Combined Spectroscopic and Computational Investigation of the Reactivation Cycle of Cobalamin-Dependent Methionine Synthase

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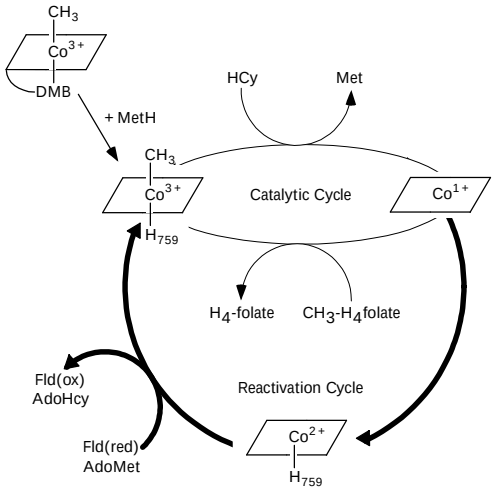
Methionine Synthase (MetH) is one of two cobalamin-dependent enzymes found in humans. During the catalytic cycle of MetH, the cobalamin cofactor alternates between cob(I)alamin (Co^{1+}Cbl) and methylcobalamin (MeCbl) as shown below. Occasionally, the Co^{1+}Cbl form is accidentally oxidized to cob(II)alamin (Co^{2+}Cbl) as shown below. The Co^{2+}Cbl form of MetH is catalytically inactive and must be reductively methylated, through a Co^{1+}Cbl intermediate, to regenerate MeCbl using flavodoxin and adenosylmethionine (AdoMet).

Upon binding to MetH, the 5,6-dimethylbenzimidazole (DMB) loop of cobalamin dissociates from the "lower" axial position and is replaced by His759 in the base-on conformation as shown in the figure below. In the base-off conformation, His759 is dissociated from the cofactor and presumably replaced by a water ligand. The H759G mutant forces the cofactor into the base-off conformation and is believed to lock MetH into the conformation corresponding to the Reactivation Cycle.[1]



We will present electronic absorption, circular dichroism (CD), magnetic circular dichroism (MCD), and electron paramagnetic resonance (EPR) spectra of H759G MetH bound species that serve as models of putative intermediates in the reactivation cycle of MetH. Our data provide evidence for the formation of an unprecedented 4-coordinate, base-off Co^{2+}Cbl in MetH, where neither water nor histidine is bound to the Co center. We will present a computationally-aided assessment of the role of the H759 ligand in the final methylation step of Co^{1+}Cbl by AdoMet.

[1]Bandarian, V. et. al *Nat. Struct. Biol.* **2002**, *9*, 53-56