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Large p*K*a Perturbations at an Enzyme Active Site, a Mechanistic Basis for Catalytic Power of Many Enzymes

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Bacterial Δ^5-3-ketosteroid isomerase (KSI) is one of the most proficient enzymes catalyzing the isomerization of a variety of Δ^5-ketosteroids to Δ^4-ketosteroids at a diffusion-controlled rate. Because of the simplicity of the reaction, the enzyme mechanism has been intensively studied as a prototype to understand enzyme-catalyzed C-H bond cleavage. Asp38 (p*K*a ~4.7) has been identified as the general base abstracting the steroid C4β-proton (p*K*a ~12.7) to form a dienolate intermediate. A key and common enigma regarding the proton abstraction is the question of how the energy required for the unfavorable proton transfer can be provided at the active site of the enzyme, and/or how the thermodynamic barrier can be drastically reduced to meet the fast reactivity. In order to provide sound ground for answering this question, the interaction between the active site and a reaction intermediate analogue was clearly elucidated along with mutational and NMR study. A LBHB formation in the course of the reaction appears to contribute crucially to the enormous rate enhancement. The formation of favorable interactions between inhibitors and catalytic residues give rise to large perturbation of the p*K*a values of both the inhibitor and catalytic groups. The results indicate that the p*K*a difference between catalytic residue and substrate can be significantly reduced in the active site environment as a result of the formation of energetically favorable interactions during the course of enzyme reactions. The reduction in the p*K*a difference should facilitate the abstraction of a proton and thereby eliminates a large fraction of activation energy in general acid/base enzyme reactions.