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Structure of coenzyme B_{12}-dependent ethanolamine ammonia-lyase in complexed with 2-aminopropanol and cyanocobalamin

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Introduction

Ethanolamine ammonia-lyase (EAL) catalyses the formation of acetaldehyde and ammonia from ethanolamine (EA) or of ammonia and propionaldehyde from 2-amino-1-propanol (2-AP). The reaction is initiated by cleavage of the cobalt-carbon bond of coenzyme B_{12} (adenosylcobalamin, AdoCbl) to form a cob(II)alamin-5'-deoxyadenosyl radical pair. The 5'-deoxyadenosyl radical abstracts a hydrogen atom from the C1 carbon atom of substrate to form a substrate radical, followed by migration of an amino group between substrate carbon atoms. Coenzyme B_{12}-dependent enzymes increase the cleavage rate of the cobalt-carbon bond by 10^{11} > compared with AdoCbl in solution. EAL is composed of six pairs of α and β-subunits, the total molecular weight reaches to ~480,000. The crystal structure of EAL has been previously determined as the ethanolamine-bound form at 2.3 Å. Although the electron density of the ethanolamine molecule was clear enough to determine the conformation of the molecule, it was unable to distinguish the two possible orientations of the molecule at the resolution due to its symmetricity. The aim of this study is to determine the orientation of the substrate molecule bound to the enzyme using 2-AP that breaks the symmetricity due to the methyl group bound to the C2 carbon atom.

Results and discussion

Crystals of EAL in complexed with cyanocobalamin (CN-Cbl) and (±)-2-AP were obtained by soaking a substrate-free crystal in a crystallization reservoir containing 10 mM (±)-2-AP. Diffraction data were collected at the BL-17A beamline, Photon Factory. The crystal diffracted to 2.05 Å resolution at 100K. The image frames were processed, scaled, and merged using the program HKL2000. The structure of the complex was obtained by refining the structure of the EA-bound form. Refinement of the structure was performed with cycles of REFMAC and manual rebuilding of the model.

The overall structure of each subunit is generally similar to the corresponding subunit of diol dehydratase [1] and the other B_{12}-dependent enzymes. The α subunit has a TIM-barrel motif in which the substrate-binding site is. The β subunit starts with the helical domain, followed by the Rossman-fold domain. The helical domain seems to be important for binding between β subunit and its two adjacent α subunits. The cobalamin molecule is bound on the interface between the TIM-barrel of α subunit and the Rossman-fold domain of the β subunit. The $\alpha_6\beta_6$ complex forms a trimer of dimer structure with a propeller-shaped, viewed along the crystallographic 3-fold axis that relates the trimer. The crystallographic asymmetric unit contains two αβ units related by a non-crystallographic 2-fold axis perpendicular to the crystallographic 3-fold axis.

The electron density map indicated that the substrate molecule was oriented so that the C1 carbon atom to which the reactive hydrogen atom is attached is closer than the C2 atom (Figure 1). The substrate molecule is held by five residues, Arg160, Gln162, Asn193, Glu287, and Asp362 through hydrogen bonds. Arg160 seems to correspond to the catalytic cation of the substrate-binding site of diol dehydratase (Fig. 1). The Co-substrate distances are 8.5 Å (Co-C1) and 9.5 Å (Co-C2). The Co-C2 distance is in good agreement with the distance estimated by EPR spectroscopy (9-10 Å) [2], whereas the Co-C1 distance is considerably shorter than that of EPR (10-12 Å) [3].

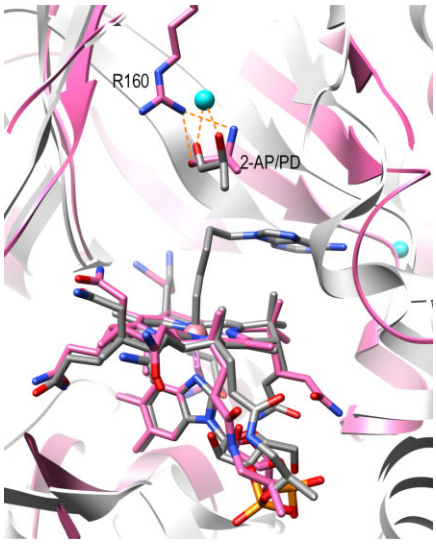

Figure 1. The active site structure between EAL (pink) and diol dehydratase (gray).

References

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