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Full Length Research Paper

Bio-nanomodeling of active site in oxidized azurin using by computational methods

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A cluster model for active site of oxidized azurin was presented and investigated the geometric structure and thermochemical parameters. Quantum-mechanical calculations were performed at the HF and B3lYP/6-31G levels of theory in the gas phase and eight solvents at four temperatures. Also, nuclear shielding parameters of the active site of oxidized azurin have been taken into account using GIAO and CSGT methods at the HF and B3LYP/6-31G levels of theory in the gas phase and in different solvents such as water, DMSO, nitromethane, methanol, ethanol, acetone, dichloroethane and dichloromethane. The results were revealed that the NMR chemical shielding parameters are strongly affected by inducing different solvent media. According to these theoretical results of energy values, some important relationships have been found between the dielectric constant and structural stability of active site of oxidized azurin. Thus, it can be drastically concluded that the dielectric permittivity of the solvent is a key factor that determines the chemical behavior of active site azurin in solution.

Key words: Azurin, IR, nuclear magnetic resonance, blue copper proteins, solvent effect.

INTRODUCTION

For more than 100 years, scientists have reported that bacterial infections can sometimes elicit remission in certain forms of cancer. A series of studies they have developed has shown that an opportunistic bacterium, *Pseudomonas aeruginosa* that grows in the soil and marshes but is often found in the lungs of cystic fibrosis patients, produces a protein, azurin, which it uses as a weapon, possibly to defend itself against cancer cells that might end up harming the microbe.

A team of researchers has shown that *P. aeruginosa* preferentially enters human melanoma and breast cancer cells, triggering apoptotic cell death. They further discovered that azurin sets off this death sequence by forming a complex with the well-known tumor suppressor protein p53, stabilizing it and activating caspases that induces apoptosis in cancer cells. P53 normally stops that are damaged from reproducing and encourages them to commit apoptosis, but a majority of cancer cells have damaged or missing p53 (Yamada et al., 2004).

Azurin is one of blue copper proteins (cupredoxins) with function of the electron transfer (Cascella et al., 2006). The blue copper proteins are characterized by an intense blue colour, distinctive electron spin resonance spectra, and unusually high reduction potentials. The copper proteins have been classified according to three types:

1. Type 1 copper proteins contain one copper ion, exhibit an unusual EPR spectrum with a hyperfine splitting appreciably smaller than that found for simple copper complexes. They exhibit an intense blue color (azurin and plastocyanin).
2. Type 2 copper proteins exhibit EPR spectra similar to those of simple copper complexes.
3. Type 3 copper proteins contain a dinuclear copper site and usually, as isolated, are EPR silent, which means that the copper atoms are either in the reduced form, or antiferromagnetically coupled. There are no pronounced features in the optical spectrum visible. The copper protein under study is azurin, a type 1 copper protein that serves as an electron transfer protein. The protein

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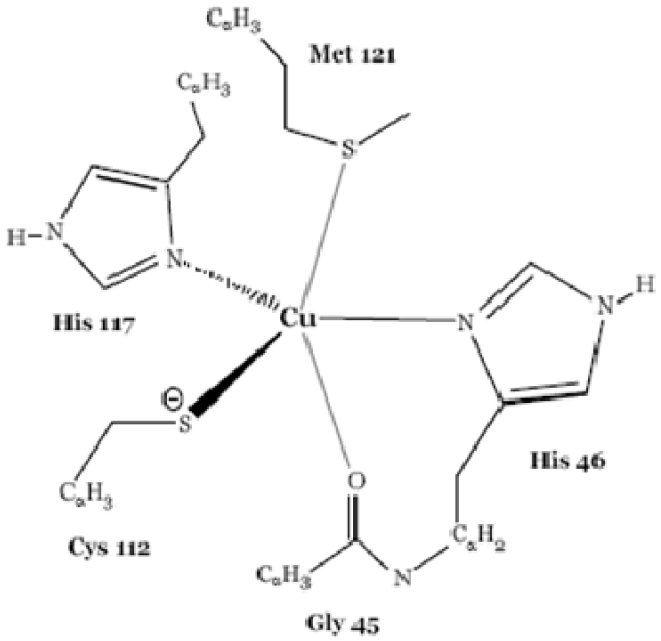

Figure 1. Cluster model for active site of oxidized azurin.

occurs in a variety of bacteria, the most commonly employed azurin are from *P. aeruginosa* (consisting of 128 amino acid) and Alcaligenes denitrificans (129 amino acid) (Olsson, 2000).

