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19. ABSTRACT (Continue on reverse if necessary and identify by block number) The primary objective of this project is to advance our understanding of the principles of biological recognition and specificity by using computer simulation approaches. We are interested in elucidating and analyzing the origin of the enormous power of biological catalysts, and exploiting this understanding in designing a new generation of highly specific molecular systems. Our computer simulation methods have approached the level where we can reproduce the effects of genetic modifications of enzymes in a semiquantitative way. We are also able to estimate in a reasonable way the overall catalytic free energies of enzymes. We are trying to utilize this progress in translating the rapidly accumulating experimental results about genetically modified enzymes into clear design principles. During the second year, we have progressed in several directions, ranging from simulations of several mutations in trypsin, subtilisin and Aspartate aminotransferase, simulations of chemical reactions in solutions, and preliminary studies of catalytic antibodies. These results and our plans for the next year are summarized in the report.					
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Annual Report: Year 2

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

Contract Number #N00014-87-K-0507

Principal Investigator: Arie Warshel
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Project Objectives:

Understanding the principle of molecular 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 Valence 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 one resonance structure to another within the catalytic active site. The EVB approach has recently entered a semiquantitative stage, reproducing the effect of genetic modifications on the catalytic activity of several enzymes within several kcal/mol. This exciting progress gives us the hope that the combination of experimental studies and computer simulation can move the field to a more advanced stage.

The objective of our research is to exploit our simulation methods to extract information about principles of biological design, and to help in transferring this information to the design of synthetic active sites.

Progress (Year 2): since July 1, 1988:

During the second year we advanced in several fronts which are listed below:

- **Simulations of Enzyme Catalysis in Genetically Modified Proteins**

(i) We completed systematic study of the catalytic reaction of serine proteases, using the 32 Asp - Ala mutation in subtilisin and the 102 Asp - Asn mutation in trypsin to demonstrate that the catalytic triad works by electrostatic stabilization and not by the so called "charge-relay" mechanism.

(ii) We explored the energetics of ion pair reversal in proteins with specific attention to genetic experiments in Aspartateaminotransferase, trypsin and Aspartatetranscarbamoylase.²

- **Chemical Reactions in Solutions**

(i) In order to verify the validity of our potential surfaces and to establish reliable references to calculations of enzymatic reactions, it is crucial to study chemical reactions in solutions. Thus, we continue our studies of key classes of chemical reactions, exploring the effect of solute solvent coupling on both the energetics and dynamics of charge transfer reactions.^{3,4}

(ii) Our studies of chemical reaction in solutions are now extended to include quantum mechanical nuclear tunneling effects and significant attention is given to isotope effect in proton transfer reactions.

- **Catalytic Antibodies**

Our studies in the exciting field of catalytic antibodies have been slow partially because of the difficulties of obtaining stable results in calculations of binding free energies. This now seems to be under control, as we developed new ways of dealing with the evaluation of long range electrostatic energies. We succeeded in reproducing the binding energies of different haptens to MCP603, and started to simulate the catalytic hydrolysis of p-nitrophenylphosphorylcholine.

Work Plan (Year 3):

The objectives of the third year are as follows:

(i) We will invest more effort in studies of catalytic antibodies, exploring hydrolysis reactions of p- nitrophenylphosphorylcholine and related molecules by MCP603 (and genetically modified MCP603). This case offers a unique chance to understand catalytic antibodies, since the structure of the binding site is known and since Dr. Andreas Plucton in Munich is performing genetic modifications on this protein and exploring their effects on the hydrolytic reaction.

(ii) We will continue our studies of chemical reactions in solutions and proteins, trying to simulate nuclear tunneling effects with special emphasis on isotope effects in proton transfer reactions. We hope to be able to determine the actual relationship between observed isotope effects and the reaction mechanism. We would like to see if the observed isotope effects in serine proteases can be use to distinguish between concerted and nonconcerted pathways.

(iii) We will continue the studies of genetically modified active sites, trying to complete our study of Ser - Cys modifications in serine proteases and to analyse the fundamental problems associated with the conversion of trypsin into artificial papain.

(iv) We will advance the simulation of inclusion compounds which are designed as artificial active sites.



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Publications (Year 2)

- 1) How do Serine Proteases really work, A. Warshel, G. Naray-Szabo, F. Sussman and J-K. Hwang, *Biochemistry*, **28**, 3629 (1989).
- 2) Ion Pair Reversal by Protein Engineering is Unlikely to Succeed, J-K. Hwang and A. Warshel, *Nature*, **334**, 6179 (1988).
- 3) Simulation of Free Energy Relationships and Dynamics of S_N2 Reactions in Aqueous Solution, J-K. Hwang, G. King, S. Creighton and A. Warshel, *J. Am. Chem. Soc.*, **110**, 5297 (1988).
- 4) Effects of Solute-Solvent Coupling and Solvent Saturation on Solvation Dynamics of Charge Transfer Reactions, J-K. Hwang, S. Creighton, G. King, D. Whitney, and A. Warshel, *J. Chem. Phys.*, **89**, 859 (1988).
- 5) Microscopic Simulations of Chemical Reactions in Solutions and Protein Active Sites: Principles and Examples, in *NATO-ASI: The Enzyme Catalysis Process*, ed. A. Cooper (in press).
- 6) Electrostatic Correlation of Structure and Function in Proteins, A. Warshel and Johan Aqvist, *Chemica Scripta*, 29A (in press).

Presentations (Year 2)

- 1) Workshop on the Molecular Basis of Biological Recognition Sodergarn, Sweden (September 1988).
- 2) Nobel Symposium on Structure and Dynamics of Biological Systems, Lund, Sweden (December 1988).
- 3) NATO ASI workshop on the Enzyme Catalysis Process, Barga, Italy (July 1988).
- 4) UCLA Symposium on Protein and Pharmaceutical Engineering, Park City, Utah (January 1989).
- 5) ACS Symposium on Computational Modeling of Molecular Systems, Dallas (April 1989).
- 6) European Symposium on Bio-organic Chemistry: Non-Covalent Interactions in Biology, Gregynog, England (May 1989).

Training Activities:

The following graduate students received partial support from the contract:

1. Steve Creighton
2. Jenn-Kang Hwang