P-2 10:40 ~ 11:20 Plenary Talk II

STRUCTURAL BIOLOGY OF A RADICAL-CATALYZED ENZYMATIC REACTION IN COBALAMIN-DEPENDENT DIOL DEHYDRATASE

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The first X-ray structure of vitamin B_{12} dependent enzyme was reported on a cobalamin-binding fragment of methionine synthase, and followed with the studies on whole enzymes of methylmalonyl-CoA mutase and glutamate mutase. These studies revealed that proteins bind cobalamin in the so-called "base-off" mode with histidine ligation. We carried out an X-ray analysis of diol dehydratase complexed with cyanocobalamin and adeninylpentylcobalamin, and found the enzyme exists as a dimeric form of heterotrimer, $(\alpha\beta\gamma)_2$, and binds cobalamin in the "base-on" mode in contrast to other enzymes. In addition, we found the location of an essential K^+ ligated with the substrate, 1,2-propanediol. The unique feature thus obtained allowed us to present a new insight on the mechnism of diol dehydratase. In the adeninylpentylcobalamin complex, the adenine ring is bound parallel to the corrin ring as in free and methylmalonyl-CoA-mutase-bound coenzyme, but with the reverse side facing to pyrrole ring C. Most of its nitrogen atoms are hydrogen-bonded to main chain amide oxygen and amide nitrogen atoms, a side chain hydroxyl group, and a water molecule. Superimposing the structure of the free coenzyme on that of enzyme-bound adeninylpentylcobalamin in a modeling study demonstrated that the tight enzyme-coenzyme interactions at both cobalamin moiety and adenine ring of the adenosyl group would inevitably lead to cleavage of the cobalt-carbon bond. Because adeninylpentylcobalamin is unstable to the visible light, a series of experiments from the preparation of crystals to X-ray data measurement were carried out in the dark environment. The effect of photoirradiation or illumination with a light source seems to cause homolysis of the Co-C bond to form an adeninylpentyl radical. In order to confirm this anticipation, X-ray data were collected at 100K with a crystal illuminated by a halogen lamp before and during the X-ray irradiation. The electron density maps of the adeninylpentylcobalamin moiety in the dark and in the illuminated states together with that of cyanocobalamin at 100K show the peaks corresponding to the upper ligand. The temperature factors of atoms comprising the adeninylpentyl group in the illuminated state are much larger than those in the dark states. These observations clearly indicate that the pentamethylene group is cleaved

from Co atom during the photoirradiation, whereas the adenine moiety remains held by hydrogen bonds with some residues in the α subunit. Formation of an adenine-anchored radical upon photoirradiation was demonstrated crystallographically with the enzyme-adeninylpentyl-cobalamin complex. Although the formation of an adenine-anchored radical under photolytic conditions mimics an initial stage of the diol dehydratase catalysis, no enzymatic reaction followed it. This would be partly because the adeninylpentyl radical could not be placed close to the substrate due to lack of the β-D-ribofuranose ring and its functional groups.