Crystallographic snapshots of TsrM, a cobalamin-dependent radical S-adenosylmethionine methylase

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TsrM methylates C2 of the indole ring of L-tryptophan (Trp) as the first step in the biosynthesis of the quinaldic acid moiety of thiostrepton, a ribosomally synthesized and post-translationally modified thiazolyl peptide natural product that exhibits potent in vitro activity against many Gram-positive bacteria of urgent clinical relevance. It is annotated as a member of the radical S-adenosylmethionine (SAM) superfamily and exhibits hallmarks of these enzymes, such as a [4Fe–4S] cluster ligated by cysteines residing in a CxxxCxxC motif and a binding site for S-adenosylmethionine. However, unlike all other characterized members of the radical SAM (RS) superfamily, TsrM does not catalyze a reductive cleavage of SAM to the almost universal 5'-deoxyadenosyl 5'-radical intermediate. Instead, recent studies suggest that it catalyzes its reaction via two polar SN2 displacements, an attack on the methyl group of SAM by a cob(I)alamin species and a subsequent attack of C2 of Trp onto methylcobalamin. Herein we report the first crystal structures of a class B RS methylase. The structure contains both the cobalamin and [4Fe-4S] cluster cofactors, each of which have unique coordination spheres. We additionally have solved structures with bound tryptophan analogs, revealing an unexpected mechanism of catalysis.