The active site of azurin consists of a copper ion and five residues, which are His 46, Cys112, His 117, Met 121 and Gly45. The copper ion is strongly connected to His 46, Cys112 and His117 with coordination bond and is weakly bonding to other residues and the structure around the copper ion is a distorted trigonal bipyramid (Figure 1) (Sugimori et al., 2005). Azurin(128 amino acids, 14 kD) consists of eight stranded -strands, arranged in a - sandwich, connected by random chains (turns) and of an a helical insertion.The copper site is at the top, or northern end, of protein, surrounded by an extensive hydrophobic patch that is the most striking surface feature of the protein (Arcangeli et al., 1999). The blue copper proteins serve as electron transfer agent. Their distorted trigonal geometry is intermediate between the tetrahedral coordination preferred by Cu(**I**) and the tetragonal geometry of most Cu(**II**) complexes(Rizzuti et al, 2007). As a results, the change in geometry when Cu(**II**) is reduced to Cu(**I**) is small, which gives a small reorganization energy and allows a high rate of electron transfer (Ryde et al., 2001). In the active site, electron transfer is with the redox reaction caused by charge transfer of S(Cys112)( ) Cu(dx^2 -y^2), which has been (investigated by resonance Raman (RR) spectra (Sugiyama et al., 2006).

Recently, the blue copper proteins including azurin have been investigated experimentally and theoretically by many groups (Lehmann, 2004; Thompson et al., 2000; Hansen et al., 2006). Experiments and computational studies are aimed at answering questions such as: To what extent are the local properties of the metal site determined by its protein and solvent environment? Which is the influence of protein internal dynamics on structural regions relevant for the protein functionality? How is protein conformation connected to solvent dynamics (Rienzo et al., 2004)?. The geometry of the active site of azurin has been estimated by density functional theory (DFT) with B3LYP method and quantum mechanics/ molecular mechanics (QM/MM) (Shuku et al., 2005)) . The hyperfine coupling constants in the active site have been calculated by DFT with B3LYP method and also by QM/MM approach(2).The electronic structure and the g-tensor of the active site of azurin have been investigated by *ab initio* multireference determinantal configuration interaction (MRD-CI) calculations (Gastel et al., 2002). Solvent effects on electronic structure of molecules have been investigated by many chemists and physicists to understand molecular structure, mechanism of chemical reactions in solution, etc. by using quantum-chemical calculations and molecular dynamics simulations. Physical properties such as geometry of molecules and charge distribution in solution often vary from those in vacuum.

Ryde et al have also performed a geometry optimization of the active site of blue copper proteins including azurin by quantum-chemical calculations with polarizable continuum model (PCM). Their results suggest that weak axial bond of Cu-S (Met 121) in solution is more elongated than that in vacuum (Ryde et al., 2001). The ability to accurately calculate solvation energies of molecules using molecular simulation methods is an important development in computational chemistry. These methods have wide applicability not only in studies of free energies of salvation, but also in studies of free energies of binding and protein and nucleic acid stability. The solvent effect is taken into account using the self- consistent reaction field (SCRF) method. This method is based on the Onsager reaction field theory of electrostatic salvation. In this model, a solvent is treated as a uniform dielectric with a given dielectric constant. A solute is placed into a cavity within the solvent. Various SCRF approaches differ in how they treat the cavity and reaction field (Tomasi et al., 2005).

