# Structure analysis of artificial proteins

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## Introduction

Periodicities are the recurring theme observed in structures of proteins, genes, and genomes. Reiteration of short DNA sequences has been thought to gave birth to rudimentary biological structures in proteins. In this study, the structures of three artificial proteins encoded by artificially reiterated short DNA sequences and created periodic DNAs that have long open reading frames (microgene polymer proteins) were investigated by using SR light source.

## Results

### *SAXS experiments*

To know whether artificial proteins fold as compact as natural protein does, small angle X-ray scattering (SAXS) analyses was performed with solution of the three microgene polymer proteins, namely, #287 ($Cu^+$), #292 ($Cu^+$) and #320 ($\alpha^+$).

SAXS experiment was carried out at the solution scattering station installed at BL-10C, Photon Factory. Proteins were dissolved in 10 mM phosphate buffer containing 0.1 M NaCl (pH 6.0) and placed in a cell (1 mm X-ray path length with 20 m thick quartz windows) for the measurements. Wavelength of incident X-ray was 1.488 Å and the size of beam cross-section was 0.5 × 3.0 $mm^2$. The sample to detector distance was 202 cm, calibrated with meridional diffraction of dried chicken collagen. The same solution without protein was measured as background. The measurements were executed at an ambient temperature.

The SAXS data have revealed that the radii of gyration (Rg) of #287 ($Cu^+$), #292 ($Cu^+$) and #320 ($\alpha^+$) were 29.0Å, 26.6Å and 26.6Å based on the calculations of Guinier plot. These values were compared with the radius of gyration of natural proteins in their native form and in their denatured form. If #320 protein, whose molecular weight is 12,306, exists as monodisperse molecule in the solution, the data suggested the protein adopt molten state. In agreement with this conclusion, the molecular weight of #320 protein in gel filtration experiments with and without denaturant were calculated as about 50K. The Krutky plot of the small angle X-ray scattering data suggested the protein has globular structure rather than elongated structure.

### *Powder diffraction experiments*

The results from small-angle X-ray scattering analyses suggested that some of the microgene polymer proteins would take a unique and compact conformation under a certain condition.

This possibility was inquired by screening for crystallization conditions for the proteins. The #320 ($\alpha^+$), #292 ($Cu^+$) and #287 ($Cu^+$) proteins were used for crystallization experiments with the reagents and methods usually applied for natural proteins. As a result, a protein crystal was obtained for #320 ($\alpha^+$).

The protein was crystallized by using the hanging-drop vapor-diffusion method. A 50 mM HEPES buffer (pH 6.5) containing 25% (w/v) PEG-4000 and 20% (v/v) glycerol was used for a reservoir (1 ml). A drop containing a 5 µl of 1% (w/v) protein solution in 50 mM phosphate buffer and a 5 µl of the reservoir solution was equilibrate against the reservoir. A lot of micro-crystals appeared in few days at a room temperature. The largest crystal had a dimensions of ~0.2 × 0.2 × 0.01 $mm^3$. The crystals were rinsed with the reservoir solution and were confirmed that they are composed of #320 ($\alpha^+$)by a SDS-PAGE.

The micro-crystals were scooped up with a cryo-loop (Hampton research) from a hanging drop and flush-frozen in a liquid nitrogen. X-ray diffraction experiment was carried out at BL-18B station, Photon Factory. ($\lambda$ = 1.00 Å). The frozen drop was kept in a 100 K nitrogen stream from a cryo-system (Oxford Cryosystem) during the experiment. The diffraction image was recoded for 10 min. by using CCD detector.

Although the #320 ($\alpha^+$) crystal did not diffract well, a powder diffraction-like pattern was observed after a long time X-ray exposure. From the observed three distinct diffractions, two possibilities remained that the unit cell is 3D with dimensions (a, b, c) = (30, 18, 15 $A^3$) or it is 2D crystal with dimensions (a, b) = (30, 18 $A^2$). In either case, the crystal has proved that the microgene polymer protein can generate a ordered structure in condition dependent manner.

## References

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