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Reply to the Comment on 'A Stationary-Wave Model of Enzyme Catalysis'

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Abstract: This letter briefly outlines the grounds that led to an expression for reaction rate coefficients based on the incomplete gamma function of Euler. The proposed law explains the catalytic activity of enzymes as an effect of the coupling of active vibrational modes to the reaction coordinate, and predicts the observed negative curvature of the relevant Arrhenius plots.

The paper '**A stationary-wave model of enzyme catalysis**'[1] aims to provide a microscopic basis for the statistical model expressed in previous papers.[2,3,4] It shows how energy is effectively transferred between coupled oscillators, and how the forces acting on the substrate are different in frequency and intensity in different media. The issue of computed versus experimental activation barriers for enzyme-catalyzed reactions had been previously addressed elsewhere. But before considering this matter and giving it the correct perspective, let us introduce a few remarks. So far, there are no experimental barriers; the lifetime of a transition structure is too short to be accessible to direct experimentation. All the so called 'experimental' barriers are the result of a fitting procedure of a theoretical expression to experimentally-determined rate coefficients at various temperature. The most common expressions in use are either the Arrhenius or the Eyring formulation. Other functional forms may be taken into consideration, and it must be made clear that different expressions would afford different values for the potential energy barrier with the same set of experimental rate coefficients.

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With this point clarified, we may proceed to a discussion of reaction barriers. Following the above-mentioned method of regression, it has been shown that the analysis based on the Eyring equation of rate coefficients for a large set of enzymes affords activation enthalpies of about 12 *kcal mol⁻¹*.[5] The computational approach to this problem usually involves the selection of a model chemistry that gives a potential energy barrier close to the value given by the regression procedure. Once this agreement is found, the computational method is considered correct, and the mechanism underlying the computational approach is proposed as the actual series of events that reduce the potential energy barrier of the uncatalyzed reaction (usually considerably higher than the 12 *kcal mol⁻¹* of the catalyzed process).

Our approach to this issue started with the observation that the calculated barriers of enzyme-catalyzed reactions are clearly dependent on the features of the model chemistry employed in the calculations, especially the net charge on the system, which is a choice made along with the selection of the computational method. In particular, calculated reaction barriers for the same reaction on systems with and without a net charge are usually different. A detailed discussion on the effect of the total electric charge of the model system is found in ref [4]. More can be found in ref [6], a paper where the problem of the net charges in the model chemistry is fully addressed, along with a detailed discussion on the effect of positive and negative charges on the reaction barriers. This position is summarized by the statement in ref. [2] "various accurate theoretical calculations on model system at the active site fail to give barriers below ~84 *kJ mol⁻¹*, once the electroneutrality of the system is satisfied".

After recognizing the failure to obtain calculated barriers close to 12 *kcal mol⁻¹* for neutral systems, we proceeded to develop a theory for the temperature dependence of rate coefficients that was able to account both for their observed high values and the potential energy barriers above the 12 *kcal mol⁻¹* limit given by calculations. The theory provided the expression

$$k_a(\zeta) = v\,\frac{z_{v,TS}}{z_v}\,\frac{\Gamma(a,\zeta)}{\Gamma(a)}\,, \quad \text{with} \quad \zeta = \frac{\Delta u_{TS}}{kT}\,, \quad (1)$$

which was shown to correctly reproduce the experimental rate coefficients for the hydrolysis of amides by α-chymotrypsin,[2,3] with a deviation between the calculated and the experimental values of the rate coefficients below the corresponding quantity given by the Eyring equation. The theory was subsequently extended to bimolecular reactions both in the gas phase[6] and in solution.[3]

The above analysis evidences two paradigms: (a) the treatment of experimental data based on transition state theory (TST) attributes the enhanced rate coefficients of enzyme-catalyzed reactions to a reduction of the potential energy barrier from the value exhibited by the uncatalyzed process to the 12 *kcal mol⁻¹* limit. (b) the model based on eq. (1) proposes the optimized coupling of active vibrations of the protein to the reaction coordinate, enhancing the rate coefficient without a need of a reduction of the potential energy barrier. Even though eq. (1) gives better fits to experimental rate coefficients, determining which paradigm is correct based only on the analysis of reaction barriers is a difficult task.

However, there are other features of the functional dependence of rate coefficients of temperature that have been considered, namely the curvature in plots of $\ln k_r(T)$ versus $1/T$, a trait predicted to be identically zero by TST. It was shown that eq. (1) correctly accounts for the negative curvature of the Arrhenius and Eyring plots exhibited by numerous reactions, both in solution and enzyme-catalyzed.[7] In all cases where a curvature was present, the deviations between the calculated and the experimental values of the rate coefficients were lower for eq.(1) with respect to the Arrhenius expression.

The judgment on which is the best paradigm to account successfully for the complex details of enzyme catalysis is now a task for the scientific community.

References

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