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# Investigation of solvent effect on the active site energy of Carbonic Anhydrase and Ribonucleotide Reductase

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## ABSTRACT

Enzymes catalyze many biological reactions. The rates of chemical reaction in the presence of enzymes are, in some cases, accelerated more than 10 orders of magnitude relative to the corresponding rates in solution.
In this paper a comparison between optimized structures of two enzyme molecules in aspect of energy and dipole moment in different conditions including presence of metallic ion, without metallic ion and in the presence of substrate molecule was performed. The stabilizing effect of metallic ion is clearly seen. The calculations were performed with three basis sets: 6-31G, 6-31G* and 6-31G** and 6 different solvents and in vacuum. We conclude that the addition of polarized functions to basis sets cause to create higher energy level of system.

**Keywords:** Enzyme; Basis sets; Dipole moment

## INTRODUCTION

Enzymatic reactions are involved in most biological processes. Thus, there is a major practical and fundamental interest in finding out what makes enzymes so efficient. Many crucial pieces of this puzzle were provided by biochemical and structural studies .Yet the actual reason for the catalytic power of enzymes is not widely understood. It is clearly not explained by the statement that "the enzyme binds the transition state stronger than the ground state" because the real question is how the differential binding can be accomplished. Similarly, it is not true that "evolution can use any factor to accelerate reactions"[1].Enzymes catalyze many biological reactions. The rates of chemical reaction in the presence of enzymes are, in some cases, accelerated more than 10 orders of magnitude relative to the corresponding rates in solution. The origins of these catalytic effects have been studied extensively for many years. One hypothesis is that enzymes reduce the activation barrier by removing water molecules and providing a gas-phase-like environment inside the enzymes. It has been argued that this hypothesis contrasts with the proposed role of the enzyme active site which provides favorable electrostatic interactions, such as hydrogen bonding, between the protein and the transition state.

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It has been proposed that enzymes may act by providing a specific polar environment that is different from the gas phase. However, the electrostatic interactions in a low dielectric medium are stronger than those in a high dielectric medium. For example, the strength of the specific hydrogen bond in a polar solvent is quite weak. Therefore, it can be proposed that enzymes may provide not only specific electrostatic interactions such as hydrogen bonding, dipole-dipole and ion-dipole interactions, but also the low dielectric environment in which they are maximized [2]. As a result of our recent studies, it is becoming clear that enzymes can vigorously function as catalysts in organic solvents, provided that some basic rules are followed. In the present work we have discovered that substrate specificities of several enzymes in organic solvents are radically different from, and sometimes opposite to, those in water [3].

To investigate fundamental features of enzyme catalysis, there is a need for high-level calculations capable of modeling crucial, unstable species such as transition states as they are formed *within* enzymes. in solution (with a variety of continuum models), at the same *ab initio* levels, allows comparison of the catalyzed and uncatalyzed reactions .Transition state stabilization (by electrostatic interactions, including hydrogen bonds) is found to be central to catalysis by the enzyme. The active site is clearly complementary to the transition state for the reaction, stabilizing it more than the substrate, so reducing the barrier to reaction [4].
Water plays an important role in enzyme structure and function in aqueous media. That role becomes even more important when one focuses on enzymes in low water media. The key factor that distinguishes molecular-level details in different media is the partitioning of hydration water between the enzyme and the bulk solvent. The enzyme surface and the active site region are well hydrated in aqueous medium, whereas with increasing polarity of the organic solvent (Aniline
→Ethanol→Methanol→Acetonitrile→DMSO)
the hydration water is stripped from the enzyme surface. Water stripping is accompanied by the penetration of organic solvent molecules into crevices on the enzyme surface and especially into the active site. More polar organic solvents (aniline and acetonitrile) replace mobile and weakly bound water molecules in the active site and leave primarily the tightly bound water in that region. In contrast, the lack of water stripping in organic solvent allows efficient hydration of the active site uniformly by mobile and weakly bound water and some structural water similar to that in aqueous solution. These differences in the active site hydration are consistent with the inverse dependence of enzymatic activity on organic solvent polarity and indicate that the behavior of hydration water on the enzyme surface and in the active site is an important determinant of biological function especially in low water media. Water plays an important role in biological structure and function. For example, hydrophobic interactions that result from the peculiar structuring of water near hydrophobic amino acids provide thermodynamic stability to folded protein structures in aqueous solution. In addition to water's role as a solvent, water molecules can mediate enzymatic catalysis either directly by taking part in the reaction or indirectly through providing a solvation medium for reactants, transition state, and products. The motivation for fundamental studies in the field of nonaqueous enzymology stems partly from a variety of potential applications. For example, by manipulating the microenvironment of an enzyme, through solvent or biocatalyst engineering, it has become possible to modulate enzyme activity, tailor biocatalyst selectivity, and alter enzyme stability. One might expect that the lack of hydrophobic interactions in a bulk nonpolar solvent such as aniline would lead to protein unfolding and therefore to a complete loss of activity. In contrast, experiments show that proteins are able to maintain their structure in many organic solvents and remain catalytically active. At low water contents, the addition of water leads to an increase in protein activity. At a high enough water content in a predominately nonaqueous environment, the protein activity drops, likely due to structural changes induced by partial denaturation in water-organic solvent mixtures. The observed decrease in enzyme activity with increasing solvent

polarity reflects the tendency of organic solvents to strip water molecules from the enzyme surface with the extent of water stripping increasing with the polarity of the organic solvent [5]. The carbonyl site is the most active center in solution and in the gas phase [6].

The origin of the catalytic power of enzymes is discussed, paying attention to evolutionary constraints. It is pointed out that enzyme catalysis reflects energy contributions that cannot be determined uniquely by current experimental approaches without augmenting the analysis by computer simulation studies. The use of energy considerations and computer simulations allows one to exclude many of the popular proposals for the way enzymes work. It appears that the standard approaches used by organic chemists to catalyze reactions in solutions are not used by enzymes.The nature of this electrostatic stabilization mechanism is far from being obvious because the electrostatic interaction between the reacting system and the surrounding area is similar in enzymes and in solution. However, the difference is that enzymes have a preorganized dipolar environment that does not have to pay the reorganization energy for stabilizing the relevant transition states [7].

## COMPUTATIONAL DETAILS

The theoretical results presented in this work were obtained by means of ab initio molecular orbital calculations as the starting point. All calculations were carried out employing the program package GAUSSIAN 98 [8] in HF level of theory with 6-31G, 6-31g*, 6-31g** and LANL2DZ basis sets. Polarized Continuum Model (PCM) with six solvents including: Water, DMSO, methanol, ethanol, aniline, and acetonitrile are used for calculations and comparison with gaseous phase. All active site amino acids in two enzymes, ribonucleotid reductaze and carbonic anhydraze, were optimized. These active sites are: His64, His94, His96, His119 and Thr 199 in carbonic anhydrase and His113, His241, Asp84, Glu115, Glu204, Glu233, tyr122 in ribonucleotide reductase.

## RESULTS AND DISCUSSION

In figure 1 relative energy of nucleotide reductase with respect to dielectric constant in the absence of Fe ion is observed without metallic ion in water solution the most energy of optimization is appeared and any important different between vaccum and other solvents has not been seen. In contrast if Fe ion would present (fig. 2) relative energy in water is the lowest and in vacuum the most instability is observed.

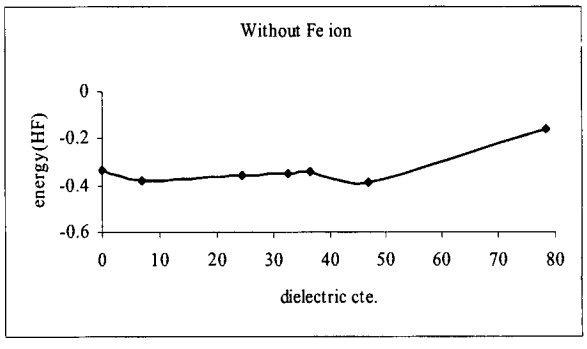


Fig.1. The values of energy (HF) versus dielectric constants for redoctaze.

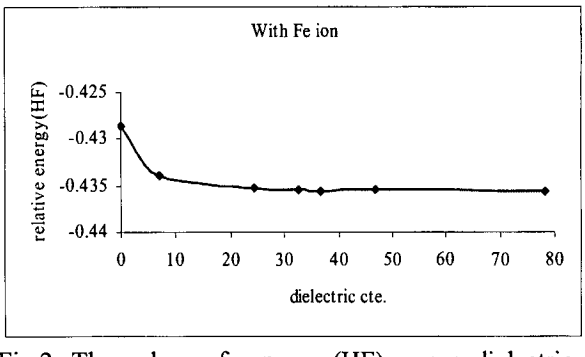


Fig.2. The values of energy (HF) versus dielectric constants for redoctaze in the presence of Fe ion.

In figure 3 relative energy of carbonic anhydrase in the presence of Zn2+ ion and substrate molecule with three basis sets including 6-31G,6-31G* and 6-31G** are observed. As it is seen addition of polarized functions in basis sets caused to higher level of energy two basis sets : 6-31G* and 6-31G** almost have same energy level but higher than 6_31G. With 6-31G basis set we have a maximum energy in ethanol and minimum in acetonitril. It means that acetonitril which has higher dielectric constant and more polar, has better interaction with enzyme molecule in the presence of substrate.

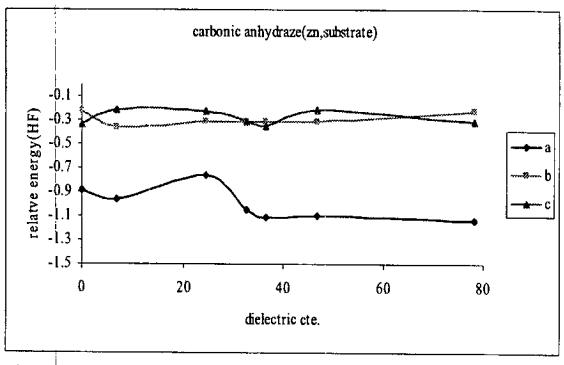


Fig.3. relative energy versus dielectric constants for three basis sets,a:6-31G,b:6-31G*,c:6-31G**.

In figure 4 relative energies of carbonic anhydrase in the presence of Zn2+ ion with three basis sets including 6-31G,6-31G* and 6-31G** were calculated. Energy level with basis set that has not polarized functions is lower than two other basis sets and a minimum in energy with DMSO solvent is seen. In 6-31G* we have a maximum with aniline that has the lowest dielectric constant. Energy in vacuum has not important difference with other solvents with basis sets 6-31G* and 6-31G** no important difference is seen.

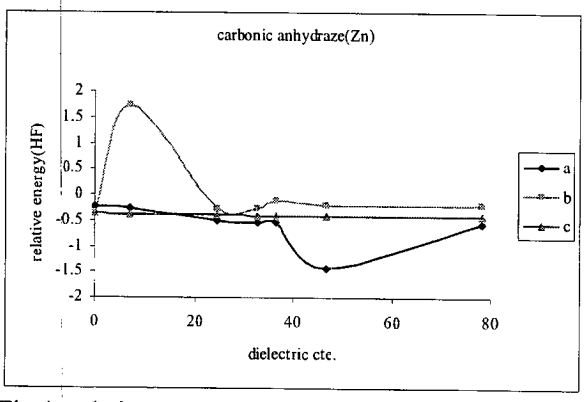


Fig.4. relative energy versus dielectric constants for three basis sets,a:6-31G,b:6-31G*,c:6-31G**.

In figure 5 relative energy of carbonic anhydrase without Zn2* ion with three basis sets are observed that the least polarizer function has lowest energy and in aniline the minimum energy is observed.

In figure 6 dipole moments of carbonic anhydrase versus dielectric constant in three different cases (with Zn2+, without Zn2+ and Zn2+/ substrate) are observed. The calculations was performed with 6-31G** basis set. In the presence of Zn2+ ion and absence of it the behavior of dipole moment is opposite; it means that the presence of Zn2+ makes the environment of the enzyme more polar.

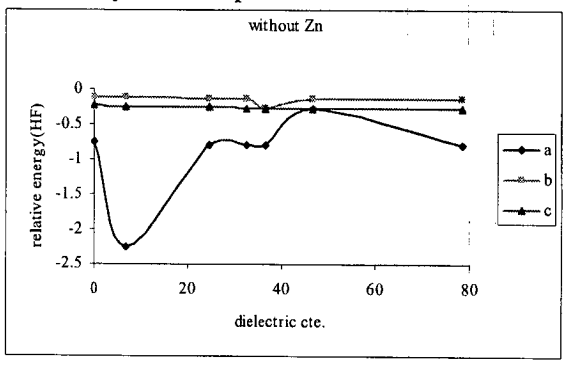


Fig.5. relative energy versus dielectric constants for three basis sets,a:6-31G,b:6-31G*,c:6-31G**.

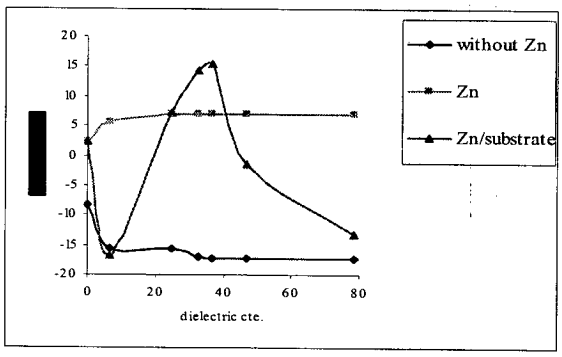


Fig.6. Values of dip. moment versus dielectric cte. for carbonic anhydraze with 6-31G**basisset.

