

C-H activation via hydrogen tunneling: toward a formalization of enzyme catalysis in the context of protein dynamics

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For almost two decades, evidence has accrued implicating hydrogen tunneling in C-H activation processes catalyzed by enzymes. These data have forced a reevaluation of the origins of enzyme catalysis that goes beyond the textbook view of “enhanced transition state binding” according to the Pauling formalism. Further, the aggregate data for enzymatic C-H activation reactions are found to be inconsistent with an interpretation of the tunneling phenomenon as a “correction” to a semi-classical barrier. Studies of the temperature dependence of the primary kinetic hydrogen isotope effect (KIE) in four different enzyme-catalyzed C-H cleavage reactions will be discussed. The properties for these systems under conditions of optimal catalysis support a full tunneling model in which both light and heavy isotopes move dominantly from the zero to zero vibrational levels of reactant and product within a compacted active site geometry. Introduction of perturbations (site specific mutagenesis, alteration in temperature and changes in the protein surface) increase the temperature dependence of the KIE, due to a decrease in the ability of the protein to achieve a close approach between donor and acceptor atoms. As a result of an observed inverse relationship between overall protein flexibility and the temperature dependence of the isotope effect, a two-tiered dynamical model is introduced that links protein conformational sampling (pre-organization) to the energetics for bond cleavage at the enzyme active site (reorganization). The relative properties for D vs. H transfer suggest that active site compaction is a general property of enzyme catalyzed reactions. The repeated observation of tunneling in enzymes may be a reflection of this property of enzyme active sites rather than a unique evolutionary feature of C-H activation reactions.