Characterization of the initial stage of refolding and reassembly of an integral membrane protein OmpF by a small-angle X-ray scattering experiment

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OmpF porin is a trimeric integral membrane protein that functions as a general diffusion pore of the outer membrane of *Escherichia coli*. The identical subunit with a molar mass of 37 kg/mol consists of a sixteen-stranded anti-parallel β -barrel structure [1]. The protein is highly resistant to protease and a denaturing surfactant such as sodium dodecyl sulfate (SDS) [2]. In this project, our attention has been paid to the refolding and reassembly mechanism of denatured monomeric Porin solubilized with SDS into the stable trimer. In order to understand well the mechanism, we have been studied about the molecular assembly of an integral membrane protein OmpF Porin by synchrotron radiation small-angle X-ray scattering experiments [3].

OmpF porin was isolated from *Escherichia coli* B and purified by HPLC. The reassembly of the denatured monomer of OmpF porin into its trimer was obtained by a refolding method with a non-ionic surfactant, as will be reported elsewhere (Y. Watanabe *et al.* in preparation and 4). Solution X-ray scattering measurements were performed with a small-angle X-ray scattering spectrometer equipped with RIGAKU R-AXIS Ⅳ (IP, 30 X 30 cm) at the BL40B2 of Spring8. The camera length and the X-ray wavelength used were 1 m and 1.3 Å, respectively.

In this study, the small-angle X-ray scattering experiment of the reassembly of a denatured monomer into the stable species of OmpF porin in the presence of surfactants was performed at a protein concentration of 0.2 mg/ml. The typical result of the X-ray scattering experiments was similar to that of the previous study [3]. The radius of gyration of the protein complexed with surfactants ranged from 38 Å to 45 Å. The radius of gyration values of the renatured dimer, native trimer and denatured monomer in SDS were estimated to be 38, 43, and 42 Å, respectively [3].Therefore, within the error limits, this result is also reasonable under the present experiment conditions. In a stopped flow experiment, the aggregates of protein species were observed at the initial phase (within 5-10 min) of refolding and reassembly at temperatures from 5 to 24 ℃. Further work is in progress elucidate the initial phase of reassembly of OmpF porin through analysis of the data of solution scattering experiments.

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References

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Crystallization and preliminary X-ray diffraction studies of *sn*-Glycerol-1-Phosphate Dehydrogenase from the Aerobic Hyperthermophilic Archaeon, *Aeropyrum pernix* K1

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G-1-P dehydrogenase is identified to be the key enzyme in the biosynthesis of archaeal enantiomeric polar lipid structures. At present, no structural information is available for any G-1-P dehydrogenase. G-1-P dehydrogenase from the hyperthermophilic aerobic archaeon, *Aeropyrum pernix K*1 has been over expressed in *Escherichia coli*, purified to homogeneity and crystallized at 298 K using the hanging-drop vapour-diffusion technique. The crystallization condition yielded crystal suitable for X-ray diffraction analysis was found. A data set to 5.18 Å resolution was obtained from a flash-cooled crystal using synchrotron radiation. The crystals grew as polygonal shape and were found to belong to space group R3, with unit-cell parameters $a = b = 126.911$, $c = 310.40$ (Table).

Table

Data collection statistics

X-ray source	BL40B2 (SPring8)
Detector	ADSC Quantum 4R
Temperature (K)	100
Distance (mm)	300
Wavelength (Å)	1.0000
Exposure time (s)	60
Resolution range (Å)	100-5.0
Space group	R3
Unit-cell parameters (Å)	$a = b = 126.911$, $c = 310.40$
No. of observed reflections	25151
No. of unique reflections	7905
R_{merge} (Å)	0.147
Completeness (%)	97.5
Redundancy	3.18