

Analysis and Optimization of Electrostatic Interactions in Enzyme Active Sites

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The Problem: In many enzyme systems, non-covalent interactions play a key role both in determining the efficiency and the specificity of the enzyme. Electrostatic interactions in particular may play an essential role in binding and catalysis of polar and charged substrates. However, due the requirement to appropriately consider differential interactions that both the enzyme and its ligand make with solvent in the bound and unbound states as well as the direct interactions made between the enzyme and the ligands in the bound state, the energetic role of electrostatic interactions is non-intuitive. An electrostatic optimization protocol developed in our laboratory provides a framework for addressing this balance in a rigorous manner, both in regards to binding affinity and to binding specificity [1, 4]. The goal of the work outlined here is to utilize the electrostatic optimization methodology alongside other tools for the analysis of biomolecular systems in order to reach a deeper understanding of the role of electrostatic interactions in modulating enzyme function.

Motivation: Understanding the role of electrostatic interactions in determining the specificity and efficiency of enzymatic catalysis is an important scientific question which remains only partially answered. In addition, such an understanding is an important foundation for the development of methods to design novel and improved enzymes and enzyme inhibitors.

Previous Work: Numerous theoretical studies of natural enzyme systems have been done using procedures involving both molecular mechanics and quantum mechanics, as well as with hybrid approaches [3, 6]. These studies have provided significant insights into the details of individual systems, but the computational cost of these methods makes this approach less than ideal. Initial studies using the electrostatic optimization procedure developed in our laboratory have shown its utility as a method for analyzing biomolecular systems [2, 5], including substrate binding and enzymatic catalysis.

Approach: Our studies of enzyme systems have focused on two primary transformations. First, analysis of the binding of enzyme substrates, by comparison of the properties of the electrostatically optimal ligand to the natural substrates, provides a means to gain insight into the role of electrostatics in modulating the energetics of the first step of enzymatic catalysis — substrate binding. In addition, we consider the role of electrostatics in directly promoting the chemical reaction in the enzyme active site, relative to the reaction in aqueous solution. Electrostatic interactions which preferentially stabilize the transition state over the substrates will lead to a reduced activation energy, and thus a faster rate of reaction. This relative stabilization can be directly analyzed by consideration of the binding energetics of the transition state and substrates, but can also be analyzed by the degree of similarity to the electrostatic optimum.

Impact: In the glutamyl-tRNA synthetase of *E. coli*, a highly specific natural system, we find that the natural substrates are very close to optimal in all the regions making close interactions in the bound state, suggesting that this system has, at least in some sense, evolved to optimize electrostatic interactions. The regions which differ most between the optimal and natural charge distributions are those areas directly involved in the chemical reactions catalyzed by the enzyme, where it is not surprising that the enzyme has not evolved for tight binding of the substrates. These results are found to be relatively invariant with respect to variations in details of the computational method.

Studies on the chorismate mutase from both *B. subtilis* and *E. coli*, enzymes catalyzing an intramolecular rearrangement involving no covalent chemistry with the enzyme, suggest an electrostatic preference for the transition state over the substrate. In the *E. coli* system, a significant electrostatic stabilization of the transition state relative to the substrate chorismate is seen. This stabilization contributes to a computed 100-fold rate enhancement within the

enzyme active site relative to the reaction in aqueous solution. These results demonstrate the utility of the approach we have taken in understanding natural enzymatic systems and validate the protocol for further investigations.

Future Work: We are currently working on extending the results described here. In particular, work on the tRNA synthetases is being extended to additional systems, and to the consideration of catalysis. In the chorismate mutase system, we are working on separating out the contributions of various groups on the enzyme to the computed stabilization of the transition state.

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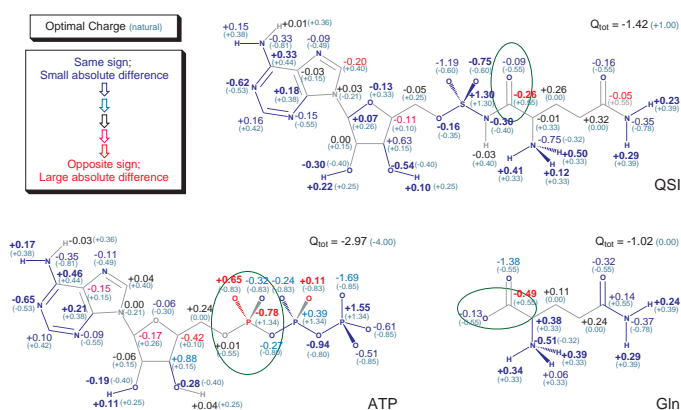


Figure 1: Partial charges at atomic positions of QSI, ATP, and glutamine that optimize binding to GlnRS are remarkably similar to the natural charges, except where chemical reactions occur.

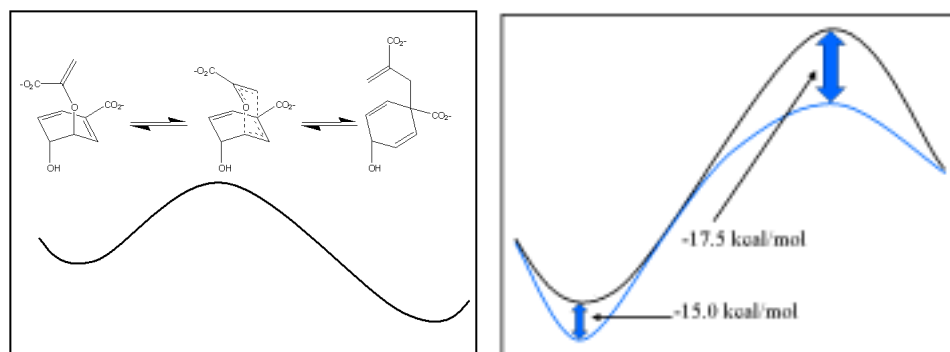


Figure 2: Electrostatic interactions account for a 100-fold rate enhancement in the transformation of chorismate to prephenate within the *B. subtilis* chorismate mutase active site.

References:

- [1] E. Kangas and B. Tidor. Optimizing electrostatic affinity in ligand–receptor binding: Theory, computation, and ligand properties. *J. Chem. Phys.*, 109:7522–7545, 1998.
- [2] E. Kangas and B. Tidor. Electrostatic complementarity at ligand binding sites: Application to chorismate mutase. *J. Phys. Chem. B*, 105:880–888, 2001.
- [3] P. A. Kollman, B. Kuhn, and M. Perakyla. Computational studies of enzyme-catalyzed reactions: Where are we in predicting mechanisms and in understanding the nature of enzyme catalysis? *J. Phys. Chem. B*, 106:1537–1542, 2002.
- [4] L.-P. Lee and B. Tidor. Optimization of electrostatic binding free energy. *J. Chem. Phys.*, 106:8681–8690, 1997.
- [5] L.-P. Lee and B. Tidor. Barstar is electrostatically optimized for tight binding to barnase. *Nat. Struct. Biol.*, 8:73–76, 2001.
- [6] G. Rastelli, B. Thomas, P. A. Kollman, and D. V. Santi. Insight into the specificity of thymidylate synthase from molecular-dynamics and free-energy perturbation calculations. *J. Am. Chem. Soc.*, 117:7213–7227, 1995.