Structure analyses of collagen model peptides,

(Pro-Hyp-Gly)n (n=9, 10, 11)

Kenji Okuyama*[1](4310), Keiichi Noguchi[2](4221), Chizuru Hongo[1](6969), Wu Guanghan[1](6095), Takafumi Ohuchi[1](6188), Shinichi Sasakura[1] (7563)

[1]Department of Biotechnology and Life Science, Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

[2]Instrumentation Analysis Center, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

The Pro-Hyp-Gly triplet is the most abundant sequence occurred in a helical region of collagen amino acid sequence. It is well known that Hyp at Y position in the X-Y-Gly sequence stabilizes triple-helical structure of collagen. On the other hand, Hyp at X position destabilizes triple helical structure. For example, the helix-coil transition temperature of (Pro-Hyp-Gly)$_{10}$ is higher than that of (Pro-Pro-Gly)$_{10}$ by about 30°C. While (Hyp-Pro-Gly)$_{10}$ could not make a triple-helix in the same condition. Three hypotheses were proposed to explain the stabilization of triple-helix by Hyp. To verify these hypotheses, the precise structures of various collagen-model peptides are necessary. In the present beam time, we collected intensity data of (Pro-Hyp-Gly)$_{11}$ at both 100K and room temperature.

X-ray diffraction experiments were performed at BL40B2 of the SPring-8 synchrotron radiation source. Diffraction data were recorded on ADSC Quantum 4 CCD detector system with a total oscillation range of 180°. The oscillation angle and exposure time per frame were 1.0° and 10sec, respectively. Intensity data were processed using CrystalClear program. The crystallographic statistics are shown below.

Out of seven, three proline rings showed the up-puckering in the structure at 100K, which is opposed to the recent structural basis hypothesis to explain stabilization of triple-helix by Hyp. On the other hand, these puckering in the structure at RT have a different tendency. The present result suggested that the structural basis hypothesis needs some reinforcement before completion.

	RT	100K
Crystallographic data		
Space group	$P2_1$	$P2_1$
Cell dimension		
a / Å	13.9	13.7
b / Å	26.1	26.2
c / Å	20.0	19.9
β / °	106.0	105.8
Refinement statistics		
Resolution range /Å	10-1.25	10-1.30
No. of peptide atoms	133	133
No. of waters	45	52
No. of reflections	3547	3126
(validation)	157	162
R / Rfree	0.13/0.18	0.12/0.17

Crystallographic Studies of ATP sulfurylase

Yuichi Taguchi (0005789)[1], Yoshimitsu Kakuta (0004757)[2]*

[1]Department of Biology, Graduate School of Science, Osaka University, Toyonaka 560-0043, Osaka

[2]Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, Fukuoka 812-8581

ATP sulfurylases (EC 2.7.7.4, sulfate adenylyltransferase) are ubiquitous enzymes that catalyse the primary step of sulfate activation. The reaction of inorganic sulfate with ATP to form adenosine-5'-phosphosulfate (APS) and pyrophosphate (PPi). ATP sulfurylase is used to generate APS from inorganic sulfate and ATP. This is the first step in the conversion of inorganic sulfate to a variety of organic sulfur compounds including sulfate esters in animals and reduced sulfur-containing biomolecules in plants and microorganisms.

The reconbinat ATP sulfurylase from *thermus thermophilus* was prepared using E.coli overexpression system. The Purified ATP sulfurylase was crystallized using hanging drop vapor diffusin meehod at 20℃. Crystals with dimensions of 0.1 x 0.1 x 0.05 mm^3 have been obtained in a few days. Se-Met substisuted crystal for the MAD phasing was crystallized under same conditions.

X-ray diffraction measurements were carried out under cryogenic condition (100K) using flash-cooling technique. The Se-Met crystal belongs to space group $P2_1$ with cell dimensions of a = 68.7 Å, b = 61.4 Å, c = 129.5 Å, α =90°, β =96.1°, γ =90°. MAD data with three wavelengths were collected up to 3.2 Å resolution using ADSC Quantum4R CCD detector. The MAD data were processed using the program HKL2000 for integration and scaling. Phase detemination is under progress.