Structure of Diol Dehydrase containing Vitamin B12 analogue

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Introduction.

Diol dehydrase is a membrane-binding enzyme that catalyzes the adenosylcobalamin dependant conversions of 1,2-diols to the corresponding deoxy aldehydes. Diol dehydrase apoenzyme is composed of three polypeptide chains with molecular weights of 60,000 (α), 24,000 (β) and 19000 (γ). The holoenzyme has two of each subunit and two adenosylcobalamins and its molecular weight is 230,000. Molecular cloning of genes encoding diol dehydrase and their sequences have been reported but a membrane binding site is remeins unknown.

Crystal structures of cobalamin-dependant enzymes, methylmalonyl-CoA mutase and methionine synthase, have been reported. Both enzymes have the structure that a cobalt-dimethylbenzoimidazole bond of a cobalamin is broken and a histidine coordinates to a cobalt ion, instead. On the contrary, diol dehydrase has been reported that it conserves the cobalt-dimethylbenzoimidazole bond. On the opposite side of a corrin ring, an adenosyl group coordinates to the cobalt ion via a Co-C bond. Activation of the Co-C bond is the initial step of the reaction. Our interest is how diol dehydrase activates the Co-C bond and how the protein is bound to membrane. We crystallized diol dehydrase to solve its crystal structure.

Results

Native data of the diol dehydrase crystal were collected by using the Weissenberg camera and the large-formatted imaging plates (40 x 80 cm). A wavelength of an incident beam was 0.708 Å and a crystal-to-detector distance was 460 mm. The crystal was sealed by a glass capillary and then stored in an ice box. The crystal was set on the camera and was kept cooled during the data collection by the Oxford Cryosystems and the temperature was set at 271 K on an indicator of the system. The crystal diffracts over 2.2 Å resolution and 22 frames with a rotation angle of 4.5° for each frame were stored from one crystal. The crystal belongs to the orthorhombic space group $P2_12_12_1$ with unit cell dimensions of a=77.0, b=122.3 and c=209.3 Å. Supposing one molecule per an asymmetric unit, a Vm value was calculated to be 2.24 $Å^3$/Da (Solvent content 45 %). The combined set gave 399,921 reflections to the 2.2 Å resolution in total, which were reduced to 87,379 unique reflections with an R_{merge} of 5.9% and the completeness of 84.4% (64.6% for 2.28 to 2.20 Å). We think the data has good quality, and hope to use shorter wavelength at next beam time.