COMMENTS

Comment on "Effect of Active Site Mutation Phe93 → Trp in the Horse Liver Alcohol Dehydrogenase Enzyme on Catalysis: A Molecular Dynamics Study"

Arieh Warshel*,† and Jordi Villà-Freixa‡

Department of Chemistry, University of Southern California, Los Angeles, California 90089, and Grup de Recerca en Informàtica Biomèdica, IMIM/UPF, C/Doctor Aiguader 80, 08003 Barcelona, Spain

Received: April 8, 2003

Recent papers of Schwartz and co-workers[1−3] may have created a somewhat distorted view of the importance of dynamical effects in enzyme catalysis and the validity of other models for the catalytic power of enzymes. They also may have created the impression that a new advanced method has been developed for evaluating vibrational modes that contribute to catalysis. To prevent further confusion, we would like to clarify the situation in the field by pointing out the problems with the above papers and related works. We will focus on the papers of Kalyanaraman and Schwartz (KS),[1] Mincer and Schwartz (MS),[2] and Antoniou and Schwartz (AS).[3]

The paper by KS starts with an apparent misunderstanding of current proposals. This includes attributing to Pauling the idea that when the reactants bind to enzymes they are close to the top of the uncatalyzed reaction barrier. It also trivializes Warshel's electrostatic preorganization proposal,[4,5] presenting it as a proposal of lowering the dielectric rather than of stabilizing the transition state by a very polar, preorganized environment. More important for the purpose of the present communication is the suggestion that previous models (including ours) are static, while Schwartz and co-workers have developed a new dynamical view.

However, our own models (e.g., ref 6) involve full configurational average in the calculations of the relevant activation free energies, consideration of the enzyme fluctuations,[7] evaluation of nonequilibrium effects,[8] transmission factors, and quantum corrections (see refs 8 and 9 for a review). These calculations cannot be considered as static. In fact, the "new" dynamical concepts that Schwartz and co-workers attribute to themselves (see references in KS and MS) were already introduced, implemented, and examined in our previous studies, including considerations of the protein reactive fluctuations.[7] Analysis of the relevant spectral distribution was introduced for electron transfer in proteins in ref 10 and for enzymes in ref 11. In this respect, it should be clarified that the coupling of different modes to the reaction coordinate is a fact that has been quantified by our spectral density calculations (see below). However, not only was the same type of coupling found to exist in the solution reaction, but also the corresponding effect was found to be noncoherent and thus statistical (see below). Thus our systematic studies (which are arguably the most consistent current studies of the dynamical hypothesis) found no significant dynamical contributions to catalysis (for a review see refs 8 and 9). It is useful to point out that our simulation studies considered any reasonable definition of the dynamical proposal (see ref. 8) and not just the rigid definition in terms of the transmission factor. Nevertheless, the corresponding results were inconsistent with the idea that dynamical effects contribute to catalysis, regardless of the definition used.

It is also important to consider the experimental studies and the presumed support of the dynamical view presented by KS. Apparently there is no experimental evidence that connects dynamical effects to catalysis in a conclusive way (see ref 8). Catalysis is defined relative to the uncatalyzed reaction in water, and the same type of reactive fluctuations that occur in enzymes also occur in solution.[7,9] The problems with the interpretation of the experimental findings that were brought to support the dynamic proposal were carefully covered by ref 9. For example, the interesting temperature effects that have been offered as evidence for dynamical effects in alcohol dehydrogenase[12] can be rationalized in a consistent way as entropic effects.[8,9] While this interpretation was not yet proven by computer simulations, it is at present the most physical interpretation.

Fluctuations similar to those that are supposed to facilitate tunneling in the protein also appear in the reference reaction in water. This point has been established by our simulations of reactions in protein active sites and in water, where it was found that the corresponding spectral densities are similar (thus the modes that are coupled to the reaction coordinate are similar).

The effects of mutations (e.g., of Val203), which also were brought as evidence for the importance of dynamical contributions to catalysis,[13] may not be relevant to studies of dynamical effects in the chemical step because the observed changes occur in k_{cat}/K_M rather than in k_{cat} (the catalytic rate constant $k_{cat} = (k_{cat}/K_M)K_M$ of ref 13 is almost unchanged). We clearly appreciate the difficulties in measuring the chemically relevant rate constant (k_4 in the notation of ref 14), which might not be equal to the apparent k_{cat}. However, any study of the origin of the chemical catalysis must focus on the activation energy of the chemical step, which does not correspond to k_{cat}/K_M but to k_4. It is important to clarify this point because all of the reported simulation studies of ADH examined only the chemical step rather than the binding process and thus should have considered k_4 rather than k_{cat}/K_M. Note that there is no experimental evidence that k_4 changes with the Val203 mutations. In fact, if k_4 could be approximated by the observed value of k_{cat}, then it does not change with this mutation.

