Activation Mechanism of Tryptophan Synthase Complex

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Protein-protein interactions play a central role in many physiologically important reactions. Tryptophan synthase complex is an excellent model system for exploring the molecular recognition mechanism in protein-protein interaction, especially in multi-functional enzymes. Prokaryotic tryptophan synthase that catalyzes the last processes in the biosynthesis of tryptophan is a multi-enzyme α2β2 complex composed of non-identical α and β subunits. The separate α and β2 subunits catalyze inherent reactions termed α and β reactions, respectively. When the α and β2 subunits combine to form the α2β2 complex, the enzymatic activity of each subunit is stimulated by 1 to 2 orders of magnitude. In 1988 the X-ray crystal structure of the tryptophan synthase α2β2 complex from *Salmonella typhimurium* was determined. However, the structure of the α or β2 subunit alone has not yet been determined. In order to elucidate the molecular basis of the mutual activation of the subunit interaction due to the formation of the α2β2 complex, we need to know the structures of the α or β2 subunits alone as well as that of the complex.

Up to now, we have determined the crystal structures of tryptophan synthase α and β2 subunits from hyherthermophile, *Pyrococcus furiosus*. Now the crystal structure determination of α2β2 complex is remained.

We have collected the 3.5 Å resolution data of α2β2 complex in the last beam time of SPring-8. Therefore, we planed to collect the higher resolution data of α2β2 complex in this beam time and tried the improvement of the crystallization condition. The qualities of many crystals were checked using a CCD detector with a 1.0 Å wavelength at 100K. The crystals belong to the orthorhombic system with the space group $P2_12_12_1$ and cell dimensions f a = 87.88, b = 218.9 and c = 289.1 Å. The best crystal diffracted to 3.2 Å resolution. The data were processed and scaled with the program HKL2000. The R_{merge}, completeness and I/sI were 8.0 %, 94.2 % and 13.2, respectively. The electron density map calculated using the positions of β2 subunits determined by molecular replacement reveals the positions of remaining α subunit. Now we are trying to get high-resolution crystals by water-mediated transformation.

In order to investigate the role of salt bridges in protein stability and mutual activation mechanism, we also collected the data of tryptophan synthase α subunit mutants, D110A, E74A, E103A, E244A and E131/132A. Their data statistics are as follows: 2.2 Å resolution, R_{merge} of 3.9-5.9 % and completeness of 81.3-99.2 %. The refinements are under way.

Structural Analysis of The Muscle Regulatory Protein Complex by X-ray Crystallography.

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Troponin (Tn), consisting of three subunits, TnC, TnI and TnT, play a key role in the regulation of striated muscle contraction. Upon calcium binding to the regulatory sites of TnC, Tn undergoes a structural change, that is transmitted to other constituents of the actin-containing thin filaments. Eventually this turns muscle filaments "off" to "on" state, resulting sliding occurs between actin and myosin filaments. For further understanding of the mechanism underlying the regulatory system, it is crucial to solve atomic structures of the protein.

We have succeeded to obtain crystals of the fragments of Tn ternary complex. Best-diffracted crystals were obtained from 46kDa fragment that still retains the regulatory activity of acto-myosin ATPase. The crystals belong to the space group $P2_1$, with the following cell dimensions, a=42Å, b=169Å, c=69Å and β=101deg. We have recently obtained MAD phases up to 3.2 Å resolution from the single osmium derivative of this crystal form that give us an interpretable electron density map. The other complex, 51kDa fragments, also crystallized under the almost identical conditions. Comparing these two crystal forms, obvious difference was found in their cell dimensions along a-axis (a=48Å, b=170Å, c=69Å and β=101deg), suggesting the existence of another folded domain within the 51kDa fragment.

We have collected diffraction data from the two crystal forms during this period at BL41XU with marCCD 165 detector at a wavelength of 1 Å. The data statistics are summarized below, and analysis is now underway.

Complex	46kDa	51kDa
Resolution	20 - 2.5 Å (2.75-2.5Å)	20 - 3.2Å (2.75-2.6Å)
Completeness	95.8% (77.7%)	98.2%(88.8%)
R-merge	5.9%(35.0%)	4.0%(37.3%)