## Sample Abstract – Biochemistry & Molecular Biology

**Abstract Title:** Stereochemical and Mechanistic Investigation of the Reaction Catalyzed by Fom3 from *Streptomyces fradiae*, a Cobalamin-Dependent Radical *S*-Adenosylmethionine Methylase

Fom3, a cobalamin-dependent radical S-adenosylmethionine (SAM) methylase, has recently been shown to catalyze the methylation of carbon 2" of cytidylyl-2-hydroxyethylphosphonate (HEP-CMP) to form cytidylyl-2hydroxypropylphosphonate (HPP-CMP) during the biosynthesis of fosfomycin, a broad-spectrum antibiotic. It has been hypothesized that a 5'deoxyadenosyl 5'-radical (5'-dA\*) generated from the reductive cleavage of SAM abstracts a hydrogen atom from HEP-CMP to prime the substrate for addition of a methyl group from methylcobalamin (MeCbl); however, the mechanistic details of this reaction remain elusive. Moreover, it has been reported that Fom3 catalyzes the methylation of HEP-CMP to give a mixture of the (S)-HPP and (R)-HPP stereoisomers, which is rare for an enzymecatalyzed reaction. Herein, we describe a detailed biochemical investigation of a Fom3 that is purified with 1 equiv of its cobalamin cofactor bound, which is almost exclusively in the form of MeCbl. Electron paramagnetic resonance and Mössbauer spectroscopies confirm that Fom3 contains one [4Fe-4S] cluster. Using deuterated enantiomers of HEP-CMP, we demonstrate that the 5'-dA' generated by Fom3 abstracts the C2"-pro-R hydrogen of HEP-CMP and that methyl addition takes place with inversion of configuration to yield solely (S)-HPP-CMP. Fom3 also sluggishly converts cytidylylethylphosphonate to the corresponding methylated product but more readily acts on cytidylyl-2-fluoroethylphosphonate, which exhibits a lower C2" homolytic bond-dissociation energy. Our studies suggest a mechanism in which the substrate C2" radical, generated upon hydrogen atom abstraction by the 5'-dA\*, directly attacks MeCbl to transfer a methyl radical (CH<sub>3</sub>\*) rather than a methyl cation (CH<sub>3</sub><sup>+</sup>), directly forming cob(II)alamin in the process.

## **KEY**

Abstract contains sufficient background to understand the problem under investigation

Abstract must contain a hypothesis, objective or statement about the problem under investigation

Abstract must contain a brief statement of the experimental methods/methodology used

Essential results must be present in summary form (even if preliminary)

Abstract must contain a conclusion that explains how the work contributes to the hypothesis, objective or statement of problem

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