In table 1 the energies and dipole moments of reductase enzyme are observed and it is seen that presence of metallic ion makes molecule of enzyme more stable.

**Table 1**. Comparison of Energy and Dipole Moment of Active Site of Redoctaze in the presence and absence of Fe ion in Vacue and Solvents

| | Without Fe | | With Fe | |
|---|---|---|---|---|
| Solvent | E(HF) | μ | E(HF) | μ |
| In Vacue | -5869.333 | -5.5973 | -3345.4286 | 0.6583 |
| Water | -5869.161 | -3.0407 | -3345.4356 | 1.9236 |
| DMSO | -5869.39 | -6.3265 | -3345.4355 | 1.8887 |
| Methanol | -5869.3514 | -6.2458 | -3345.4354 | 1.844 |
| Ethanol | -5869.3536 | -5.3057 | -3345.4352 | 1.8338 |
| Aniline | -5869.3777 | -6.1625 | -3345.4339 | 1.5602 |
| Acetonitrile | -5869.3399 | -6.6928 | -3345.4356 | 1.8686 |

In table 2 energies and dipole moments of carbonic anhydrase with three basis sets (with Zn2+, without Zn2+ and Zn2+/ substrate) were compared and the stabilizing effect of metallic ion is clearly seen.

**Table 2.** Comparison of Energy and Dipole Moment of Active Site of Carbonic Anhydraze in the presence and abcence of Zn ion and Substrate in Vacue and Solvents

| | without Zn | | Zn and substrate | | Zn | |
|---|---|---|---|---|---|---|
| solvent | E(HF) | μ | E(HF) | μ | E(HF) | μ |
| a | -2707.7525 | -8.8178 | -4212.8856 | -1.7537 | -4017.2446 | 3.9993 |
| In Vacue b | -2709.1034 | -8.1533 | -4214.2208 | -13.6579 | -4018.3727 | 2.2366 |
| c | -2709.228 | -8.2261 | -4214.3412 | 2.2454 | -4018.3727 | 2.2366 |
| | -2709.2596 | -17.3999 | -4213.1324 | 6.5341 | -4017.5608 | 5.0229 |
| Water | -2709.1473 | -8.1735 | -4214.2202 | -13.6577 | -4018.208 | 2.5285 |
| | -2709.258 | -17.2919 | -4214.2126 | -13.3235 | -4018.431 | 6.9283 |
| | -2707.7949 | -17.9269 | -4213.0995 | 4.4641 | -4018.4205 | 6.9318 |
| DMSO | -2709.1463 | -15.2746 | -4214.31 | 14.4971 | -4018.2085 | 2.529 |
| | -2709.258 | -17.2919 | -4214.2205 | -1.4229 | -4018.4205 | 6.9318 |
| | -2707.7935 | -17.7363 | -4213.0503 | 1.4883 | -4017.5299 | 4.4646 |
| Methanol | -2709.1295 | -14.2231 | -4214.3133 | 18.3161 | -4018.2746 | 2.6038 |
| | -2709.2679 | -17.0317 | -4214.3108 | 14.1681 | -4018.4094 | 6.9329 |
| | -2707.7927 | -17.673 | -4212.7611 | -8.8818 | -4017.5199 | 4.7787 |
| Ethanol | -2709.2669 | -15.7488 | -4214.3112 | 16.9214 | -4018.2731 | 2.6867 |
| | -2709.2669 | -15.7468 | -4214.3459 | 7.0773 | -4018.3975 | 6.9323 |
| | -2707.2807 | -7.6793 | -4218.1331 | 6.2289 | -4017.2767 | 5.3094 |
| Aniline | -2709.1403 | -14.9454 | -4214.3614 | 5.4134 | -4017.7235 | 2.2765 |
| | -2709.2696 | -15.6022 | -4214.216 | -16.844 | -4018.3892 | 5.4981 |
| | -2070.7941 | -17.8439 | -4213.1076 | 4.6295 | -4017.5404 | 4.4989 |
| Acetonitrile | -2709.1317 | -14.3989 | -4214.3111 | 13.34 | -4018.119 | 2.9773 |
| | -2709.268 | -17.2522 | -4214.3116 | 15.2797 | -4018.4134 | 6.9327 |

a: 6-31G, b:6-31G*, c:6-31G**

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