In this paper, we use non empirical calculation in one of the first detailed studies of active site, geometries, energies, enthalpies, free energies, entropies and other thermochemical properties with special emphasis on solvent effect on it. We optimized the geometries of the active site of oxidized azurin in various solvents using the Onsager model at the Hartree- Fock, B3LYP levels of theory and compared our results with those obtained for the gas phase in addition, the effect of the permittivity of solvents on the stability of this structure was explored and discussed .The calculations were performed using the 6-31G basis set. In our current research, we have also theoretically studied the effects of DMSO, nitromethane, methanol, ethanol, acetone, dichloroethane,

dichloromethane, water and gas phase on the chemical shielding parameters of C^{13}, N^{15}, ^{64}Cu, ^{32}S nuclei involving in active site and its structural stability. The gauge including atomic orbitals (GIAO) and continuous set of gauge transformations (CSGT) approaches within the SCF-Hartree- Fock and B3LYP approximation have been used in order to investigate the influence different solvent media on the magnetic shielding tensors through Hartree-Fock and B3LYP approximation using 6-31G basis set.

THEORETICAL METHODS

IR approach

The Onsager-SCRF code elaborated by Wiberg and co-workers for the Gaussian computational code has been fairy popular in the past years. The Onsager model is the simplest version of the MPE approach. Solvation is described in terms of a dipole moment drawn iteratively from QM calculations of the molecule. The appealing feature of the Onsager-SCRF method was the ability of directly exploiting almost all the computational facilities of the Gaussian package (Tomasi et al., 2005).
Following the Onsager model, the interaction energy of a dipole in a solvent is:

$$E^{solv}(t) = -m(t)R(t), \quad (1)$$

Where R(t) is the reaction field at time t caused by the surrounding solvent that acts on the dipole of the solute.
The Onsager model describes the system as a molecule with a multipole moment inside a spherical cavity surrounded by a continuous dielectric. In some programs, only the dipole moment is used, and calculations therefore, fail for molecules with zero dipole moment. The results obtained using the Onsager model and HF calculations as a rule is qualitatively correct. Accuracy increases significantly with the use of MP2 or hybrid DFT functional. This is not the most accurate method available, but it is stable and fast.This makes the Onsager model an attractive alternative when a PCM calculation fails (Monajjemi et al., 2008).

In this study, a theoretical analysis at the HF and B3LYP /6-31G level of theory was performed to characterize all the stationary points of the potential energy surface as minima and obtain thermodynamic corrections. Solvation effects were modeled by the Onsager method as implemented in the Gaussian98 program. The equilibrium free energy of salvation can be divided into smaller contributions corresponding to cavitations, universal salvation effect such as solute-solvent electrostatic, dispersion,
and repulsion interactions and non universal specific interaction, such as intermolecular hydrogen bonding or electron donor-electron acceptor interactions (Schleyer, 1998).

G (solvation) = G (electrostatic) + G (dispersion) + G (repulsion) + G (cavitations) + G (specific).

Electrostatic solute-solvent interactions are usually introduced into quantum-mechanical calculations by means of the self-consistent reaction field (SCRF) approach.

In the Onsager model, a solute is placed in a spherical cavity immersed in a continuous solvent and the full classical multipole expansion of the total solute charge distribution is truncated after the dipole term, that is, it only includes solute monopole and dipole interactions with the continuum. Despite the simplicity of the Onsager approach, its applicability was proved for many systems.

The advantage of this model is analytic calculations of the solvent reaction field for spherical cavities, which speed up the process and allow geometric optimizations in solution to be performed for compact molecules at a modest computational cost. Although this method includes significant improvements of the Onsager approach, a new problem arises. For the definition of the density surface, an isodensity level that fixes the amount of electron density within the built cavity must be specified. However, a small percentage of charge density extends outside the cavity. This causes serious problems in the dielectric continuum treatment of solvents by affecting the charge inside the cavity.

Nuclear magnetic resonance approach

The calculation of nuclear magnetic resonance (NMR) parameters using semi-empirical, *ab initio* and DFT techniques has become a major and powerful tool in the investigation to look at how variations in the molecular structure occurs. The ability to quickly evaluate and correlate the magnitude and orientation of the chemical shielding anisotropy tensor with variations in bond length, bond angles and local coordination and nearest neighbor interactions has been a number of recent applications in the investigation of molecular structure. Nuclear magnetic resonance was shown that it is possible to calculate chemical shifts of individual amino acid residues of proteins without a detailed knowledge of the complete protein structure. The calculations also provide valuable information for exploring the experimental NMR chemical shifts with the molecular geometry and environment. Also NMR chemical shifts are quite sensitive to intermolecular interactions.

