Crystal Structure Analysis of Diol Dehydratase-Cyanocobalamin Complex Crystallized in the Absence of Substrate

***Tetsuo Toraya[1] 6198, Mamoru Yamanishi[1] 1799, Koichi Mori[1] 7065, Masaki Fukuoka[1] 7064, Kyoko Suto[2] 3016, and Noritake Yasuoka[2] 3009**

[1]Okayama University [2]Himeji Institute of Technology

Vitamin B_{12} coenzyme (adenosylcobalamin) serves as a cofactor for the enzymatic radical reactions. The cobalt-carbon bond of the coenzyme undergoes activation and cleavage upon binding to apoenzyme. This is the initial step common to all the adenosylcobalamin-dependent enzymatic reactions. We have carried out the structural studies of diol dehydratase, one of the representative adenosylcobalamin-dependent enzymes. First, we determined the crystal structure of the inactive complex of the enzyme with cyanocobalamin. Secondly, we succeeded in the crystal structure analysis of the complex with adeninylpentylcobalamin, an adenine-containing inactive analog of the coenzyme. These structures opened a new horizon for our understanding of the mechanism of action of this enzyme. From the structures, it becomes also possible to discuss about the mechanism of activation of the cobalt-carbon bond.

From the biochemical data, it has been established that the substrate binding to the holoenzyme triggers the homolysis of the coenzyme C-C bond. In order to elucidate the role of the substrate-induced conformational change in the catalysis, it is essential to determine the structure of the enzyme in the absence of substrate and compare the structure with that of the substrate-containing complex.

Recently, we have succeeded in the crystallization of the enzyme-cyanocobalamin complex in the absence of substrate. In the last beam time, we measured diffraction on BL40B2. In this beam time, we collected the diffraction data at a cryogenic temperature on BL41XU to compare the structures of the substrate-containing enzyme that was determined on BL41XU. The crystals gave good diffraction data, and we obtained the crystal structure of the complex with about 2.0-Å resolution.

Crystal Structure Analysis of Diol Dehydratase-Cyanocobalamin Complexes in the Presence of Optically Active Substrates

***Tetsuo Toraya[1] 6198, Mamoru Yamanishi[1] 1799, Koichi Mori[1] 7065, Masaki Fukuoka[1] 7064, Kyoko Suto[2] 3016, and Noritake Yasuoka[2] 3009**

[1]Okayama University [2]Himeji Institute of Technology

Vitamin B_{12} coenzyme (adenosylcobalamin) serves as a cofactor for the enzymatic radical reactions. The cobalt-carbon bond of the coenzyme undergoes activation and cleavage upon binding to apoenzyme. This is the initial step common to all the adenosylcobalamin-dependent enzymatic reactions. We have carried out the structural studies of diol dehydratase, one of the representative adenosylcobalamin-dependent enzymes. First, we determined the crystal structure of the inactive complex of the enzyme with cyanocobalamin. Secondly, we succeeded in the crystal structure analysis of the complex with adeninylpentylcobalamin, an adenine-containing inactive analog of the coenzyme. These structures opened a new horizon for our understanding of the mechanism of action of this enzyme. From the structures, it becomes also possible to discuss about the mechanism of activation of the cobalt-carbon bond.

It is well known that the enzyme acts on both (*R*)- and (*S*)-1,2-propanediol. This phenomenon is very interesting, because most enzymes are specific for one of the optically active isomers of substrates. To solve the molecular basis of this apparent lack of stereospecificity, we attempted to determined the X-ray structures of diol dehydratase·cyanocobalamin complexes which were crystallized in the presence of each optical isomer.

Recently, we have succeeded in the crystallization of the enzyme-cyanocobalamin complex in the presence of (*R*)- or (*S*)-1,2-propanediol. We collected the diffraction data of the complexes crystallized in the presence of each isomer. The crystals gave good diffraction data, and we obtained the crystal structures of the complexes with about 2.0-Å resolutions.