Spatio-Temporal Resolution of the Thermal Initiation of Enzyme activity

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Abstract: The measurement of temperature-dependent hydrogen deuterium exchange into protein scaffolds, coupled to Stokes shifts analyses of surface attached chromophores, uncovers long range networks for the thermal activation of enzyme chemistry.

Methodology: The vast majority of known enzymes depend on thermal (rather than photochemical) initiation of their activities. A two-tier experimental protocol has been developed, to probe for productive heat transfer pathways from the solvent bath to enzyme active sites. This begins with measurements of time averaged, temperature dependent hydrogen deuterium exchange (TDHDX) into native and mutant forms of enzymes, leading to the identification of functionally relevant spatial connections between protein/solvent interfaces and distal active sites. The positional information available from TDHDX guides the subsequent attachment of fluorescent chromophores to the enzyme surface and measurement of temperature-dependent Stokes shifts (kss). Comparison to the properties of kss to enzymatic rate constants yields the time constants and activation energies for protein motions that extend from discrete distal protein/solvent surfaces to buried active sites.

Results: The outlined methodology was first pursued with an enzyme catalyzing C-H cleavage via a deep H-tunneling mechanism. The data indicate an anisotropic and transient protein restructuring that takes place over long distances (ca. 20Å) and on rapid time scale (>ns), acting to reduce internuclear distances and optimize electrostatic interactions between bound substrate and enzymatic cofactor. Extension of a similar suite of experimental protocols to a completely different class of enzyme reaction reveals analogous behavior. We conclude that site specific, long range and rapid dynamical thermal networks are likely a general feature of all enzyme reactions.

Discussion: The collected data indicate the same enthalpies of activation for dynamical motions that occur at selective protein surfaces and enzyme active sites, yet the measured rate constants for these processes differ by ~ 10^9-fold. This discrepancy supports a formalism for observed rate constants that combines a shared, very fast (>ns) temperature dependent protein restructuring, k_{int}, with a very low and variable probability for productive barrier crossings, P_{conf}.

Going forward, there is a need to develop detailed and predictive theoretical/computational frameworks of the observed behaviors. Understanding the structural and mechanistic determinants for functional heat flow in proteins has the potential to transform the field of *de novo* catalyst design, prompting rational modifications to both active site residues and defined regions within the protein scaffold.

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