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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The main objective of this project is to advance our understanding of the principles of biological recognition and specificity by using computer simulation approaches. Such approaches are expected to be essential for detailed elucidation of the origin of the enormous power of biological catalysts and to help in exploiting the resulting insight in designing a new generation of highly specific molecular systems. Our computer simulation models have progressed to the level where we can reproduce the effect of genetic modifications of enzymes on its catalytic power in a semiquantitative way. We are also able to estimate in a reasonable way the overall catalytic effect of some enzymes. In the three years of this contract we have tried to exploit the fast accumulation of experimental information about genetically modified enzymes in developing clearer design principles. We have progressed in several directions toward this aim: (i) we explored a significant number of genetically modified enzymes (trypsin, subtilisin Aspartateiminotransferase and staphloccocal nucleases) and obtained more confidence in our predictive power. These studies strongly pointed toward electrostatic factors as a key requirement for the design of effective catalysts (ii) we progressed in studies of chemical reactions in solutions and in establishing reliable references for the enzyme calculations (iii) we started studies of catalytic antibodies and performed simulations of the hydrolysis of p-nitrophenyl N-trimethylammonioethyl by McPC603. This demonstrated the practicality of such simulations but more studies are clearly needed.

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Final Report:

Contract Title: Computer Simulation of Chemical Reactions in Synthetic del Compounds and Genetically Engineered Active Sites

Contract Number: #N00014-8'-K-0507

Principle Investigator: Arieh Warshel
Department of Chemistry
University of Southern California
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Project Objectives:

Understanding the principles of biological recognition is one of the most fundamental problems in biochemistry. In particular, it is important to understand the origin of the enormous selectivity and specificity of biological catalysts, and to use the relevant principles in the designing of a new generation of chemical catalysts. The emergence of genetic engineering has started to provide key information about the relation between the sequence of catalytic sites and their activities. Converting this information into design principles can be accomplished at present only in a qualitative way. A more systematic analysis should be based on some quantitative form of structure-function correlation. One of the promising options of obtaining quantitative correlation is provided by our Empirical Valance Bond (EVB) method. A combination of the EVB method with a free energy perturbation method can yield the activation free energy of enzymatic reactions through the evaluation of the reversible work needed to "transform" the system from its reactant to product state.

The objective of this research is to examine the performance of our simulation methods by reproducing the rapidly emerging information from genetic engineering of enzymes. Once the quantitative value of the simulations is established we can use them to extract information about principles of biological design, and to help in transferring this information to the design of synthetic active sites.

Progress Report (Since July 1, 1987):

During the last three years we advanced on several fronts which are discussed below.

Simulations of Chemical Reactions in Solutions:

In order to obtain reliable potential surfaces for calculations of enzymatic reactions, it is crucial to invest a significant effort in studies of the corresponding reactions in solutions. We progressed in this direction through studies of key classes of chemical reactions including S_N2, proton transfer and electron transfer reactions. We explored the validity of the linear free energy relationship, and the dynamical effects of solute-solvent coupling [1,2]. We also improved our Surface Constrained All Atom Solvent (SCAAS) model [3] and demonstrated its accuracy.

We progressed in developing new methods to study quantum mechanical nuclear tunneling effects [4]. This was done by extending our dispersed polaron model from diabatic reactions (e.g. electron transfer reactions) to adiabatic reactions (e.g. proton transfer reactions). This extension uses the second order diabatic free energy as a reference state for the adiabatic calculations. The approach provides a practical way for simulating isotope effects in solution and proteins, and will likely provide a very significant help in extracting more unique mechanistic information from observed isotope effects in enzymatic reactions.

Simulations of Enzyme Catalysis in Genetically Modified Proteins

i. We completed a systematic study of the catalytic reaction of serine protease, using the 32 Asp $\rightarrow$ Ala mutation in subtilisin and the 102 Asp $\rightarrow$ Asn mutation in trypsin to examine the so called "charge-relay" mechanism. Our studies [5] indicated that the catalytic triad works by an electrostatic stabilization mechanism, and not by the charge-relay mechanism. These findings should provide a useful guide in the design of model compounds that mimic serine protease.

ii. We explored the energetics of ion pair reversal with specific attention to genetic experiments in Aspartateaminotransferase, trypsin and Aspartatetrancarbonylase [6]. This study gave clues about interesting design principles, showing that ion pair reversal will not work without "inverting" the entire polarity of the environment around the ions. Apparently, biological recognition of ion pairs is not associated with the vacuum charge-charge interaction between the ions (which is the same for a given distance in all environments) but with the surrounding of the ions.

iii. We conducted studies of the effects of mutations in metalloenzymes. We simulated the Glu 21 → Asp mutation in staphylococcal nuclease, reproducing the observed effect in a semiquantitative way [7]. Also we simulated the effect of "mutating" the metal in staphylococcal nuclease, reproducing the observed trend. This study provided some general conclusions about the role of metals in metalloenzymes [8] and the principles for an effective selection of the metal and its conjugated general base. This relationship is expected to provide a useful design principle in metal base catalysts.

iv. We completed a study of the $His^{+}Cys^{-}$ ion pair in papain and started to explore the 221 Ser → Cys mutation in serine protease. This should help in understanding the requirements for the conversion of trypsin into an artificial papain.

Catalytic Antibodies

Our studies of catalytic antibodies have progressed in two directions

(i) We spent significant effort trying to obtain reliable results for binding free energies. We succeeded in obtaining a reasonable estimate for the difference in binding energy of different haptanes to McPC603 and in obtaining qualitative results for the absolute binding energies [12]. The calculations were performed by both the very fast Protein Dipoles Langevin Dipoles (PDLD) method and the much slower Free Energy Perturbation method. In view of the speed of the PDLD model, we incorporated it into an interactive docking program that now gives us the ability to calculate binding free energies (including solvation energies) in real time. This is expected to help in exploring alternative binding sites.

(ii) We started an exploratory study of the hydrolysis of p-nitrophenyl N-trimethylammonioethyl carbonate by McPC603. The calculation produced a minor catalytic effect $\Delta\Delta g$ = -2.±1.5 kcal/mol in agreement with the experiments of Dr. A. Flukthun. However, in view of the small catalytic effect in this system (which is at present the only catalytic antibody with an available X-ray structure), it is important to search for mutations that can lead to larger catalytic activity. Such a search can benefit from our computer modeling approach. It will also be important to study antibodies with larger catalytic power such as MOPC167. In such cases, however, one will have to exploit the sequence homology to McPC603 and to use model building approaches.

Publications Acknowledging ONR Contract N0014-87-K-0507

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