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# Crystallization and preliminary X-ray diffraction studies of the neurotoxic, heterodimeric phospholipase $A_2$ from the Taiwan viper (*Vipera russelli formosensis*)

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*This work is dedicated to Professor Wolfram Saenger on the occasion of his 60th birthday*

The 28 kDa heterodimeric complex from Taiwan viper (F4/F7 complex) is composed of a neurotoxic phospholipase $A_2$ (F4) and a non-toxic $PLA_2$-like component (F7). Despite a high sequence identity (65%), the biological and pharmacological activities of F4 and F7 are contrasting. The complex is a structural analogue of Vipoxin found in the venom of the Bulgarian viper *Vipera ammodites meridionalis*. It is unclear how and why such varied bioactivities are expressed in these similar components. The F4/F7 complex has been crystallized using hanging-drop vapour diffusion and macroseeding techniques. The space group is monoclinic $P2_1$ with unit-cell dimensions $a = 74.92$, $b = 85.13$, $c = 78.16$ Å and $\beta = 95.12^\circ$. X-ray intensity data to 2.0 Å resolution have been collected at 120 K and the structure has been solved using the molecular-replacement method. There are four F4/F7 complex molecules in the asymmetric unit, which do not exhibit any local point-group symmetry.

## 1. Introduction

The heterodimeric complex from Taiwan viper (*Vipera russelli formosensis*) (hereafter F4/F7 complex), a 28 kDa molecule, is composed of a neurotoxic phospholipase $A_2$ ($PLA_2$, designated F4) and a non-tox $PLA_2$ like component (F7) (Tsai *et al.*, 1996; Wang *et al.*, 1992). Despite a high sequence identity of 65%, the biological and pharmacological activities of F4 and F7 are contrasting. While F4 shows $PLA_2$ enzymatic activity the one of F7 is very weak, despite the presence of the catalytic residues. F7 acts as an inhibitor of F4 in reducing the $PLA_2$ activity by up to 60%. F4 is neurotoxic while F7 is not. Interestingly, F7 increases the lethal potency and neurotoxicity of F4 by up to threefold. This complex is reminiscent of the Vipoxin complex found in the Bulgarian viper (*Vipera ammodytes meridionalis*), a heterodimeric complex of a basic, highly toxic $PLA_2$ (Vp) and an acidic, catalytically inactive $PLA_2$-like natural inhibitor (Inh) (Aleksiev & Shipolini, 1971; Mancheva *et al.*, 1987). The primary structures of F4 and F7 are 92% identical to those of Vp and Inh in the Vipoxin complex, respectively (Wang *et al.*, 1992). Hence, the F4/F7 complex can be considered as the first structural analogue of Vipoxin. In spite of these similarities, these are marked differences in the biochemical and pharmacological properties of the F4/F7 complex and Vipoxin. For example, Vipoxin is a postsynaptic neurotoxin while the F4/F7 complex acts on presynaptic sites of the neuromuscular junction. The Inh decreases the toxicity of the Vipoxin complex by around fivefold while F7 potentiates the toxicity of the F4/F7 complex. The Inh in Vipoxin lacks the active-site residue His48 and has no enzymatic activity while F7 has His48 and exhibits very weak enzymatic activity. Vipoxin is readily soluble at neutral pH while the F4/F7 complex is not. Hence the F4/F7 complex appears like a natural variant of the Vipoxin complex with altered biochemical and pharmacological properties. The three-dimensional structure of the F4/F7 complex may provide a structural basis for these differences.

Phospholipase $A_2$ enzymes are extensively studied in terms of structure and function (Arni & Ward, 1996; Dijkstra *et al.*, 1981; Scott *et al.*, 1990; Kini, 1997; Lee *et al.*, 1998). There is significant therapeutic interest in the design of inhibitors for these enzymes (Schevitz *et al.*, 1995). The crystal structure of the Vipoxin complex has been reported recently (Perbandt *et al.*, 1997) providing valuable insights into the mechanism of $PLA_2$ inhibition, being the only structural data available to date on the Vipoxin class of $PLA_2$ complexes. In this paper we report the crystallization and preliminay X-ray diffraction studies of the heterodimeric neurotoxic $PLA_2$ complex from Taiwan viper (*Vipera russelli formosensis*), the first $PLA_2$ analogue of Vipoxin known so far.