In view of the above, it is not clear that the MD studies of the effect of Val203 on the ground-state geometry have much bearing on the catalytic effect. Other calculations that KS cite as support for the presumed dynamical role of Val203 did not reproduce any change in catalysis.[15]

Finally, we would like to comment on theoretical treatment of KS and MS and the impression that it involves a new way of identifying promoting modes of the protein. The use of simulated spectral densities for identifying promoting vibrations was introduced previously by Warshel and co-workers (see

* To whom correspondence should be addressed. E-mail: warshel@usc.edu.
† University of Southern California.
‡ IMIM/UPF.

10.1021/jp034932b CCC: $25.00
Published on Web 10/10/2003

above) and was even applied to the same enzyme considered subsequently by KS (alcohol dehydrogenase).[8] Our treatment, however, used the actual reaction coordinate and reaction surface of the enzyme, rather than an arbitrary distance as was done by KS. More specifically we used the spin boson (dispersed polaron) model,[10,16] which finds the contribution from any mode to the reaction coordinate, reorganization energy, and activation barrier. This is done by evaluating the spectral density of the energy gap between reactant and product states. By contrast, KS considered the spectral density of the ground-state distance between the donor and acceptor, which cannot be related quantitatively to the activation barrier or the rate constant. We found that some vibrations do have projections along the reaction coordinate (as must be the case for any reaction) but that similar effects also occur in the reference solution reaction. Because catalysis is related to the difference between the reaction in solution and the enzyme, the calculations do not provide any evidence of dynamical contribution to catalysis.

The study of Phe93 mutations described by KS found no dynamical effect, and a study of the Val203Ala mutation by the same group[15] found only a factor-of-2 difference in the projection on the donor−acceptor distance. Even if this difference is relevant to the actual reaction coordinate, it does not provide direct evidence of a dynamical effect; it indicates only that the reaction coordinate can be represented by different normal modes in the native and mutant protein. A recent study by Hammes-Schiffer and co-workers[17] found that Val203 has no significant dynamical effect and that its effect on the rate constant is associated with an increase of the activation free energy.

To our knowledge, there is no current consistent simulation that supports the hypothesis that an enzyme excites vibrations that are coupled to the reaction coordinate in a coherent way, which, if observed, would qualify as a dynamical effect. We examined this possibility by running downhill trajectories and showing that they correspond to energy-randomized reactive trajectories rather than to coherent excitations (see Figure 7 in ref 8). Thus, we can state that consistent calculations have led to the conclusion that enzyme catalysis is governed by the same Boltzmann probability law as other reactions in condensed phases. Furthermore, simulations that actually examined the origin of enzyme catalysis indicate that the enzyme reduces the activation barrier by providing a preorganized polar environment that stabilizes the transition state.[5]

We do not mean to suggest that one should stop exploring theoretically and experimentally the possibility of concerted dynamical contributions to enzyme catalysis. This, however, should be done in the light of previous works in the field.

References and Notes

(1) Kalyanaraman, C.; Schwartz, S. D. *J. Phys. Chem. B* **2002**, *106*, 13111−13113.
(2) Mincer, J. S.; Schwartz, S. D. *J. Phys. Chem. B* **2003**, *107*, 366−371.
(3) Antoniou, D.; Schwartz, S. D. *J. Phys. Chem. B* **2001**, *105*, 5553−5558.
(4) Warshel, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5250−5254.
(5) Warshel, A. *J. Biol. Chem.* **1998**, *273*, 27035−27038.
(6) Warshel, A. *Computer modeling of chemical reactions in enzymes and solutions*; Wiley: New York, 1991.
(7) Warshel, A. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 444−448.
(8) Villà, J.; Warshel, A. *J. Phys. Chem. B* **2001**, *105*, 7887−7907.
(9) Warshel, A.; Parson, W. W. *Q. Rev. Biophys.* **2001**, *34*, 563−679.
(10) Warshel, A.; Chu, Z. T.; Parson, W. W. *Science* **1989**, *246*, 112−116.
(11) Hwang, J. K.; Chu, Z. T.; Yadav, A.; Warshel, A. *J. Phys. Chem.* **1991**, *95*, 8445−8448.
(12) Kohen, A.; Cannio, R.; Bartolucci, S.; Klinman, J. P. *Nature* **1999**, *399*, 496−499.
(13) Bahnson, B. J.; Colby, T. D.; Chin, J. K.; Goldstein, B. M.; Klinman, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12797−12802.
(14) Sekhar, C.; Plapp, B. *Biochemistry* **1990**, *29*, 4289−4295.
(15) Caratzoulas, S.; Mincer, J. S.; Schwartz, S. D. *J. Am. Chem. Soc.* **2002**, *124*, 3270−3276.
(16) Hwang, J. K.; Warshel, A. *Chem. Phys. Lett.* **1997**, *271*, 223−225.
(17) Billeter, S. R.; Webb, S. P.; Agarwal, P. K.; Iordanov, T.; Hammes-Schiffer, S. *J. Am. Chem. Soc.* **2001**, *123*, 11262−11272.