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CRYSTALLOGRAPHIC STUDY OF THE PROTEIN COMPLEX OF SIGMA FACTOR AND ANTI-SIGMA FACTOR

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Introduction

Transcription in bacteria is facilitated by a complex of proteins called RNA polymerase which is a heteromultimeric complex of five essential protein subunits. Four subunits, $\alpha_2\beta\beta'$, are tightly associated and known as the core RNA polymerase. Association of core RNA polymerase with one of two families of homologous σ subunits forms the holoenzyme. In σ^{70} family, multiple σ factors are presented and share high sequence homology at amino acid level. Regulation of promoter transcription in flagella biosynthetic pathway organizes the flagellar genes into a transcriptional hierarchy of three classes. σ^{28} is a flagellar-specific alternative σ factor and is necessary for transcription from class 3 promoters. FlgM acts, by binding directly to σ^{28}, to prevent both the interaction with core RNA polymerase and the interaction of free σ^{28} to class 3 promoter DNA. NMR spectroscopy has showed that FlgM was mostly unstructured by itself in solution and that C-terminal amino acids from residues 47 through 94 of FlgM became structured in the presence of σ^{28}.

This result has awaken us to the fact that there are any biological importance in an unfolded protein. On the basis of this information, we purified and crystallized σ^{28} and the σ^{28}-binding domain of FlgM in order to obtain a more detailed information for understanding the regulatory mechanism by the anti-σ^{28} factor, FlgM.

Crystallization and Data collection

Crystals were obtained at 277 K by the hanging-drop vapour-diffusion technique. The protein solution was mixed in a 1:1 ratio with the reservoir solution. Clusters of very small needle crystals (maximum dimensions 5 x 5 x 50 μm) appeared in two weeks and were successively micro-seeded, which gave single crystals suitable for X-ray diffraction analysis. All data were collected from frozen crystals at 100 K. The crystals belong to the space group $P3_121$ or $P3_221$, with unit-cell parameters a=b=106.0, c=51.5 Å. A complete data set was collected to 3.2 Å resolution using a single crystal. To facilitate structure determination of the protein complex by the multiple-wavelength anomalous dispersion (MAD) technique, selenomethionine-substituted proteins for both σ^{28} and His-tagged σ^{28}-binding domain of FlgM have been prepared and crystallized.

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High-resolution X-ray Crystallographic Analysis on Toropomodulin C-terminal Domain

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Tropomodulin (40 kDa) is the unique P-end (slow growing end) capping protein of the actin filament. Actin-capping proteins attract interests of many scientists because the capping proteins not only determine the length of actin filament, but also these are involved in the actin dynamics (through polymerization –depolymerization cycles) commonly found in many motile system in live cells. To know the mechanism of capping / de-capping of actin filament, atomic structure of the capping proteins is of crucial importance. To date no atomic structure is known of any of these proteins. The structural information of tropomodulin would shed light on the mechanism how it could cap the P-end of actin filament.

Our systematic structural characterization of this protein (limited proteolysis, circular dichroism, differential scanning microcalorimetry and small angle X-ray scattering) revealed that the C-terminal half represents a compact globular cooperatively melting domain whereas the N-terminal half is elongated and has no definite tertiary structure in solution. The C-terminal half is so far reported that it is required for capping activity. Based on these results, we concentrated on crystallization of the C-terminal half domain, and successfully obtained a crystal. The crystal belongs to the space group of $R3$, with the cell dimensions of a = b = 69.3 Å and c = 101.2 Å.

Recently, we could obtain phase information of this crystal up to 1.8 Å by multi-wavelength anomalous dispersion (MAD) experiment at BL44B2, Spring-8, using Zn^{2+} incorporated into the native crystals during crystallization. In order to obtain higher resolution data to refine the structure more precisely, we collected three sets of diffraction data during this beam time at BL41XU with marCCD165 at a wavelength of 0.7 Å. The table shows data statistics of two merged data, one for high-resolution and another for low-resolution data collection. Structure analysis is now underway.

Table. Data statistics

wavelength (Å)	1.0
resolution (Å)	20.0 – 1.3
observed reflections	880319
unique reflections	44639
completeness (%)	99.6
R-merge (%)	5.8