## 2. Methods and results

About 200 mg of the Russell's viper venom were dissolved in 1 ml of distilled water and all 200 µl were gel-filtrated through a Superdex G75 HR 10/30 column on an FPLC system (Pharmacia) while the column was pre-equili-

**Table 1**
Data-collection statistics.

| Crystal data | |
|---|---|
| Space group | $P2_1$ |
| Cell parameters (Å, °) | $a = 74,92, b = 85.13,$ $c = 78,16, \beta = 95.12$ |
| Data collection | |
| Resolution (Å) | 2.0 |
| Number of observations | 147003 |
| Number of unique reflections | 65221 |
| $R_{merge}$ (%) | 6.1 |
| $R_{merge}$, last resolution shell (%) | 28.1 |
| Completeness (%) | 98.1 |
| Completeness, last resolution shell (%) | 97.9 |

brated in 0.1 *M* ammonium acetate buffer of pH 6.0. The major peak rich in proteins of 28 kDa was collected and lyophilized. These proteins were rechromatographied on the same column, now equilibrated and eluted with acetic acid in the presence of 0.6 *M* NaCl (pH 3.5). The major peak corresponding to the 14 kDa protein was harvested and dialyzed before lyophilization. This enzyme purification resulted in a 1:1 mixture of the $PLA_2$ subunits F4 and F7.

The lyophlized protein was found to be poorly soluble at neutral pH. Decreasing the pH to 4.5 using 50 m*M* sodium acetate buffer increased the solubility of the complex, which was crystallized using the hanging-drop vapour-diffusion method at 289 K. The hanging drops were 4 µl in volume and contained 2 µl of 24 mg ml$^{-1}$ protein in 50 m*M* sodium acetate buffer pH 4.5 and 2 µl of reservoir solution. These drops were equilibrated against 300 µl reservoirs containing 7% MPD, 1% PEG 4000 and 1 m*M* $CaCl_2$ at pH 4.5. After 45 d one crystal appeared in a drop. The rest of the drops were clear without crystals. However, macroseeding the clear drops was very successful and seeds grew to diffraction quality crystals within 24 h.

Three-dimensional X-ray intensity data were collected at X11 beamline of the EMBL Outstation (DESY, Hamburg) equipped with a MAR Research image plate scanner. The X-ray wavelength was 0.9063 Å. A total of 105 frames each with 1° of rotation were collected from one crystal cryo-cooled to 120 K using 25% MPD as cryo-protectant. $CaCl_2$ was found to be a necessary ingredient for proper cryo-cooling, absence of which increased the mosaicity to unacceptably high values. The frames were indexed and processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). Data-collection statistics are given in Table 1. Packing-parameter calculations based on a molecular weight of 28 kDa per heterodimer F4/F7 suggested the presence of four dimers in the asymmetric unit. This corresponds to a Matthews coefficient of 2.22 Å$^3$ Da$^{-1}$ which is within the observed range for protein crystals (Matthews, 1968).

No significant peak could be identified in self-rotation functions calculated using the program *POLARRFN* [Collaborative Computational Project, Number 4, 1994 (*CCP*4)] with various resolution ranges. However, this did not hinder the structure determination by molecular-replacement methods because of the availability of a very good model *i.e.* the Vipoxin complex (Perbandt *et al.*, 1997). The Vipoxin model was modified to match the sequence of the F4/F7 complex using the rotamer database of the program *O* (Jones *et al.*, 1991) and was used as the initial search model. The program *AMoRe* (Navaza, 1994) was applied for molecular-replacement calculations. Cross-rotation search using the data in the resolution range 20–3.4 Å resulted in 18 peaks higher than 0.5 times the highest peak, which were passed on to the subsequent translation search. The translation search was carried out using the data between 8 and 3.5 Å. Searching for the position of the subseqent dimer, while keeping the position of previously found dimers fixed, resulted in the location of four dimers designated as AB, CD, EF and GH. The correlation for the first dimer was 16% (*R* factor = 52.7%) which increased to 51.2% (*R* factor = 40.9%) for four dimers. Rigid-body refinement using the data in resolution range 20–3.5 Å resulted in a correlation of 65.2% and an *R* factor of 36.7%. Five cycles of refinement using *REFMAC* (Murshudov *et al.*, 1997) using the data between 25 and 2 Å reduced the *R* factor to 35.7% and *R* free to 40.4% (4% *R*-free set). The resulting electron-density map is clear for most of the residues. Although an approximate twofold local symmetry can be found between AB and CD dimers as well as between AB and EF dimers, there is no obvious symmetry between all four dimers, as also earlier evidenced by a featureless self-rotation map. It is known that local symmetry can often be rather complex when there are more than two molecules in the asymmetric unit (Wang & Jenin, 1993). It is not yet clear if the F4/F7 complex includes $Ca^{2+}$ which was a necessary ingredient for crystallization. The preliminary model of the F4/F7 complex shows a high structural homology to that of the Vipoxin complex. Further structure analysis and refinement is in progress and will allow a closer comparison between the F4/F7 complex and Vipoxin which may provide clues to their different biochemical, biological and pharmacological activities.

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