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Preliminary X-ray diffraction studies of triflin, a potent voltage-gated Ca^{2+} channel blocker derived from the venom of Habu snake

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Numerous toxic proteins have been isolated from several venomous organisms, such as snakes, marine mollusks, scorpions, spiders, and sea anemones, and these proteins have been well characterized. Neurotoxins are the most famous and lethal toxins commonly contained in the venom.

Recently, we have isolated and characterized novel neurotoxins from the snake venom. These proteins have larger size (around 25 kDa) compared with the known neurotoxic peptides. There are few reports that such larger proteins have neurotoxicity in the venom. Recently we found that the homologous proteins are widely contained in the snake venom. Our isolated proteins have potency to block the voltage-gated calcium channel at 0.1-1 μM range. We named these proteins "meizoneurotoxins", because they form a new category due to their unique structures and activities. Meizoneurotoxins have possibilities to act as novel receptors and to appreciate the mechanisms of channels. As the first step to grasp their character, we focused on triflin which is one of the meizonerotoxins derived from the venom of habu snake recently we isolated.

Preliminary X-ray diffraction study was carried out using in house instrument, but the crystals diffracted to 7 Å. We searched for the crystallization condition to prepare larger size, but it did not contribute to the improvement of resolution.

Here we report the crystallographic study of the triflin by using the BL41XU. Crystals were cryoprotected by adding glycerol to mother liquor to 30%. Crystals of native triflin diffracted to 2.7Å. Crystals belong to the space group $P4_12_12$ with unit cell constants of a=b=83.25, c=85.25Å. The data set was processed using the program HKL2000, and observed 35,714 unique reflections with an merging R-factor of 6.5% and a completeness of 94.7 % up to 2.7 Å resolution.

Now, we are investigating a heavy atom search for collecting the diffractions at an appropriate wavelength to enable MAD phasing.

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X-ray diffraction studies blood coagulation factor IX gla domain complexed with its binding protein

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Coagulation factor IX (FIX) is a crucial component of the blood coagulation system and its deficiency causes haemophilia B. Activated FIX associates with factor VIIIa on phospholipid membrane in the presence of Ca^{2+} ions and converts factor X to its activated form. FIX is known to possess Ca^{2+} binding sites in the Gla domain, which on binding Ca^{2+} ions helps to sustain its structure. Recently, we showed that physiological concentrations of Mg^{2+} ions greatly augmented the biological activity of factor IXa even in the presence of excess concentration of Ca^{2+} ions, suggesting that Mg^{2+} ions also bind to the Gla domain and cause structural changes that contribute to an increase in activity. Here, we report attempts to solve the structure of the FIX Gla domain in the presence of Mg^{2+} and Ca^{2+} ions to elucidate the binding sites of Mg^{2+} ions and the contribution of Mg^{2+} ions toward the structural changes.

We prepared a complex of bovine FIX Gla domain (GD46, residues 1-46) and FIX binding protein (IX-bp), isolated from the venom of *Trimeresurus flavoviridis*, and crystallized the complex in the presence of Mg^{2+} and Ca^{2+} ions.

Preliminary X-ray diffraction study was carried out using Photon Factory (PF, Tsukuba) beam line 6A, but the crystals diffracted to 2.5 Å. We attempted to prepare the larger size crystal, but it was too difficult to distinct Ca^{2+} and Mg^{2+} ions in the structure due to the insufficient improvement of the resolution.

Here we report the crystallographic study of the complex by using the BL41XU. Crystals were cryoprotected by adding PEG 6000 to mother liquor to 30%. Crystals of the complex diffracted 1.6 Å. Crystals belong to the space group $C2$ with unit cell constants of a=128.69, b=37.28, c=62.69Å, β=103.56°. The data set was processed using the program HKL2000, and observed 39,489 unique reflections with a merging R-factor of 3.7% and a completeness of 93.6 % up to 1.6 Å resolution.

Now, we are attempt to prepare the complex with Ca^{2+} but without Mg^{2+} condition for the estimate the contribution of Mg^{2+} ions toward the structure.