Radical Rearrangement Catalysis in an Enzyme at 190-207 K: Mechanistic Features Revealed by Substrate $^1$H/$^2$H Isotope Effects

CHEN ZHU, KURT WARNCKE, Emory University — The decay kinetics of both the natural abundance and [1,1,2,2-$^2$H$_4$]-aminoethanol generated Co$^{II}$-substrate radical pair catalytic intermediate in ethanolamine ammonia-lyase (EAL) from Salmonella typhimurium have been measured by using time-resolved, full-spectrum X-band continuous-wave electron paramagnetic resonance (EPR) spectroscopy in frozen aqueous solution from 190 to 207 K. The decay reaction proceeds through sequential radical covalent rearrangement and hydrogen atom transfer (HT) steps. In the temperature range from 190 to 207 K, the decay is biexponential, and the two phases correspond to distinct populations [1]. The $^1$H/$^2$H isotope effects (IE) on the fast phase and slow phase are 1.3 and 0.8, respectively. These IE are not caused by a primary kinetic IE. Therefore, HT is rapid, relative to rearrangement. We propose that the fast phase is rate-determined by the rearrangement step, and that the slow phase is rate-determined by a step after rearrangement that is associated with protein guidance of the reactions. The results reveal microscopic features of the core reaction chemistry and protein dynamics participation in the reaction, which are not accessible at ambient temperatures.