“Eppur si muove” (yet it moves)

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In the 17th century, the problem of the relative movement of the Earth with respect to the sun was an issue of central importance, and in fact, the man who has been considered responsible for the birth of modern science was committed to house arrest for defending the heliocentric hypothesis. Four centuries after the (never-confirmed) legend of Galileo Galilei’s rebellious phrase, a problem of relative motion, now between the protein and substrate in enzyme catalyzed reactions, is once again a hot topic in the scientific community. In this regard, Warshel and coworkers’ study in PNAS (1) reports a very interesting study on the relationship between enzyme catalysis and protein conformational motions.

Although the stabilization of transition states by means of electrostatic interactions with the protein (Fig. 1) is the most accepted hypothesis to account for the origin of the amazing enhancement of chemical kinetics in enzyme catalyzed reactions (compared with its counterpart reaction in solution), other hypotheses have been suggested to explain the origin of this feature of enzymes. One of the most controversial proposals raised in the past is the role of enzyme dynamics. Thus, although it is broadly accepted that protein conformational movements are indispensable to enzyme function throughout the catalytic cycle, in substrate recruiting, chemical transformations, and product release, nevertheless, as pointed out by Benkovic and coworkers (2), knowledge of the coupling between protein movements and the chemical reaction, as well as the chronological order of these events, is still in an early stage of its development, despite being indispensable for understanding these amazing catalysts. In this regard, Warshel and coworkers (3) have stated that, in examining dynamical proposals, there is a tendency to describe different views of the catalytic role of the enzyme dynamics as semantic issues. The origin of this lack of agreement is probably in the fact that protein dynamics are enormously complex, and it can be difficult to determine their exact effect on the reaction rate constant without going into clear physical definitions. In fact, the impact of protein dynamics on chemical reactivity can range over quite different phenomena. For instance, it is well known that substrate binding or product release controls many enzymatic processes. Here, protein mobile loops can act as the active site’s gates, and then their motion can be the rate-determining step. In other enzymes, the binding of the substrate can promote conformational changes in the enzyme that are needed to correctly place some catalytic residues. Single-molecule experiments have shown that different conformational states of a particular enzyme can actually function as independent enzymes with noticeably different reaction rate constants (4, 5). In this case, the dynamics associated with the interconversion among conformational states can determine the global rate constant. However, the most intriguing question is probably whether the dynamics of the protein structural fluctuations are on the same timescale as the chemical step, and therefore coupled to the reaction coordinate and influencing chemical catalysis. In this regard, the role of protein motions in enzyme catalysis has been the topic of many experimental and theoretical studies in recent years, with no consensus of whether they can be considered crucial for the catalysis (1–3, 6–19). Thus, the key question would be on quantifying the contribution of “protein-promoting vibrations” (18) (Fig. 1) toward reducing the free energy barrier of the chemical reaction. Also, a topic of debate is the implicit assumption that the conformational motion transfers energy to the chemical coordinate in an inertial way, and the fact that restriction of some of the dynamical fluctuations could change the sampling of the transition state.

Warshel and coworkers’ study (1), based on empirical valence bond calculations on the wild type (WT) and two dihydrofolate reductase (DHFR) mutants, claims that dynamics do not contribute to catalysis, but rather that the reorganization free energy (which basically results from electrostatic changes for the WT) can rationalize the entire effect of the mutants. Their results are in contradiction with a recent study of Bhabha et al. (17), which suggested that conformational fluctuations limitations on DHFR mutants are the reason for the slightly reduced activity. The authors claim to provide direct experimental evidence for a dynamical contribution to catalysis in the same enzyme, DHFR, in which blocking a relevant conformational coordinate was identified as suppressing the motion toward the occluded conformation (17). Warshel and coworkers (1) point out that it is necessary to distinguish between orthogonal conformational fluctuations and those toward the chemical transition state, with the contribution to catalysis of the former being negligible. A change in flexibility along the conformational coordinate can lead to minimal changes in the activation entropy, which is not what it is usually understood as a dynamical effect. Any conclusion about dynamical contributions to catalysis should be based on clear definitions and measurement of these two different...

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phenomena by examining the conformational coordinate and the chemical coordinate (for instance). Warshel and coworkers (1) point out that the changes in the reaction potential surface change the reorganization free energy (which includes entropic effects), and such changes in the surface also alter the motion.

In this regard, we have recently published a study on the temperature dependence of the kinetic isotope effects in thymidylate synthase (19). Downhill modes allow a study on the temperature dependence of the kinetic isotope effects in the reaction coordinate. We found a study on the temperature dependence of the kinetic isotope effects in the reaction coordinate.

Catalysis may have a signature in some motions, but it is not caused by them. calculated across the same temperature range examined experimentally, revealed a temperature independent behavior, in agreement with experimental findings. The protein–substrate equilibrium approach would in this case provide an over-estimation of the catalyzed rate constant although, according to the results of the environmental motions in the reaction coordinate can be different at different stages of the reaction progress. Free-energy landscapes for both WT EcDHFR, as well as the N23PP/S148A mutant are obtained by Warshel and coworkers (1) to examine the energetics along the conformational coordinate, as well as the transition between the closed and occluded conformations, independently from the chemical coordinate itself. Their assertion that “protein motion is never the reason for enzyme catalysis, but rather simply a reflection of the shape of the surface itself” (1) is based on quantitative estimates of protein dynamical contributions to catalysis. Of course, we should still assert, as Galileo did, that “yet it moves,” and such movements are essential for enzymes to work. However, and again in analogy to Galileo, we now know that the motion of the planets is determined by gravitational forces, and is not the reason for the forces. Thus, catalysis may have a signature in some motions, but it is not caused by them.

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