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Can the local enzyme scaffold act as an H-donor for a Co(I) – H bond formation? The curious case of methionine synthase-bound cob(I)alamin

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ABSTRACT

The density functional calculations and analysis of the existing X-ray crystallographic data have been carried out to gain mechanistic insight into the reactivation cycle of methionine synthase (MetH) enzyme. The calculations were carried out on the cobinamide-type model complexes of cob(I)alamin (Co(I)Cbx) testing H_2O and PhOH as possible β-axial ligands. The PhOH motif was used to mimic the tyrosine (Y1139) residue that has been found in the active site of the MetH-bound cob(II)alamin (Co(II)Cbx). The calculations indicate that the β-axial PhOH ligand forms stronger Co(I) – H bonds than H_2O ligand due to its better H-donor capacity. The calculated redox tuning of Co(I) – H interactions on the reduction potential of Co(II)/Co(I) couple (60–800 mV vs standard hydrogen electrode (SHE)), irrespective of the β-axial ligand considered, is significantly higher than the biological redox gap between the reduction potential of Co(II)/Co(I) couple and that of the biological reducing agents (50 mV vs SHE). The analysis of existing crystallographic data for the reactivation conformation of MetH enzyme (1K7Y (@3.0 Å); 1K98 (@3.8 Å) and 3IVA (@2.7 Å)) indicates that the Y1139 residue and the β-axial H_2O ligand in the MetH-bound Co(II)Cbx complex are equidistant from the Co(II) ion (Y1139 – Co(II) = 3.97 Å; H_2O – Co(II) = 3.96 Å). Taking into account that the Y1139-induced Co(I) – H linkages are thermodynamically more stable than the H_2O-induced ones, the present calculations suggest that the Y1139 residue may serve as the β-axial ligand in the reactivation conformation of MetH enzyme.

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1. Introduction

E. coli methionine synthase (MetH) is a cobalamin-dependent modular methyltransferase that carries out the methyl group transfer from CH_3-folate to homocysteine (Hcy) forming methionine (Met) [1]. The reaction cycle of MetH enzyme contains the main catalytic cycle and the reactivation cycle (Scheme 1), respectively. The main catalytic cycle involves the transfer of a methyl group from CH_3-folate to Hcy resulting in the formation of Met with methylcobalamin (MeCbl) cofactor providing the intermediate platform for this reaction. Precisely, during the first half of the catalytic cycle, the methyl group is transferred from MeCbl to Hcy resulting in the formation of Met and cob(I)alamin (Co(I)Cbx), while during the other half of the catalytic cycle, Co(I)Cbx is methylated by CH_3-folate and catalytically competent MeCbl form is re-generated [2–5]. Note that Co(I)Cbx and Co(II)Cbx in the present study have been used as abbreviations for the full cob(II)alamin and cob(I)alamin respectively, while Co(II)Cbi and Co(I)Cbi represent their mimics where only the nucleotide loop of the corrin ring has been terminated at the phosphodiester end. Co(I)Cbx is an unusually strong nucleophile, which is often referred to as a "supernucleophile" due to its very high Karl Pearson constant (14) [6]. However this supernucleophilicity of Co(I)Cbx under microaerophilic conditions also makes it a prime target for sporadic oxidation which results in a catalytically incompetent Co(II)Cbx form. This deactivation reaction is reported to occur once every 2000 turnover cycles [7]. Note that in a related methyltransferase namely Fe–S corrinoid [8], the similar deactivation occurs every 100 catalytic cycles [9]. In order to maintain the efficient reaction cycle, the activated MeCbl form must be re-generated. This mandatory reactivation which is achieved by the reductive methylation of Co(II)Cbx, involves a two-step process: (i) initially, the lower axial histidine (His759) ligand is displaced from the MetH-bound Co(II)Cbx [10] and is substituted by the axial H_2O ligand on the β ("upper")-face of the cofactor [11]. (ii) In the subsequent step, Co(II)Cbx is reduced to Co(I)Cbx (i.e., Co(II)/Co(I)) by the physiological electron donors such as flavodoxins, followed by the subsequent methylation by S-adenosyl-L-methionine (AdoMet) [12].

Though the molecular basis of the first step of the reactivation cycle is relatively well understood, yet the mechanistic details of the second step continue to remain elusive. This is mainly attributed to the fact that the Co(II)/Co(I) reduction is an endergonic process under the cellular conditions due to its inaccessible redox chemistry [10]. The reduction potential of Co(II)/Co(I) couple is −500 mV vs standard hydrogen electrode (SHE), which is more cathodic than that of the common biological reducing agents (−280 mV to −440 mV vs SHE) operational inside methyltransferases [13,14]. Based on the simple electrochemical

