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Crystal structure of ethanolamine ammonia-lyase complexed with coenzyme B_{12} analog Adeninylpentylcobalamin and substrate

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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. The reaction is initiated by cleavage of the cobalt-carbon bond of adenosylcobalamin (AdoCbl) to form cob(II)alamin-5'-deoxyadenosyl radical pair. The 5'-deoxyadenosyl radical abstracts a hydrogen atom from the C1 carbon atom of the substrate to form a substrate radical, followed by the migration of an amino group from C2 to C1 of the substrate. AdoCbl-dependent enzymes accelerate the cleavage rate of the cobalt-carbon bond by $>10^{11}$ compared with AdoCbl in solution. EAL is composed of six pairs of α subunit (EutB protein, Mr = 49k) and β-subunit (EutC protein, Mr = 31k), and the total molecular weight reaches to ~480,000. The aim of the structural work of EAL is to investigate how the enzyme activates the cobalt-carbon bond of AdoCbl and catalyses the reaction by a radical mechanism.

Experimental Procedures

Data collection, structure determination, and refinement

X-ray diffraction data collections were performed at the Photon Factory BL-17A beamline for the Adeninylpentylcobalamin (AdePeCbl)/EA complexes. Prior to diffraction experiments, the crystals were flash-cooled with a nitrogen-gas stream at 100 K. Diffraction data sets were indexed, integrated, and scaled with the HKL2000 program. The EAL/AdePeCbl/EA complex crystal diffracted to up to 2.3 Å resolution. The space group belongs to $P6_3$ and the unit cell parameters of the native form were $a = b = 242.73$ Å and $c = 76.66$ Å. For structure determination, the model of cyanocobalamin/EA complex was used as the initial model. The model was improved by iterative rounds of refinement using REFMAC5 and model rebuilding using COOT until the R_{free} value decreased to less than 30 %. At this stage, TLS refinement using REFMAC5 was applied to the model, and several further refinement cycles yielded the final model.

Results and Discussions

Adenine ring-binding site and its vicinity

The adenine ring of AdePeCbl shares the same site in complexes with both EAL and diol dehydratase, which is sitting on the methyl group on C12 of the pyrrole ring C. Like in free AdoCbl, the adenine ring of AdePeCbl is bound to EAL almost parallel to the corrin ring, but with the other side facing the pyrrole ring C. This mode of adenine binding is similar to that observed in diol dehydratase [1].

One of the central questions to be answered is how the Co-C bond of AdoCbl is cleaved upon its binding to EAL apoenzyme. When the cobalamin moiety of AdoCbl is superimposed on that of the EAL-AdePeCbl complex, the adenine ring of the coenzyme is positioned in a different direction from the adenine ring-binding site and cannot be superimposed on the site without cleaving the Co-C bond (Fig. 1A), as in the case of diol dehydratase [1]. If both the cobalamin moiety and the adenine ring were superimposed on those of the enzyme-bound AdePeCbl with the Co-C bond cleaved and the Co-C distance kept at a minimum, then the Co-C distance has to be elongated to 3.5 Å and the Co-C bond has to lean toward the nitrogen atom of pyrrole ring B to the C5'-Co-N (ring B) bond angle of 61° (84° for free AdoCbl) and the Co-C5'-C4' bond angle of 150° (124° for free AdoCbl) (Fig. 1B). Therefore, it is evident that marked angular strains and tensile forces induced by tight interactions of the coenzyme with the enzyme cobalamin-binding site and the adenine ring-binding site inevitably break the Co-C bond.

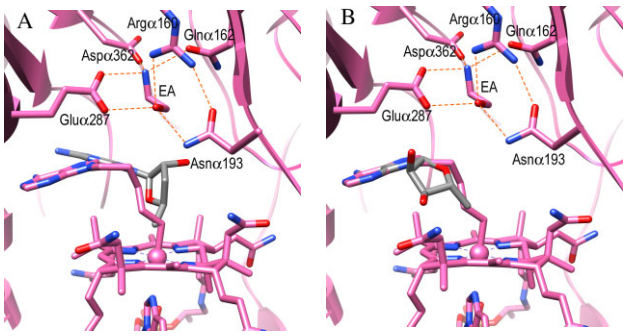

Fig. 1. AdoCbl-bound model of EAL based on the AdePeCbl-complexed structure. A, Without cleavage of the Co-C bond. B, Superimposed on the adenine-rings of AdePeCbl and AdoCbl so that the Co-C distance is kept at a minimum.

References

[1] Masuda, J. *et al*.. (2000) *Structure* **8**, 775-788

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