More is Always Better: Measuring Many KIEs to Characterize Enzymatic Transition States

Paul J. Berti

Department of Chemistry, Department of Biochemistry, McMaster University

Enzymes catalyze reactions by stabilizing transition states. If we want to understand how they work, we need to understand their transition states. The challenge in experimental studies of transition states is that they exist for ~ 0.1 ps, which obviously creates experimental challenges. Our lab uses kinetic isotope effects (KIEs) to determine enzyme transition states. By measuring KIEs at many different atoms, we can determine the structures of transition states at resolutions that, in the best cases, equals x–ray crystallography of stable compounds. We recently measured 28 ^{13}C KIEs on acid-catalyzed and enzyme-catalyzed hydrolyses of α– and β–methyl glucosidases with a new NMR method. This has allowed us to characterize the transition states of these four reactions in detail. The acid– and α–glucosidase-catalyzed reactions were stepwise (S_N1), while the β–glucosidase reaction was concerted (S_N2). α–Glucosidase distorted the sugar ring during catalysis, but β–glucosidase showed no signs of ring distortion. We are also studying the catalytic mechanism of MutY, an DNA repair enzyme, using KIEs.

^{13}C KIEs: