Computational Investigation of the Reaction Thermochemistry and Kinetics of TTQ Cofactor

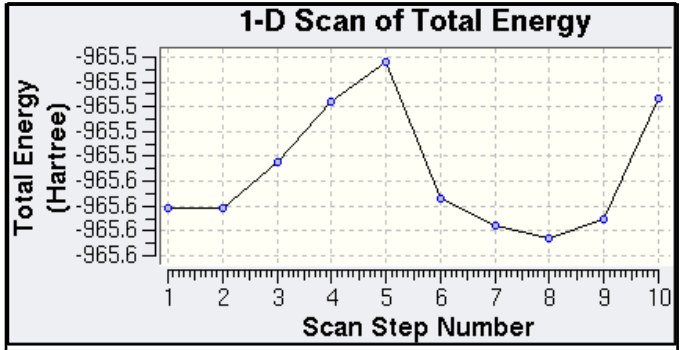
Belynda Sanders, James Hart, Chris Estela, and Andrew Cooksy
Department of Chemistry and Biochemistry, SDSU

Abstract:
Among the simplest electrobiochemical pathways to characterize experimentally is a series of electron transfer reactions that provide the mechanism for dehydrogenation of methylamine. We describe a computational investigation of the chemical reaction mechanism for the enzyme activity of methylamine dehydrogenase in converting methylamine to ammonia and formaldehyde, focusing on the activity of the cofactor tryptophan tryptophylquinone (TTQ). The free energies, reaction rate constants, and related effects of temperature, pH, and isotopic substitution are being computed for comparison against experimental observations. Electronic structure calculations are carried out by density functional methods shown to be effective in the study of simpler chemical systems involving the dynamics of conjugated pi-electron systems. The reaction energies of several reaction steps have been mapped along selected reaction coordinates, and additional reaction steps are currently being probed to determine the minimum energy path. The COSMO-RS model will then be applied to account for the considerable solvent effects in ion-mediated reaction dynamics, and will allow determination of the influence of pH on the reaction system.

Introduction:
The purpose of this project is to probe the reaction of TTQ, a non-protein component of the enzyme methylamine hydrogenase, to metabolize methylamine. The enzyme is present in various anaerobic bacteria, and converts methylamine (CH_3NH_2) into formaldehyde (H_2CO) and ammonia (NH_3) by reaction with water, in a reaction of several steps. The computational study is focused on the effects of intermolecular interactions upon the energies, rate constants, and molecular structures. The most stable molecular geometries are calculated at the beginning and end of each step in the reaction, to find the reactant and product energies. Starting from these, basic thermodynamic properties such as the ΔH (heat of reaction) and ΔG (free energy of reaction) for the reaction can be calculated. To obtain the reaction rate constants, it is necessary to graph the energy of the reacting molecules as the reaction progresses. The reaction rate will be determined mainly by the energy of the transition state – the highest energy point along this graph. The higher the energy of the transition state, the slower the reaction. This procedure must be carried out for each reaction step.

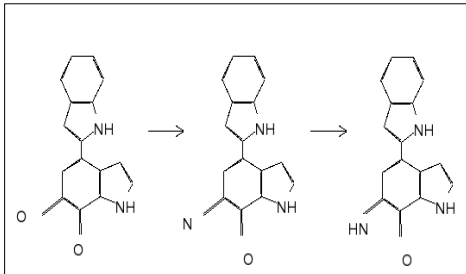
Upon completion, we will be able to model all the steps of the complete reaction, including all of the intermediate steps, demonstrating the ability of these computational tools to predict the stability and structures of this biochemical system.

Methods:
The initial molecular structures were constructed using the program *GaussView*, and the molecular energies were calculated with *Gaussian 03*. We initially used the Hartree Fock method, which is a common approach that allows the electron-electron interactions to be calculated using a large series of two-electron double integrals, rather than a smaller group of quadruple integrals. The calculations produce a list of eigenvalues which are calculated using the Schrödinger equation. Using the Hartree-Fock calculations as a guide, we are now predicting the energies with more accurate methods from density functional theory. This set of calculations will use the B3LYP method and cc-pVDZ as a basis set. This combination of method and basis set has been found to be an accurate and computationally affordable combination, meaning relatively fast calculations (several geometries per week) with reasonable results. The B3LYP method is a *hybrid* functional because it it includes Hartree-Fock calculations. Final calculations will be carried out using a non-hybrid BP86 density functional method with a larger TZVP basis set. This method is compatible with the COSMO-RS methof of correcting the energies for solvation effects.

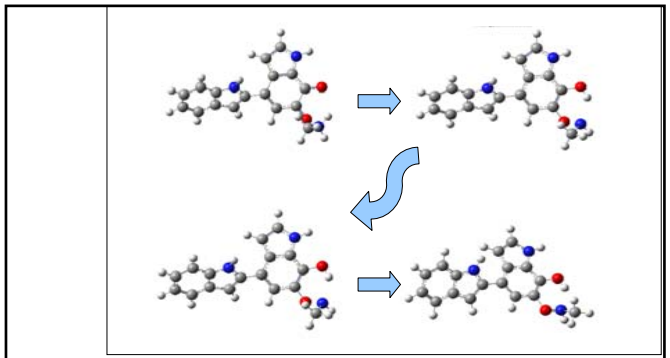


Results:
This is a graph of the Hartree-Fock energies during the first step in the metabolic reaction, attachment of the methylamine to the TTQ. The peak energy activation of the molecule corresponds to most unstable part of the reaction step, the transition state of the molecule. The reactant energy corresponds to the energy at scan step 1, and the most stable product structure is associated with the base of the curve in scan step 8. Thus, it takes more energy for the molecule to move back to reactants over the unstable transition state. The energy of activation is the energy required to reach the transition state from the reactant structure.

Summary:
Complete studies of TTQ electron transfer will require a massive amount of calculations. These calculations take several weeks and run constantly. When the calculations finish we will have the optimal structure of the methylamine-TTQ interaction at each particular step. The complete sequence of these steps will give the complete reaction process and allow us to further explain how the mechanism works (for example, which oxygen is preferred for attack by the methylamine).



This is the basic reaction that is taking place. The methylamine attacks the TTQ cofactor, with the amine N atom taking the place of one of the oxygen atoms. It is also the basic schematic for the electron transfers that are taking place



These are the structures obtained during the first step of the reaction, corresponding to the graph at the left. The methylamine molecule approaches the TTQ cofactor. In this step, methylamine loses one H atom to a carbonyl group and then becomes connected to the opposite carbonyl group. The alternative step in the reaction is also explored where the methyl amine attaches to the opposite carbonyl group.

Acknowledgments: We thank Ray Wight, Olga Noris, and Ray Lui for assistance with these calculations, and the Blasker Fund of the San Diego Foundation for financial support. CE thanks the Computational Sciences Research Center for support under an NIW fellowship from the National Institutes of Health.