Structure and Dynamics in B_{12}-Dependent Enzyme Catalysis

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Coenzyme B_{12}-dependent enzymes catalyze radical-mediated molecular rearrangement reactions. The steps of the catalytic cycle that bracket the core rearrangement chemistry involve *radical migration* between cobalt in cobalamin and the substrate binding site, and *hydrogen atom transfers* that sequentially activate the substrate and quench the product radical. These steps are common to metalloenzymes that promote reactions by using electron-deficient radical species. The key intermediates in the B_{12} enzymes are Co^{II}-radical pair states. To provide a structural basis for describing the molecular mechanisms of the component reactions, we have used electron paramagnetic resonance (EPR) and powder and orientation-selection electron spin echo envelope modulation (ESEEM) spectroscopies and simulations to determine the three-dimensional geometry of reactant centers in the active site of the Class II coenzyme B_{12}-dependent enzyme, ethanolamine deaminase, from *Salmonella typhimurium*, in cryotrapped, disordered samples of the Co^{II}-substrate radical pair and Co^{II}-product radical pair states.[1-3] Ethanolamine deaminase converts ethanolamine [H_2N**C2**(H_2)**C1**(H_2)OH] to acetaldehyde and ammonia. The Co^{II}-C1• distance is 11.1 Å (range, 10.9-11.5 Å) in the substrate radical state, and the Co^{II}-C2• distance is 9.3 Å (range, 8.5-10.5 Å) in the product radical state. The separations between the C5' carbon of 5'-deoxyadenosine and C1• (substrate radical state) and C2• (product radical state) are 3.2 Å and 3.3 Å (±0.1 Å), respectively. Therefore, C5' is the direct mediator of radical migrations, over a significant distance through the protein interior, both to (~6 Å) and from (~4 Å) the substrate/product binding site. The C5'-methyl group maintains van der Waals contact with the radicals during the rearrangement reaction. The C2-H_s-C5' angle with the strongly-hyperfine-coupled hydrogen, H_s, and the C5'-H_s orientation relative to the C1-C2 axis, are consistent with a linear hydrogen atom transfer coordinate and an in-line acceptor *p*-orbital orientation. Further insight into the molecular mechanisms of radical migration and hydrogen atom transfer is emerging from correlation of the high resolution structural information with results of protein structure studies and measurements of the reaction dynamics.

[1] Canfield, J.M.; Warncke, K. *J. Phys. Chem. B* **2002**, *106*, 8831-8841; [2] Warncke, K. *Biochemistry* **2005**, *44*, 3184-3193; [3] Canfield, J.M.; Warncke, K. *J. Phys. Chem. B* **2005**, *109*, 3053-3064. Supported by NIH DK54514.