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Perspective

Perspective on "Theoretical studies of enzymic reactions: dielectric, electrostatic and steric stabilization of the carbonium ion in the reaction of lysozyme"

Warshel A, Levitt M (1976) J Mol Biol 103:227–249

Jiali Gao

Department of Chemistry, State University of New York, Buffalo, NY 14260, USA

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Abstract. This paper provides an overview of the title paper by Warshel and Levitt. Its important contributions and influence to present-day studies of enzymatic processes are discussed.

Key words: Enzymatic reactions – Electrostatic effects – Hybrid quantum mechanical and molecular mechanical methods

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This paper by Warshel and Levitt was one of the first describing "realistic" molecular modeling of enzymatic reactions, and paved out the way of exploring one of the central questions in molecular biology, the origin of the catalytic power of enzymes, through computer simulations. Transition-state stabilization by electrostatic interactions for the reaction of lysozyme was supported, whereas steric factors, such as the ground-state strain due to substrate–enzyme bonding, were found to be insignificant in catalysis. Although the rationale of the catalytic cleavage of a glycosidic bond by lysozyme was interesting, the study turns out to be even more fundamental because electrostatic effects are now recognized to be one of the most important factors in enzyme catalysis [1]. The paper also introduced several theoretical methods for studies of other aspects of molecular biology. The following are the new ideas that were introduced in this work.

1. Hybrid quantum mechanical (QM)/molecular mechanical (MM) methods. An important contribution of this work was the introduction of a hybrid QM/MM method that can be used to study the mechanism and energetics of enzymatic reactions. Recognizing the difficulty of treating the entire enzyme–substrate complex quantum mechanically, Warshel and Levitt proposed partitioning the whole enzyme–substrate system into a "QM" region directly involved in the reaction, and a "classical" region consisting of the rest of the enzyme residues and surrounding solvent. An important feature of this model is that microscopic dielectric effects are included in the QM calculation of enzymatic reactions by computing the electrostatic field due to the dipoles induced by polarizing the protein atoms and the dipoles induced by orienting the solvent water molecules. Consequently, along with the classical force field, all important factors that may contribute to the potential-energy surface of enzymatic reactions are adequately described. The effectiveness of the hybrid QM/MM model is evidenced by numerous recent enhancements of the methodology and applications to studies of reactions in solutions and in enzymes [2–5].

2. Computer modeling of enzymatic reactions. Another important contribution of this paper is to demonstrate the capability of computer modeling in the understanding of enzyme actions. This along with molecular dynamics simulations of proteins reported in the following year [6] provided the opportunity to explore the free-energy surface and dynamic effects in enzymatic reactions. Although extensive biochemical and structural studies, including X-ray crystallography had at that time provided useful information about the mechanisms of many enzymatic reactions, and numerous factors that may contribute to enzyme catalysis had been proposed, their specific contributions and relative importance were not known. In the mid-1970s, it was also far less clear how to use theoretical methods to treat a system as large as the whole enzyme–substrate complex using QM methods. In addition, it was not obvious whether or not it was essential to include the complete system including the surrounding solvent in these calculations since previous theoretical studies had mainly used cluster models in the gas phase. Modeling of the entire system with the newly developed hybrid QM/MM approach allowed Warshel and Levitt to examine the actual chemical process of bond-forming and bond-breaking in the enzyme active site, and to explore various factors influencing the enzyme's catalytic power. This led to the recognition that

electrostatic effects are the most important factors in enzyme catalysis and, in subsequent studies, that a preorganized dipole orientation can more effectively stabilize the transition state than water does [8].

3. Electrostatic energy of macromolecules. The consistent treatment of electrostatic interactions that was introduced in the hybrid QM/MM method in principle provided a procedure to accurately determine electrostatic energies in proteins. Because the method is a microscopic model, the difficult task of assigning a dielectric constant to the enzyme active site was completely circumvented [7]. Furthermore, the authors immediately recognized that the pK_a of ionizable groups in proteins can be estimated using computer simulations. The ability to adequately determine electrostatic energies allowed the authors for the first time to estimate the electrostatic stabilization of the carbonium ion intermediate in the reaction of lysozyme. It was found that electrostatic effects are the most important catalytic factor in lysozyme, whereas the effect of steric strain in the ground state is not important in the enzyme action.

The impact of the 1976 paper on computational studies of enzymatic reactions and on the QM treatment of large molecular systems has been enormous. Although this was not the first computer application aimed at elucidating the relationship between the structure of an enzyme and its activity, the Warshel and Levitt study did provide an early glimpse of biological supercomputing, signaling the arrival of a new era in which an unprecedented amount of information and a detailed understanding of biological processes can be obtained from computer simulations. The finding that lysozyme and perhaps most other enzymes work by an electrostatic effect has had a profound influence on the thinking about enzyme catalysis. The proposal will likely continue to be tested and validated.

Technically, the representation of the polarization of the solvent water by a grid of Langevin dipoles was used later by Warshel to develop a self-consistent-reaction-field model for the treatment of aqueous solvation. In addition, the development of consistent, polarizable intermolecular potentials for proteins, some of which are analogous to the approach used in the 1976 study, is currently being actively pursued by many research groups. In view of the importance of electrostatic interactions in macromolecular systems, more effective and accurate methods will certainly continue to emerge in the twenty-first century, taking advantage of the ever-increasing power of computers.

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