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Crystallographic analysis of cytokine BMP-10

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1 Introduction

Bone morphogenetic protein 10 (BMP-10) is a member of the TGF-β superfamily and plays a critical role in heart development. A substitution variant of BMP-10 gene has been found to be associated with hypertensive dilated cardiomyopathy. [1] BMP-10 is translated as a precursor protein with an N-terminal propeptide and a C-terminal region (active BMP-10) that is cleaved by a protease such as furin. [2] The mature protein consists of both the propeptide and BMP-10 as a complex, and interactions of this mature protein with the target receptors would promote releasing the activated BMP-10. To elucidate the detailed molecular mechanisms of maturation, activation, and pathogenesis of BMP-10, we have initiated crystallographic studies for human BMP-10. In this study, we report the preliminary X-ray diffraction analysis of human BMP-10.

2 Experiment

Human BMP-10 were expressed in HEK293-EBNA cells and purified from the culture supernatants. Initial crystallization conditions were screened by the sparse matrix method [3] using commercially available screening kits (Hampton Research) with the hanging-drop vapor diffusion method. Heavy atom derivative crystals were prepared by soaking in reservoir solutions containing 1-10 mM $HgCl_2$, Methylmercury(II) chloride, or K_2PtCl_4. Prior to X-ray diffraction experiments, the crystals were soaked in the mother liquor containing 20-25% glycerol as a cryoprotectant and flash-frozen in a nitrogen gas stream at 95 K. X-ray diffraction experiments were performed at beameline NW12A at PF-AR, KEK. The data were processed and scaled using the HKL2000 package [4] and were truncated using the CCP4 program suite. [5] Self-rotation function was calculated using MOLREP. [6]

3 Results and Discussion

The crystals of BMP-10 (Fig. 1) appeared within 7-14 days with typical dimensions of 0.2 × 0.2 × 0.2 mm^3. For the best set, the crystal diffracted X-rays up to 3.6 Å resolution. The detector, camera distance, wavelength, oscillation range, and exposure time were ADSC Q210r, 279.4 mm, 1.0000 Å, 0.5 degree, and 20.0 sec without an attenuator, respectively. A total of 180 frames were obtained.

The crystal belongs to the space group $I222$ or $I2_12_12_1$, with unit-cell parameters a = 183.0, b = 184.6, c = 186.0 Å. Assuming that 3 or 6 molecules are contained in an asymmetric unit, the Matthews coefficient V_M [7] is calculated to be 5.5, 2.7 $Å^3$/Da, corresponding to 77.5, 55.0% solvent content, respectively. Self-rotation function clearly show that BMP-10 molecules have non-crystallographic 2, 3, and 4 fold axes. These facts suggest that BMP-10 forms 12 or 24-meric oligomer with an octahedral pseudo-symmetry.

Since no clear solutions were obtained by the molecular replacement (MR) method, the heavy atom derivatives were prepared for the multiple isomorphs replacement (MIR) method. We have collected the data from the $HgCl_2$ derivatives at 4.2 and 5.0 Å resolution, and K_2PtCl_4 derivatives at 6.5 Å resolution. Due to the severe non-isomorphism of these derivative crystals, no interpretable peaks were observed in the difference Patterson maps. Combination of the MR and MIR methods is in progress.

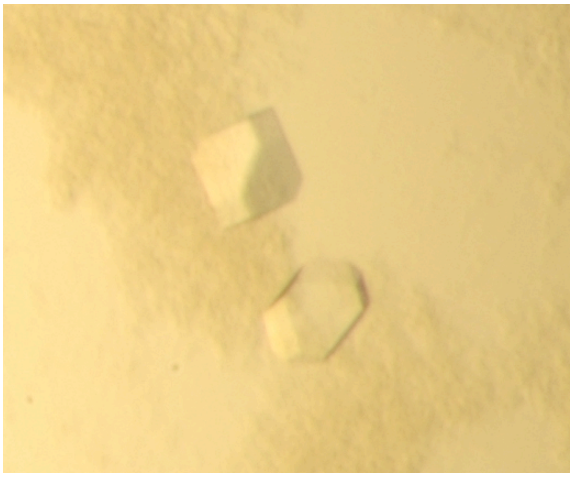

Fig. 1: Crystals of BMP-10.

References

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