NMR is based on the quantum mechanical property of nuclei. The chemical shielding refers to the phenomenon which associated with the secondary magnetic field created by the induced motions of the electrons that surrounding the nuclei when in the presence of an applied magnetic field for chemical shielding (CS) tensors, which describes how the size of shielding varies with molecular orientation, we often use the following convention for the three principle component:

$$112233 \quad (2)$$

The three values of the shielding tensor are frequently expressed as the isotropic value ($_{iso}$), the anisotropy shielding ( ) and the asymmetry parameter ( ). In our current study, extensive quantum mechanical calculation of electronic structure of the active site of oxidized azurin and solvent effects on C^{13}, N^{15}, O^{17}, ^{64}Cu, ^{32}S-NMR parameters have been performed in different solvent media and in two available methods using GAUSSIAN 98 program.

A common feature among the blue copper proteins is the geometry around the Cu center. It has an approximately tetragonal symmetry, which is indeed distorted trigonal. The metal atom is coordinated by three strong atom ligands lying on a distorted trigonal plane, which are the S atom of a deprotonated cysteine and the N atoms of two histidines and by a weak axial atom ligand, namely the S atom of a methionine. In azurin from *P. aeruginosa*, these ligands belong, respectively, to Cys112, His46, His117, and Met121, and there is an additional weak axial ligand, that is, the O atoms of Gly45. After fully optimization of active site, we have calculated NMR parameters using the density functional B3LYP and HF method by Gauge Including Atomic Orbitals (GIAO) and continuous set of gauge transformations (CSGT).

RESULTS AND DISCUSSION

IR results

Quantum-chemical solvent effect theories give a self con-sistent description of the electronic structure of solutes,

which is closely related to the polarizable environment. Such calculations are indispensable for getting insight into the molecular properties and reactivity of condensed phases. This goal is usually achieved by means of a solute-solvent Schrödinger equation corresponding to some simplified representation of the solvent. In particular, the electronic structure of solute molecules can be closely related to the solvent structure and vice versa. This effect can be of key importance, for example, for the understanding of the microscopic mechanism of certain reactions in solutions. We should note that there is interaction energy between solutes and solvents. Because of this, solute properties that depend on energy and several other factors, such as geometry, vibrational frequencies, total energy and electronic spectrum, also depend on the solvent.

The presence of a solvent, particularly a polar solvent, can also stabilize charge separation within a molecule. This not only changes energy, but also causes electron density shifts and influences associated properties. In reality, this is a result of quantum mechanical interactions between the solute and solvent, which must be averaged over all possible arrangements of solvent molecules according to the statistical mechanics principles. The energy of solvation can further be divided into terms that describe the bulk solvent and terms that specify the first solvation shell. The bulk solvent contribution is primarily the result of dielectric shielding of electrostatic charge interactions. In the simplest form, it can be included in electrostatic interactions by means of the dielectric constant , as in the Coulomb interaction equation (Young, 2001).

$$= q_i q_j / r_{ij} \quad (3)$$

There are several effects in the region where a molecule meets its solvent shell. The first one is referred to as cavitations energy, which is the energy required to push aside solvent molecules to produce a cavity for the solute molecule. The second effect is related to forces attracting the solute molecule to the solvent. These are van der Waals, dispersion and hydrogen bonding interactions. Finally, the solvent molecules in the first shell can rearrange in order to maximize interactions with the solute. The largest amount of hydrogen bonding energy is usually related to solvent rearrangement to the preferred hydrogen bonding orientation. By solving the corresponding electrostatic equations inside and outside the sphere and applying proper boundary conditions, one can find the potential at any point inside the cavity and the total electrostatic energy of the interaction of molecular charge distribution with polarizable medium. In the quantum-chemical application of this theory, this interaction energy is represented by additional molecular Hamiltonian terms corresponding to nuclear-nuclear, nuclear-electronic, and electron-electron contributions. Because of the unavoidable deficiencies of the Onsager model, further insight into the nuclear polarization effects, that is, geometric optimization in solution would not yield better results. Therefore, the use of multipole expansions up to infinite order and a more realistic description of the solute cavity are necessary.

Solvent effects on relative stabilities

Active site of azurin was studied in the gas phase (= 1) and various solvent media with dielectric constants of water (= 80), dimethylsulfoxide (= 46.7), nitromethane (= 38.2), methanol (= 32.63), ethanol (= 24.55), acetone (= 20.7), dichloroethane (= 10.36) and dichloromethane (= 8.93) at 25, 27, 35 and 37°C. First, the active site was fully optimized by the HF and DFT (B3LYP) methods using the 6 - 31G basis set to obtain minima of the potential energy surfaces of the active site. The influence of the solvent on the relative stability of active site was studied by means of the Onsager approach. The values listed in Table 1 show that interactions between water molecules and active site reduce the energy of the whole system (*E*) . The only exception is nonbonding dispersion energy; this may imply that, in aqueous solutions, the import of polarized water molecules reduces the rate of active site polarization. The effect of solvents on the stabilization of the active site is of interest, it plays a major role in their activities.

The standard Onsager approach (the SCRF method) to active site with 6 - 31G basis set, as is used here, appears to be a good first step in theoretical investigations of solvent effects. In this paper, we studied the solvation of the active site of oxidized azurin. The influence of the dielectric constant on the standard geometry of active site in water, DMSO, nitromethane, methanol, ethanol, acetone, dichloroethane and dichloromethane was studied. We found that the relative energies (*E*) of active site in solution are smaller than in the gas phase, because interactions in solution are stronger than in the gas phase. The interaction energies of the active site decrease as the dielectric constant of solvents increases according to HF and B3LYP calculations. The results obtained at the B3LYP/6-31G level are more negative than those of the HF/6 -31G calculations because these methods differently take correlation energy into account (Table 1). As regards the high dielectric constant of water molecules surrounding the hydrophilic part of amino acid chains, we optimized these parameters much better than for the other solvents. We found that there was no significant difference between thermochemical parameters at 25, 27, 32 and 37°C (Figure 2).

Solvent effects on thermochemical parameters

Dielectric constant values ( ) and thermochemical parameters have a great influence on the mechanism of

Table 1. Relative thermochemical parameters of active site of azurin obtained in water and other solvent using different methods.

