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S-SAD phasing trials of human afamin

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1. Introduction

Afamin (AFM) is a secreted glycoprotein found in blood plasma at~30 μg/mL [1] and belongs to the albumin superfamily proteins, which also comprises serum albumin, Vitamin D binding protein, and alpha-fetoprotein. It is reported that AFM can bind to alpha-tocopherol (vitamin E) and is suggested to function as its carrier protein [2]. However other biological functions or physiological importance of this relatively abundant plasma protein has not been explored and no crystal structure is available so far. Recently, we found that AFM has a unique capability to bind lipidated Wnt proteins [3]. AFM forms 1:1 complex with Wnt3a and keeps this water-insoluble hydrophobic protein in a solution, while maintaining its biological activities. In order to clarify the molecular mechanism of this "Wnt protection" activity, we aim to determine the crystal structure of AFM at atomic resolution.

2. Experiment

Recombinant human AFM (hAFM) was produced using HEK293S GnTI- cells by stably transfecting expression plasmid coding for hAFM N-terminally tagged with a PA tag developed in our laboratory [4]. PA-hAFM was purified from the culture supernatants using Sepharose immobilized with the anti-PA tag NZ-1 (Wako Pure Chemical Co.). After the removal of the N-terminal PA-tag and N-linked glycans by TEV protease and Endoglycosidase H (New England Biolab), respectively, hAFM was finally purified by size exclusion chromatography on a Superdex 200 10/300 column and was concentrated with the ultrafilteration devise to approximately 10.0 mg/mL.

The crystallization conditions were screened with several commercially available screening kits, and the diffraction quality crystals were obtained under the condition of 100 mM Hepes-NaOH pH7.0, 200 mM ammonium sulfate, 5%(v/v) isopropanol, and 17.5-23.0%(w/v) PEG 8,000. The diffraction dataset were collected at BL1A, Photon Factory under the cryogenic helium stream of 100 K mounted by the CrystalCap Magnetic (Hampton Research) and the Litholoop (Protein Wave Corporation). The diffraction dataset were collected on 3-4 paths from three different crystals using the wavelength of 2.7 Å. For one path, the data range of 270° with the oscillation angle of 0.2° was collected. The collected data were processed using XDS[5], and the search procedure for anomalous scatterer was performed by SHELXC and SHELXD[6].

3. Result and Discussion

Our initial attempts to solve the phase of the hAFM diffraction data by molecular replacement method using serum albumin structure had failed, forcing us to seek for other means. Thus we tried to apply S-SAD phasing. Each dataset from single path showed reasonable values of R_{sym}, and $CC_{1/2}$ indicated the good quality of each dataset (Table1). The statistics for anomalous data, such as the correlation between random half-sets of anomalous intensity differences (AnomalCorr) and the mean anomalous difference divided by its estimated standard deviation (SigAno), indicated poor anomalous contribution. Merging the data of several paths from one crystal improved the anomalous statistics in all crystals (Fig). However, the position search trials of anomalous scatterers by SHELXD for the merged data failed with any resolution cutoff values. Although merging the data from several different crystals improved the statistics value slightly for the anomalous data (Fig), the search trials also failed. Based on these results, we suspect that the apparent signal difference appeared in the statistics of data from single crystal was not entirely originated from the true anomalous signal. In fact, the crystals of human AFM used in the current experiments appeared as stacked plates, and some diffraction images apparently contained several independent diffraction patterns, likely originated from different crystalline parts. The overlaps of

