Enzyme Dynamics: Consensus and Controversy

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A vigorous contemporary debate spotlights the role of protein dynamics in enzyme catalysis [1-7]. By the end of the day, the controversy goes down to statements that obviously do not agree with each other, such as "enzyme dynamics do (or do not) contribute to catalysis". However, as the researchers studying this issue come from different fields (kinetics, spectroscopy, theory, computer simulations, etc.) it is important to distinguish between "semantical disagreements" resulting from different terminologies, and matters of scientific merit (i.e., how enzymes work). Below I try to clarify points of agreement that sometimes go unrecognized, and sharpen the disagreements on matters of merit.

While there is a broad consensus that enzymes dynamically change their conformations upon binding of ligands and substrates ("induced-fit"), different views and approaches invoke different roles for motion in catalysis. Several key issues seem to be included in the consensus, e.g. enzymatic functions require a broad range of protein, ligand, and solvent motions at various time scales (seconds to femtoseconds); and proteins in solution, including globular enzymes in complex with their reactants (substrates), occupy a diverse conformational space, where only some of these conformations lead to the catalyzed chemistry.

So where is the controversy? First, several leading experimentalists have implied (mostly based on the relationship between NMR relaxation dynamics and enzyme kinetic parameters) that enzyme dynamics are directly coupled to the catalyzed chemical step [5,8-10]. The claim that the relationship between the measured dynamics and the kinetic parameters indicate coupling between the protein motions and the catalyzed chemical conversion is problematic for H-transfer reaction, as the time scales of the two phenomena are very different. The C-H cleavage event, which occurs on picoseconds to femtosecond timescales, is so much faster than the measured motions, which occur at millisecond to nanosecond timescales, that the motions appear rigid within the life time of the reactive state, thus prohibiting direct coupling. Consequently, it appears that the conclusions from the experimental studies in references 5 and 8 stretched beyond what the data could support when it comes to demonstrating direct coupling between the motion and the chemistry. While this point is almost certainly true for H-transfer reactions (as H-tunneling is very fast), it might not be a problem for isomerization reactions which may require re-positioning of large moieties slowing down the chemical step [9,10].

Second, other experimentalists measured the temperature dependence of kinetic isotope effects (KIEs) and analyzed their findings using models where statistical dynamics lead to the formation of the reactive state [4,11,12]. We classify these phenomenological models as Marcus-like models (also known as Promoting Vibrations, Environmentally Coupled Tunneling, and more). The controversy in these cases arose from the distinction between "statistical dynamics", that are motions in thermal equilibrium with their environment, and "non-statistical dynamics" that are motions not in equilibrium with their environment. In the condensed phase (e.g., solvent or enzyme) non-statistical dynamics dissipate on the femtosecond-picosecond timescale toward a statistical distribution [13]. While the Marcus-like models have never invoked or required any non-statistical effects, at times, some authors (including us) used loose language that could have been understood as suggesting coupling between non-statistical dynamics and the catalyzed chemistry. Several theoreticians thus assumed that those kinetic experiments and their interpretation made a claim for non-statistical dynamics coupled to the H-transfer step [7,14]. The truth of the matter, though, is that these Marcus-like models are based on rigorous theories that only involve statistical dynamics [15-19]. Furthermore, several high level computer simulations (i.e., hybrid quantum mechanics/molecular mechanics, or QM/MM) examined the same enzymatic C-H→C transfers and extracted parameters that are very relevant to Marcus-like models [20-24]. Interestingly, figures from these papers are in excellent and obvious agreement with similar figures summarizing Marcus-like models, further illustrating that there is actually no substantial disagreement between those two concepts. It appears that this part of the controversy was ignited due to different terminologies used by the experimentalists and the theoreticians, but both groups have reached similar conclusions on the physical nature of the enzyme catalyzed reaction.

Third, only a few calculations and computer simulations involve methods that may include non-statistical dynamics [25-27]. In one case, a simulation that applied a procedure that allowed non-statistical coupling to affect a C-H→C transfer reached similar conclusions to a simulation that directly examined the question of non-statistical coupling [13,28]. For that model system (i.e., dihydrofolate reductase), neither study found significant coupling between the chemistry and protein dynamics. Most other simulations of the same system did not employ non-statistical dynamics in the first place, and could still reproduce experimental findings [21,22,29-31]. The Ockham's razor approach may suggest that for the time being, such effects need not be invoked [3]. At the same time, there is no evidence that such effects do not contribute to the catalyzed reaction [2]. To resolve this issue, more calculations and experiments are needed that directly address possible coupling between non-statistical dynamics and the chemical step (e.g., bond formation or cleavage).

Lastly, the term "catalysis" refers to the relationship between the catalyzed and the uncatalyzed reactions. Many researchers studying enzyme catalyzed reactions use the term "contribution to catalysis" in vain. Since they usually do not compare their findings to a relevant uncatalyzed reaction (which is very challenging), they undoubtedly meant "contribution to the enzyme catalyzed reaction", not "catalysis". Nevertheless, much confusion arose when other researchers assumed the studies did mean "catalysis", despite the
fact that the uncatalyzed reaction was not studied. This issue led to some literature that argued for substantial controversy, which again is probably semantic rather than substantive. Probably more attention to words on the one hand, and more willingness to assume that loose language does not indicate bad faith or stupidity on the other hand, could better focus the discussion and advance the field.

In summary, part of the apparent controversy regarding the role of dynamics in catalysis is indeed substantive and requires further investigation by both experimentalists and theoreticians. This part includes pico-femtosecond dynamics across the system that might be non-statistically coupled to the catalyzed chemistry, and comparison to non-enzymatic reactions. Other parts of the controversy are likely semantic, where closer interactions between the communities addressing these problems could mediate and focus that debate. This part includes all models that do not assume non-statistical dynamics and focus on the catalyzed enzymatic reaction. The last type of debate is not always scientifically productive as both “schools” actually see eye to eye on the nature of the catalyzed chemistry. What’s next? To the best of my knowledge no current experimental data directly examine the role of non-statistical dynamics in enzyme catalyzed H-transfer reactions. Studies aiming at this question could have high impact in the future. Also, closer comparisons of the physical nature of the same chemical transformation in an enzyme and in solution would address the role of various physical features in catalysis.

References


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