COENZYME BIOSYNTHESIS AND DELIVERY IN THE ETHANOLAMINE UTILIZATION METABOLOSOME

ABSTRACT

Corrinoids are characterized by a tetrapyrrole ring coordinating a central cobalt ion. The coenzymic form adenosylcobalamin (AdoCbl) is required by species in all domains of life. However, only a few prokaryotes including *S. enterica* can produce AdoCbl *de novo*. Additionally, organisms that scavenge incomplete corrinoids must encode an ATP:Co(I)rrinoid adenosyltransferase (ACAT) to activate the vitamin form to the coenzymic form.

There are three families of ACATs, which are named CobA, PduO and EutT. Structural and mechanistic understanding of the CobA and PduO families of ACATs is extensive, however relatively little is known about the EutT family of ACATs. In *S. enterica,* EutT (*Se*EutT) is a metalloenzyme, and requires Fe(II). The residues that bind Fe(II) in *Se*EutT are not conserved in the EutT homologues of *Firmicutes* such as *Listeria monocytogenes*. Biochemical characterization of the metal-less EutT from *L. monocytogenes* (*Lm*EutT) revealed that *Lm*EutT belongs to a second class of EutT-type ACATs whose mechanism proceeds in a metal-independent manner.

Analysis of *Lm*EutT revealed sequence homology to the *Lactobacillus reuteri* PduO (*Lr*PduO) corresponding to residues that form the ATP binding site and formation of an intersubunit salt bridge in *Lr*PduO. Mutation analyses of these residues in *Lm*EutT and characterization of the resulting variants suggests a substrate binding mechanism for *Lm*EutT analogous to *Lr*PduO.

The product of EutT, AdoCbl, is required in the catabolism of ethanolamine. The ability to utilize this metabolite provides *S. enterica* a competitive advantage over the course of infection. *S. enterica* metabolizes ethanolamine in a proteinaceous compartment (metabolosome) that sequesters the reactive intermediate acetaldehyde. In *S. enterica,* the functions for ethanolamine catabolism are encoded in the ethanolamine utilization (*eut*) operon, including EutT, the AdoCbl-dependent enzyme ethanolamine ammonia-lyase (EAL), and the reactivase EutA. *In vivo* and *in vitro* studies described herein suggest that EutT, EutA and EAL are localized to the metabolosome, and that EutA and EAL interact.