A New Paradigm in Biological Co–C Bond Formation: Spectroscopic Studies of Human ATP:Cobalamin Adenosyltransferase

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In humans, exogenously derived vitamin B_{12} must be adenosylated before it can serve as the cofactor for the enzyme methylmalonyl-CoA mutase (MMCM). Malfunction of the human adenosyltransferase (hATR) that catalyzes this conversion can result in harmful accumulation of methylmalonyl-CoA, leading to the potentially fatal disease methylmalonic aciduria. Formation of the adenosylcobalamin (AdoCbl) cofactor is achieved by transferrin g the adenosyl group from co-substrate ATP to a transiently formed Co^{1+}cobalamin (Co^{1+}Cbl) species. A particularly puzzling aspect of hATR function is that the midpoint potential for $Co^{2+}\rightarrow Co^{1+}$ reduction of the free Cbl cofactor is below that of readily available biological reductants suggesting that the redox active orbitals of Co^{2+}Cbl are significantly perturbed by hATR. To probe potential changes in the Co^{2+}Cbl geometric and electronic structures caused by binding to hATR in the absence and presence of the co-substrate ATP we have employed magnetic circular dichroism (MCD) and EPR spectroscopies. Our data (see figure), interpreted within the theoretical framework established in our earlier work on free Co^{2+}corrinoids, reveal that in the absence of ATP, the interaction between Co^{2+}Cbl and hATR promotes partial conversion of the cofactor to its "base-off" form in which a water molecule occupies the lower axial position. This protein-induced perturbation of the cofactor becomes much stronger in the presence of ATP, leading to the formation of an unprecedented Co^{2+}Cbl species with spectroscopic signatures consistent with an essentially four-coordinate, square planar Co^{2+} center. This unusual Co^{2+}Cbl coordination is expected to raise the $Co^{2+/1+}$ reduction potential well into the physiological range. Interestingly, our preliminary data suggested that formation of this activated four-coordinate Co^{2+}Cbl may be universal to all enzymes that catalyze Co–C bond formation.