Enzymatic Acceleration of Co–C Bond Homolysis by AdoCbl-Dependent Isomerases: Spectroscopic and Computational Insights from Active Site Electronic Structure Studies

Amanda J. Brooks[1], Monica Vlasie[2], Ruma Banerjee[2], Christel C. Fox[3], E. Neil G. Marsh[3], and Thomas, C. Brunold[1]

[1] Department of Chemistry, University of Wisconsin-Madison, [2]Department of Biochemistry, University of Nebraska, [3]Department of Chemistry, University of Michigan

Methylmalonyl-CoA Mutase (MMCM) and Glutamate Mutase (GM) are two examples of carbon skeleton isomerases that require adenosylcobalamin (AdoCbl) as a cofactor for catalytic turnover. These radical rearrangement reactions are initiated by homolytic cleavage of the cofactor's Co–C bond, generating Co^{2+}Cbl and an adenosyl radical. Understanding the enzymatic mechanism for activation of the Co–C bond for homolytic cleavage is of considerable interest, as the rate of bond cleavage shows a spectacular degree of acceleration (as much as 10^{12}-fold over the free cofactor) under physiological conditions. Through a combination of spectroscopic and computational studies, we have elucidated the electronic structures of AdoCbl and several of its biologically relevant derivatives in aqueous solution and in complex with MMCM and GM. Examination of changes in the electronic structure upon cofactor incorporation into the enzyme active sites and at various stages along the reaction coordinate have revealed only minor perturbations to the Co^{3+} forms of the cofactor, whereas significant changes to the electronic structure are observed for enzyme-bound Co^{2+}Cbl compared to its unbound form. Thus, it appears that enzymatic activation of homolytic Co–C bond cleavage primarily involves stabilization of the Co^{2+} intermediate state and not destabilization of the Co^{3+} ground state.

To investigate possible protein-induced Co^{2+}Cbl stabilization mechanisms, QM/MM calculations were performed on a variety of free cofactor (analogues) in the absence and presence of active site residues. Our calculations represent the first comprehensive QM/MM study that accurately predicts the structural and electronic properties of a wide range of free cobalamins and cobinamides. The extension of these model systems to include active site residues suggests that the ligand triad plays an important role in the enzymatic stabilization of the post-homolysis product Co^{2+}Cbl.