Ensemble-function relationships from cryo and room temperature X-ray crystallography provide new insights into enzyme catalysis

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Understanding how enzymes provide the enormous rate enhancements required for all life has been and remains a major goal of Biochemistry. Enzymes use functional groups that carry chemical transformations, but these so-called “catalytic residues” provide substantial rate enhancements only when appropriately positioned with respect to reactants in the context of a folded enzyme. Although the notion of precise positioning of catalytic groups is at the heart of enzymology, we do not know how precisely these groups are positioned, and we do not know the relationship between positioning and function. To address these questions, we must transition from traditional single conformation structural descriptions of enzymes to ensembles of structures, from which we can start building models of their energy landscapes. We approached these questions using the ketosteroid isomerase (KSI) because i) it contains an oxyanion hole (OAH), a classic catalytic motif; ii) high-quality structural information is attainable and iii) there is a wealth of functional information available. We used two complementary approaches, building a cryo-pseudo ensemble from ~50 pre-existing cryo structures at < 2 Å resolution, and obtaining ‘room temperature’ X-ray data and corresponding ensemble information for WT and mutant KSIs in different enzyme states and at resolutions of 1.1–1.3 Å. While the two approaches uncovered similar scales of positioning, some differences were also obvious, presumably arising from cryo cooling effects. The two OAH residues, Y16 and D103, exhibited motions on the scale of 1 Å in two dimensions but considerably smaller deviation in the third dimension. These motions, if uncoupled, would yield a wide range of hydrogen bond lengths, but the cryo-pseudo ensemble indicate coupled positioning. Thus, while KSI active site allows considerable motion, it does so in a coordinated fashion that maintains effective hydrogen bonding. To question the relationship between ensembles and function, we considered two KSI mutants, Y57F and Y32F/Y57F, that reduce catalysis by 10- and 5-fold, respectively. Whereas both show altered Y16 positioning in single conformation structures when compared to WT, Y32F/Y57F has a similar Y16 conformational ensemble, and functional studies revealed that its defect arises from altered catalytic contribution of KSI’s general base. In contrast, the Y16 ensemble in Y57F has little or no overlap with WT Y16 ensemble and very different directions of motion. Overall, our ability to visualize ensembles provides previously inaccessible insights into catalysis and the relationship between positioning and function.