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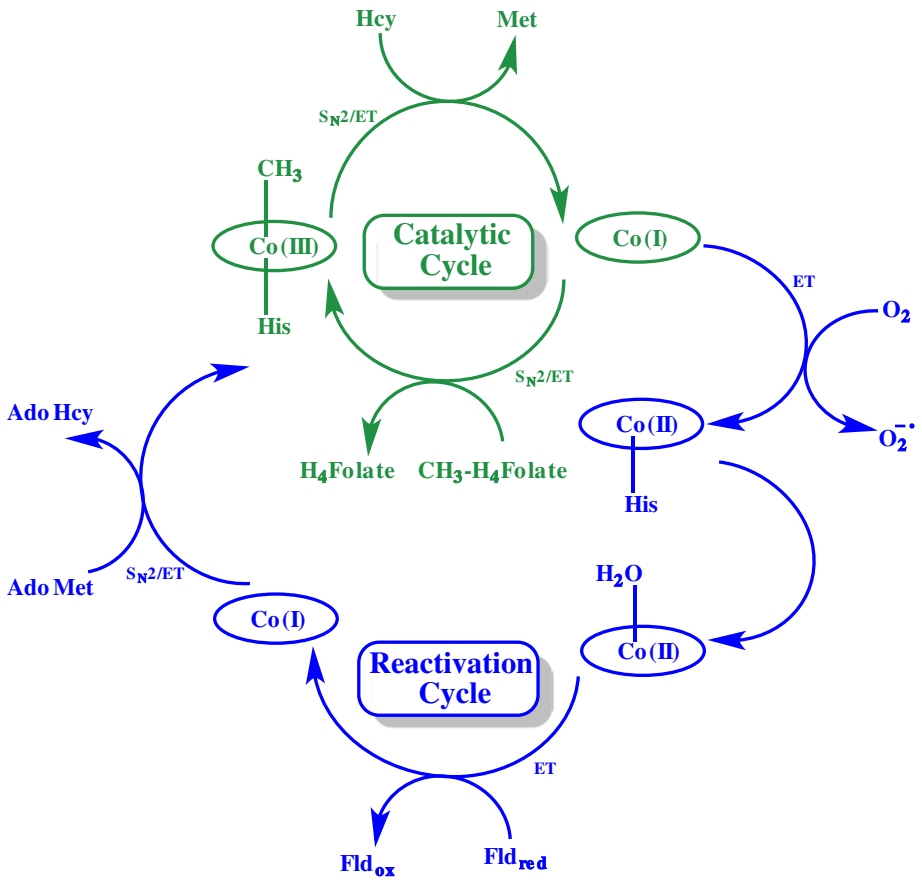
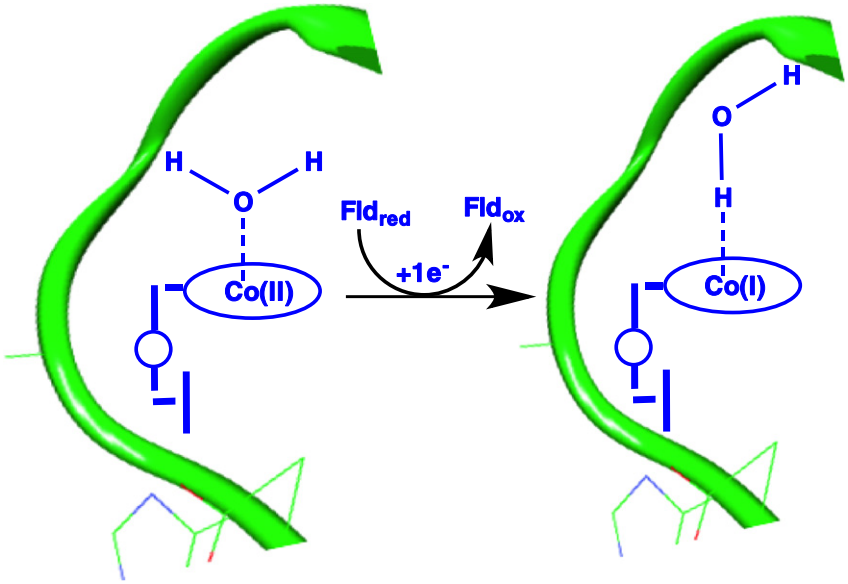


Scheme 1. Reaction cycle of MetH enzyme. The main catalytic cycle and the reactivation cycle are shown in green and blue colors respectively.

considerations, this reaction should not take place. But this reaction is indeed observed inside the enzymes indicating the precise control of the enzyme over the reduction process. Previously reported computational, structural and spectroscopic studies suggest that the formation of tetracoordinated square planar Co(I)Cbx drives the Co(II)/Co(I) reduction [11,15–18]. Especially, the catalytic role of Y1139 residue in the MetH-bound Co(II)/Co(I) reduction is well documented in literature [11,17,18]: the Y1139 residue serves to weaken the communication between the axial H_2O ligand and the Co(II) ion of the cofactor and thus promotes the generation of square planar Co(I)Cbx. The existing structural and spectroscopic data have been interpreted that the MetH-bound Co(II)/Co(I) reduction involves the conversion of nearly square planar Co(II)Cbx into square planar Co(I)Cbx. Though the Co(II)/Co(I) reduction would occur in a spontaneous fashion if the participation of the square planar Co(II)Cbx and Co(I)Cbx is assumed, it is plausible that the supernucleophile Co(I)Cbx may then engage in the



Scheme 2. Alternate mechanistic pathway for MetH-bound Co(II)/Co(I) reduction.