Structure and dynamics in B12 enzyme catalysis revealed by electron paramagnetic resonance spectroscopy

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Challenges to the understanding of how protein structure and dynamics contribute to catalysis in enzymes, and the use of time-resolved electron paramagnetic resonance (EPR) spectroscopic techniques to address the challenges, are examined in the context of the coenzyme B12-dependent enzyme, ethanolamine ammonia-lyase (EAL), from Salmonella typhimurium. EAL conducts the homolytic cleavage of the coenzyme cobalt-carbon bond, intraprotein radical migration (5-6 A), and hydrogen atom transfers, which enable the core radical-mediated rearrangement reaction. Thermodynamic and activation parameters are measured in two experimental systems, which were developed to isolate sub-sequences from the multi-step catalytic cycle, as follows: (1) A dimethylsulfoxide (DMSO)/water cryosolvent system is used to prepare the kinetically-arrested enzyme/coenzyme/substrate ternary complex in fluid solution at 230 K.[1] Temperature-step initiated cobalt-carbon bond cleavage and radical pair separation to form the Co(II)-substrate radical pair are monitored by using time-resolved, full-spectrum EPR spectroscopy (234 ≤ T ≤ 250 K).[1] (2) The Co(II)-substrate radical pair is cryotrapped in frozen aqueous solution at T < 150 K, and then promoted to react by a temperature step. The reaction of the substrate radical along the native pathway to form the diamagnetic bound products is monitored by using time-resolved, full-spectrum EPR spectroscopy (187 ≤ T ≤ 217 K).[2] High temporal resolution is achieved, because the reactions are dramatically slowed at the low temperatures, relative to the initiation and spectrum acquisition times. The results are combined with high resolution structures of the reactant centers, obtained by pulsed-EPR spectroscopies,[3] and the protein, obtained by structural proteomics[4] and EPR and electron spin echo envelope modulation (ESEEM) in combination with site directed mutagenesis,[5] to approach a molecular level description of protein contributions to catalysis in EAL.


1Supported by NIH grant DK54514.