Dielectric constant	Method	E (Kcal/mol)		H(Kcal/mol)		G(Kcal/mol)		S(Kcal/ molK)		SCF(Done) (Kcal/mol)
		25°C	27°C	25°C	27°C	25°C	27°C	25°C	27°C	
		32°C	37°C	32°C	37°C	32°C	37°C	32°C	37°C	
80	HF	0.0001	0.000088	0.000104	0.000088	0	0.000039	0.044915	0.045458	-183.67437
		0.000045	0	0.00004	0	0.000134	0.000229	0.04692	0.048377	
	B3LYP	0.513413	0.513396	0.513414	0.513396	0.513229	0.513263	0.042086	0.042664	-184.184521
		0.51335	0.513303	0.513349	0.513301	0.513356	0.51345	0.044223	0.045777	
46.7	HF	0.000161	0.00014	0.000162	0.000145	0.000044	0.000078	0.044305	0.044848	-183.67435
		0.000102	0	0.000101	0.000056	0.000172	0.000267	0.046311	0.047768	
	B3LYP	0.513409	0.513392	0.51341	0.513392	0.513213	0.513247	0.041663	0.042242	-184.18452
		0.513346	0.513295	0.513345	0.513293	0.51334	0.513429	0.043801	0.045355	
38.2	HF	0.00011	0.00009	0.00011	0.00009	0	0.00004	0.044769	0.045312	-183.67433
		0.00005	0	0.00005	0	0.00014	0.00023	0.046775	0.048231	
	B3LYP	0.51341	0.51339	0.51341	0.51339	0.51324	0.51327	0.042489	0.043067	-184.18452
		0.51334	0.5133	0.51334	0.51329	0.51336	0.51346	0.044627	0.046181	
32.63	HF	0.000114	0.0001	0.000117	0.0001	0.000011	0.000046	0.044737	0.04528	-183.67432
		0.000057	0.000013	0.000056	0	0.000101	0.000235	0.046743	0.0482	
	B3LYP	0.513409	0.513392	0.51341	0.513393	0.513178	0.513212	0.040485	0.041063	-184.18452
		0.513346	0.5133	0.513346	0.513298	0.513304	0.513397	0.042622	0.044174	
24.55	HF	0.00032	0.0003	0.00032	0.0003	0.00016	0.00019	0.043041	0.043585	-183.67429
		0.00025	0.00021	0.00025	0.00021	0.00028	0.00038	0.04505	0.046509	
	B3LYP	0.51341	0.51339	0.51341	0.51339	0.51324	0.51327	0.04251	0.043088	-184.18452
		0.51334	0.5133	0.51335	0.5133	0.51336	0.51346	0.044648	0.046202	
20.7	HF	0.000127	0.000111	0.000128	0.000112	0.000018	0.000053	0.044596	0.045139	-183.67428
		0.000068	0.000024	0.000067	0.000023	0.000147	0.000242	0.046602	0.048059	
	B3LYP	0.513412	0.513395	0.513413	0.513395	0.513253	0.513288	0.042928	0.043507	-184.184525
		0.513349	0.513302	0.513348	0.5133	0.513381	0.513475	0.045066	0.04662	
10.36	HF	0.00015	0.00013	0.00015	0.00013	0	0.00007	0.044345	0.044888	-183.67416
		0.00009	0.00006	0.00009	0.00004	0.00016	0.00026	0.046351	0.047808	
	B3LYP	0.51341	0.51339	0.51341	0.51339	0.51323	0.51326	0.042217	0.042796	-184.18456
		0.51335	0.5133	0.51334	0.5133	0.51336	0.51345	0.044355	0.045909	
8.93	HF	0.00016	0.00014	0.00016	0.00014	0.00004	0.00007	0.044305	0.044848	-183.67413
		0.0001	0	0.0001	0	0.00017	0.00026	0.046311	0.047768	
	B3LYP	0.51346	0.51344	0.51346	0.51344	0.51309	0.51312	0.035934	0.036501	-184.18458
		0.5134	0.51335	0.51339	0.51335	0.51321	0.5133	0.038027	0.039548	
1	HF	0.00034	0.00032	0.00034	0.00033	0.00018	0.00021	0.042699	0.043243	-183.67313
		0.00028	0.00024	0.00028	0.00024	0.0003	0.0004	0.046167	0.046167	
	B3LYP	0.5134	0.51266	0.51341	0.51266	0.51321	0.51125	0.041728	0	-184.18452
		0.51262	0.51259	0.51262	0.51259	0.51132	0.51139	0.001189	0.002378	