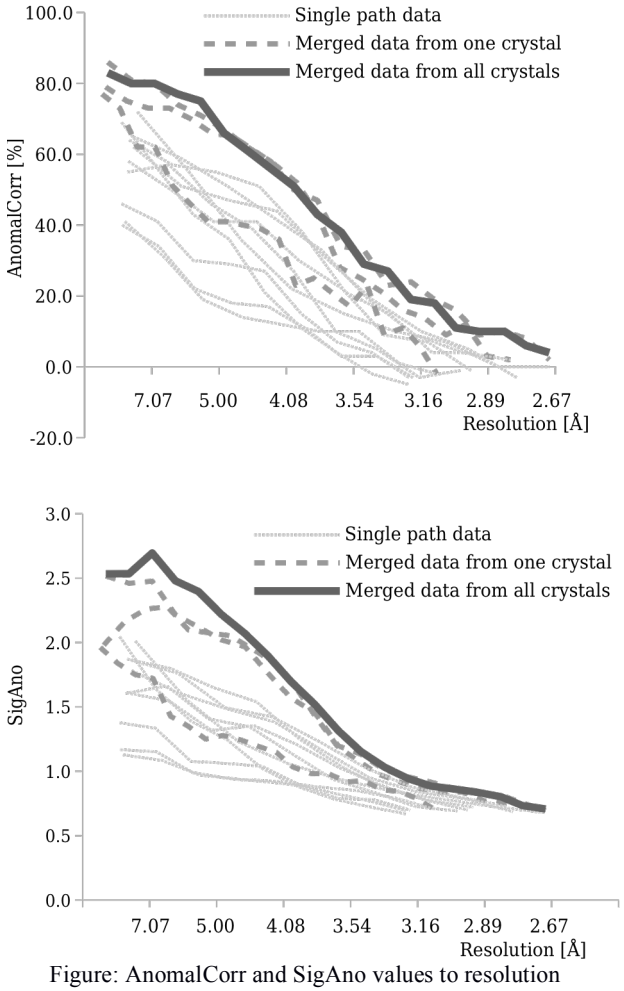

Figure: AnomalCorr and SigAno values to resolution

Table: Diffraction data statistics

Crystal	Crystal 1				Crystal 2					Crystal 3				merge
Dataset	Path 1	Path 2	Path 3	merge	Path 1	Path 2	Path 3	Path 4	merge	Path 1	Path 2	path3	merge	
Resolution limits [Å]	49.3-3.09 (3.27-3.09)	49.4-3.20 (3.39-3.20)	49.4- 3.22 (3.42-3.22)	49.3-3.09 (3.17-3.09)	49.1-2.93 (3.11-2.93)	49.4-2.79 (2.96-2.76)	49.4-2.74 (2.90-2.74)	49.4-2.69 (2.85-2.69)	49.1-2.69 (2.76-2.69)	49.7-2.99 (3.17-2.99)	49.4-2.81 (2.98-2.81)	49.3-2.95 (3.13-2.95)	49.6-2.81 (2.89-2.81)	49.3-2.69 (2.76-2.69)
No. Unique Reflections	49354 (7405)	44942 (6856)	43714 (7419)	50720 (3240)	56082 (8619)	65401 (9743)	69630 (10567)	73320 (11132)	74532 (5156)	55191 (8100)	65903 (10236)	56633 (8506)	66798 (4089)	76918 (4307)
Multiplicity	2.5 (2.3)	2.6 (2.3)	2.6 (2.3)	6.9 (2.3)	2.3 (2.1)	2.3 (2.2)	2.3 (2.2)	2.3 (2.1)	8.1 (2.7)	2.3 (2.1)	2.3 (2.1)	2.3 (2.0)	6.1 (1.9)	17.8 (2.3)
Completeness [%]	94.3 (88.0)	94.8 (89.8)	94.5 (89.5)	96.9 (84.0)	94.0 (89.5)	92.9 (86.0)	93.0 (87.4)	93.1 (87.4)	96.3 (90.4)	95.1 (86.9)	95.2 (91.4)	95.4 (88.8)	95.9 (79.3)	97.0 (73.6)
R_{sym}	0.152 (0.586)	0.157 (0.511)	0.148 (0.476)	0.171 (0.650)	0.105 (0.464)	0.106 (0.444)	0.098 (0.427)	0.097 (0.435)	0.158 (0.506)	0.123 (0.542)	0.117 (0.560)	0.134 (0.551)	0.159 (0.579)	0.208 (0.736)
<I/σI>	5.7 (1.6)	5.5 (1.8)	5.7 (1.8)	9.2 (1.4)	6.9 (1.6)	6.7 (1.7)	7.2 (1.7)	7.1 (1.7)	10.6 (1.7)	6.2 (1.8)	6.2 (1.8)	5.8 (1.7)	8.6 (1.8)	11.9 (1.6)

diffraction intensities from different crystals might be the source of the errors. In the case of S-SAD, it is crucial to obtain the diffraction images for a single path dataset from single crystal. Otherwise the weak anomalous difference of sulfur atoms will be easily masked by the overlapped intensities. For the success of S-SAD phasing for our human AFM crystal, it is necessary to collect more dataset from several paths of multiple crystals, and combine a part of them correctly based on the anomalous statistics.

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