. The folded peptide was purified by reversed-phase HPLC and further lyophilized. The synthetic La1 peptide showed the same molecular mass and retention time as native La1 on LC/MS analysis (unpublished data), indicating that the synthetic peptide is identical to native La1. The synthesis of La1 will be described in detail elsewhere.

2.2. Crystallization

La1 solution was prepared by dissolution of the lyophilized powder in distilled water to a final concentration of 10 mg ml⁻¹. Crystallization conditions were initially searched using the commercially available screening kit Crystal Screen HT (Hampton Research). Crystallization trials were performed using the sitting-drop vapour-diffusion method by mixing 1 μ l La1 aqueous solution with an equal volume of crystallization solution. The drops were then equilibrated against a 100 μ l reservoir of crystallization solution in a 96-well plate at 277 K. In the initial crystallization trials, La1 was crystallized in two different forms. Small needle-shaped crystals were obtained using solution No. 31 of the screen, which consisted of 0.2 M ammonium sulfate, 30% (w/v) PEG 4000. Crystals were grown using a lower concentration of precipitant, 24% (w/v) PEG 4000, to maximal dimensions of approximately 0.2 $\times$ 0.05 $\times$ 0.05 mm in one week. However, the crystals diffracted weakly to a maximal resolution of 4.5 Å. With condition No. 68, consisting of 0.1 M MES buffer pH 6.5,

1.6 M MgSO₄, well formed crystals grew to maximal dimensions of approximately 0.4 $\times$ 0.08 $\times$ 0.05 mm in three weeks (Fig. 1).

2.3. Data collection and processing

Preliminary X-ray diffraction studies were carried out at 100 K using an R-Axis IV⁺⁺ image-plate area detector mounted on an RA-Micro7HF rotating-anode X-ray generator with VariMax optics at 40 kV and 30 mA with Cu K α radiation. The crystals of La1 were mounted in LithoLoops (Molecular Dimensions) and flash-cooled in a nitrogen-gas stream at 100 K. The low-viscosity oil LV CryoOil (MiTeGen) was used to remove the excess of buffer solution surrounding the crystal. A complete data set was collected on an ADSC CCD detector using synchrotron radiation of wavelength 1.500 Å at the BL38B1 station of SPring-8, Japan. The crystal-to-detector distance was 150 mm and 360 images were recorded at 1° intervals with an exposure time of 2 s per image. The intensity data were processed and scaled using the *HKL-2000* package (Otwinowski & Minor, 1997).

3. Results and discussion

An X-ray diffraction data set was collected to 1.9 Å resolution on beamline BL38B1 at SPring-8 (Fig. 2). The crystal was determined to be monoclinic, space group *C2*, with unit-cell parameters $a = 63.0$, $b = 30.2$, $c = 32.3$ Å, $\beta = 108.5^\circ$. The statistics for the intensity data are shown in Table 1. Assuming one molecule of La1 (7.8 kDa) in the asymmetric unit, the V_M value was calculated to be 1.87 Å³ Da⁻¹, corresponding to a solvent content of 34.1%. This is within the range of values tabulated by Matthews (1968).

Molecular-replacement calculations with *MOLREP* in the *CCP4* suite (Winn *et al.*, 2011) using the structure of the N-terminal VWC domain (67 amino-acid residues, 23.6% sequence identity; PDB entry 3bk3; Zhang *et al.*, 2008) of CV-2 from zebrafish and the structure of the VWC module (73 amino-acid residues, 20.0% sequence identity;

Figure 1

A typical crystal of venom peptide La1 of *L. australasiae*. Its dimensions were approximately 0.4 $\times$ 0.08 $\times$ 0.05 mm.

Figure 2

An X-ray diffraction pattern of the La1 crystal (oscillation width of 1°). The edge of the diffraction frame corresponds to approximately 1.9 Å resolution.

Table 1

Data collection and processing.

Diffraction source	BL38B1, SPring-8
Wavelength (Å)	1.5000
Temperature (K)	100
Detector	ADSC CCD
Crystal-to-detector distance (mm)	150
Rotation range per image (°)	1
Total rotation range (°)	360
Exposure time per image (s)	2
Space group	C2
<i>a</i> , <i>b</i> , <i>c</i> (Å)	63.0, 30.2, 32.3
α , β , γ (°)	90.0, 108.5, 90.0
Mosaicity (°)	0.31
Resolution range (Å)	50–1.9 (1.97–1.90)
Total No. of reflections	31104
No. of unique reflections	4658
Completeness (%)	98.6 (100.0)
Multiplicity	6.9 (6.6)
$\langle I/\sigma(I) \rangle$	59.7 (42.3)
$R_{\text{r.i.m.}}^{\dagger}$ (%)	6.9 (22.7)
Overall <i>B</i> factor from Wilson plot (Å ²)	20.7

$^{\dagger} R_{\text{r.i.m.}}$ was estimated by multiplying the conventional R_{merge} value by the factor $[N/(N-1)]^{1/2}$, where N is the data multiplicity. $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity and $\langle I(hkl) \rangle$ is the average intensity for multiple measurements.

PDB entry 1u5m; O'Leary *et al.*, 2004) from collagen IIA as search models were unsuccessful. The C^{α} r.m.s.d. between the model and the structure to be solved must be less than 2 Å for successful molecular replacement (Abergel, 2013). Thus, we considered that the overall structure of La1 peptide (73 amino-acid residues) is considerably different from those of the two model peptides, although La1 was presumed to have the VWC domain like these two peptides. La1 has nine sulfur-containing residues [3.7% (w/w); one methionine and eight cysteines]. Thus, we attempted to determine the structure using the sulfur single-wavelength anomalous diffraction (S-SAD) method. However, the anomalous difference Patterson map calculated using

anomalous signal from S atoms (S-SAD) did not give any significant peaks. Therefore, heavy-atom derivatives of La1 crystals are currently being prepared for phase determination.

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