LETTER

Role of conformation transitions in adenylate kinase

A recent paper by Pisliakov et al. (1) asserts that it is a response to several papers, which, according to the authors (1), imply that "motions along conformational coordinates play an important role in the chemical step" (1). Because ref. 1 and most of the cited papers in ref. 1 [references 3,6,7, and 14 in Pisliakov et al. (1)] are concerned with adenylate kinase (AdK), my comments refer to this enzyme. AdK has an open state, to which the substrates bind and from which the products are released, as well as a closed state, in which catalysis takes place. None of these cited references are concerned with the chemical reaction or suggest that there is coupling between the closing/opening conformational transitions and the chemistry; catalysis in these papers refers to the overall rate and not to the chemical step. They show that the opening transition after the chemical reaction is the rate-determining step [reference 14 in (1); i.e., in AdK, like triosephosphate isomerase, the chemistry has reached perfection] (2). The two other papers, of which I am a coauthor, study the closing transition encoded in the structure [references 6 and 7 in (1)] and the role of fast motions as lubricants (3) for hinge regions.

Although there is no relation between the objectives of these papers and the calculations in ref. 1, some comments on the method used therein are appropriate. It reduces the multidimensional system to 2D, one a "chemical coordinate" and the other a "conformational coordinate." Such reductions have served in the past for qualitative insights (4), but ref. 1 does simulations on the 2D surface to try to obtain quantitative results. An essential element of the model is the use of experimental data to calibrate parameters. The model fits one experimental opening rate (k_{open} = 6,500 s^{-1} at 20 °C) "with a barrier of 14–15 kcal/mol for the conformational coordinate" (1). Such a barrier would require a preexponential factor of ~10^{15} s^{-1}, a value that is orders of magnitude too large for this type of conformational transition (5), suggesting a problem with the model. In addition, although (1) treats the mesophilic (*Escherichia coli*) AdK, the cited opening rate is for the thermophilic enzyme. Furthermore, reference 1 mistakenly assigns the overall mesophilic rate (260 s^{-1}) to the chemical step; to our knowledge, the latter has not been measured.

Understanding the role of enzyme dynamics in the chemical step is both important and complex. Ref. 6 describes possible equilibrium and nonequilibrium contributions and discusses motions of the enzyme that can lower the free energy of activation of enzyme reactions. Crossing of the transition state region is usually very fast (in the fs to ps range) in enzyme reactions (7), and the reaction seems slow only because the activation barrier makes it improbable to reach the transition state. Thus, fs to ps motions must play a role, although whether they contribute to the faster rate of the reaction in the enzyme versus that in solution has yet to be determined. Short-time simulations with all-atom representations can be used for such studies (7, 8), avoiding the need for the approximate coarse-grained approach in ref. 1. It would be of interest to compare the two approaches for specific systems.

Martin Karplus[1]
Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138; and Laboratoire de Chimie Biophysique, Institut de Science et d'Ingénierie Supramoléculaires, Université de Strasbourg, 67000 Strasbourg, France

1. Pisliakov AV, Cao J, Kamerlin SCL, Warshel A (2009) Enzyme millisecond conformational dynamics do not catalyze the chemical step. *Proc Natl Acad Sci USA* 106: 17359–17364.
2. Knowles JR, Albery WJ (1977) Perfection in enzyme catalysis. Energetics of triosephosphate isomerase. *Acc Chem Res* 10:105–111.
3. Karplus M, McCammon JA (1983) Dynamics of proteins: Elements and function. *Annu Rev Biochem* 53:263–300.
4. Agmon N, Hopfield JJ (1983) CO binding to heme-proteins. A model for barrier height distributions and slow conformational changes. *J Chem Phys* 79:2042–2053.
5. McCammon JA, Karplus M (1977) Internal motions of antibody molecules. *Nature* 268: 765–766.
6. Garcia-Viloca M, Gao J, Karplus M, Truhlar DG (2004) How enzymes work: Analysis by modern rate theory and computer simulations. *Science* 303:186–195.
7. Neria N, Karplus M (1997) Molecular dynamics of an enzyme reaction: Proton transfer in TIM. *Chem Phys Lett* 267:23–30.
8. Quaytman SL, Schwartz SD (2007) Reaction coordinate of an enzymatic reaction revealed by transition path sampling. *Proc Natl Acad Sci USA* 104:12253–12258.

Author contributions: M.K. wrote the paper.

The author declares no conflict of interest.

[1]E-mail: marci@tammy.harvard.edu.