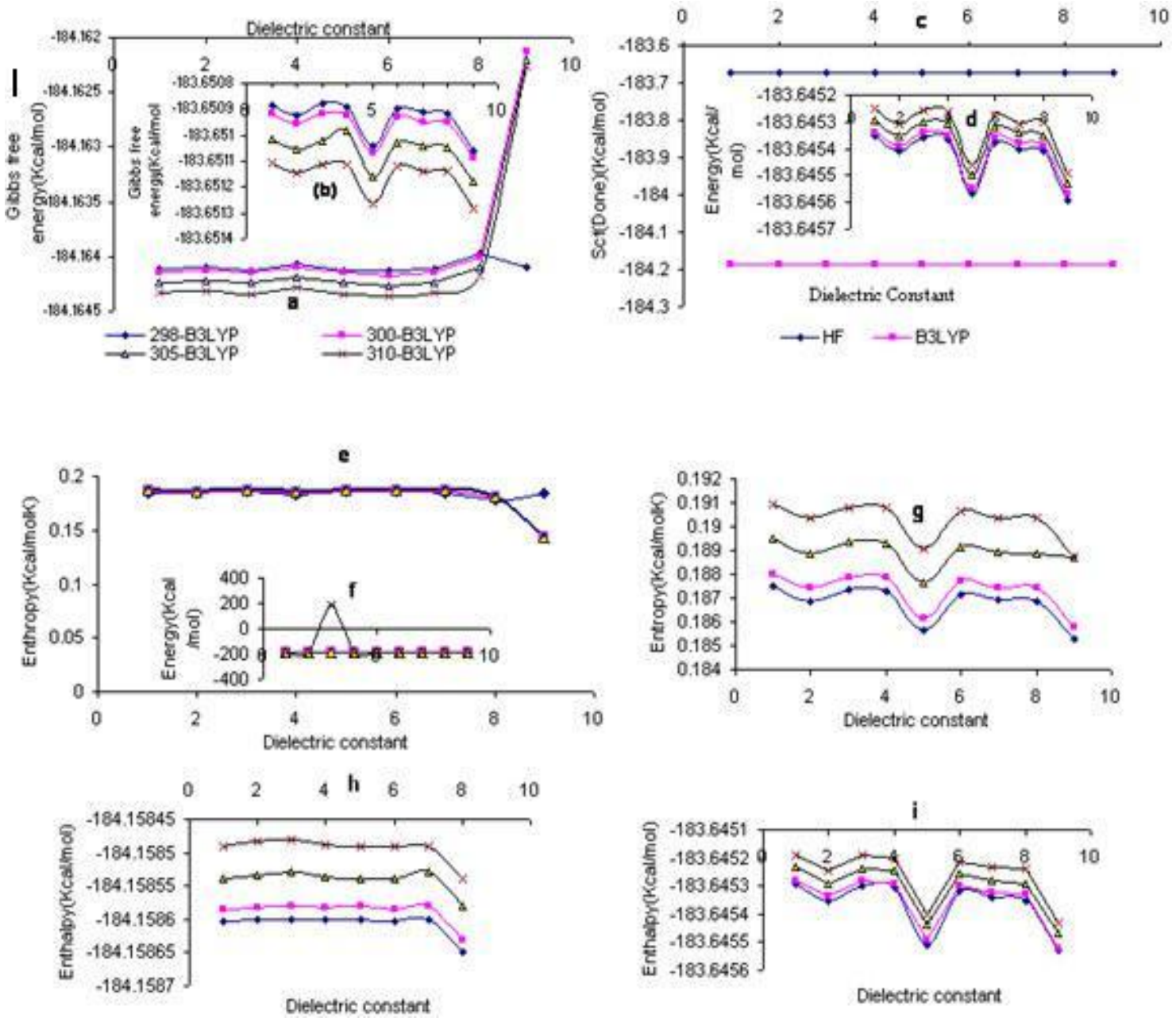

Figure 2. Comparison of energy(E),enthalpy (H),Gibbs free energy(G) (Kcal/mol),and entropy (S) (Kcal/ molK),of active site of oxidized azurin versus dielectric constants obtained using the HF and DFT methods at four temperatures (25,27,32,37˚C).(a) B3LYP- G, (b) HF- G, (c) SCF(Done)=E, (d)HF- E ,(e) B3LYP- S ,(f) B3LYP- E ,(g)HF- S, (h) B3LYP- H, (i)HF- H.

electron transfer in complexes and their studies could provide a deeper advance in quantitative treatment of charge transfer reactions in proteins (Chamorovsky et al., 2007). The standard enthalpies (*H*), entropies (*S*) and free energies (*G*) of active site was obtained by theoretical methods using the GAUSSIAN 98 package (Table 1). We found that there was some difference between these functions obtained by the HF and B3LYP methods. A study of hydrogen bonding between the active site of oxidized azurin and water, DMSO, nitromethane, methanol, ethanol, acetone, dichloroethane and dichloromethane was performed for optimized structure in solution at 25, 27, 32 and 37°C (Table 1) .The best results in various solvent media were obtained at the B3LYP level of theory. Free energy variations in terms of dielectric constant at two levels of theory and four temperatures obtained using 6 - 31G basis set are plotted in Figure 2 in terms of the dielectric

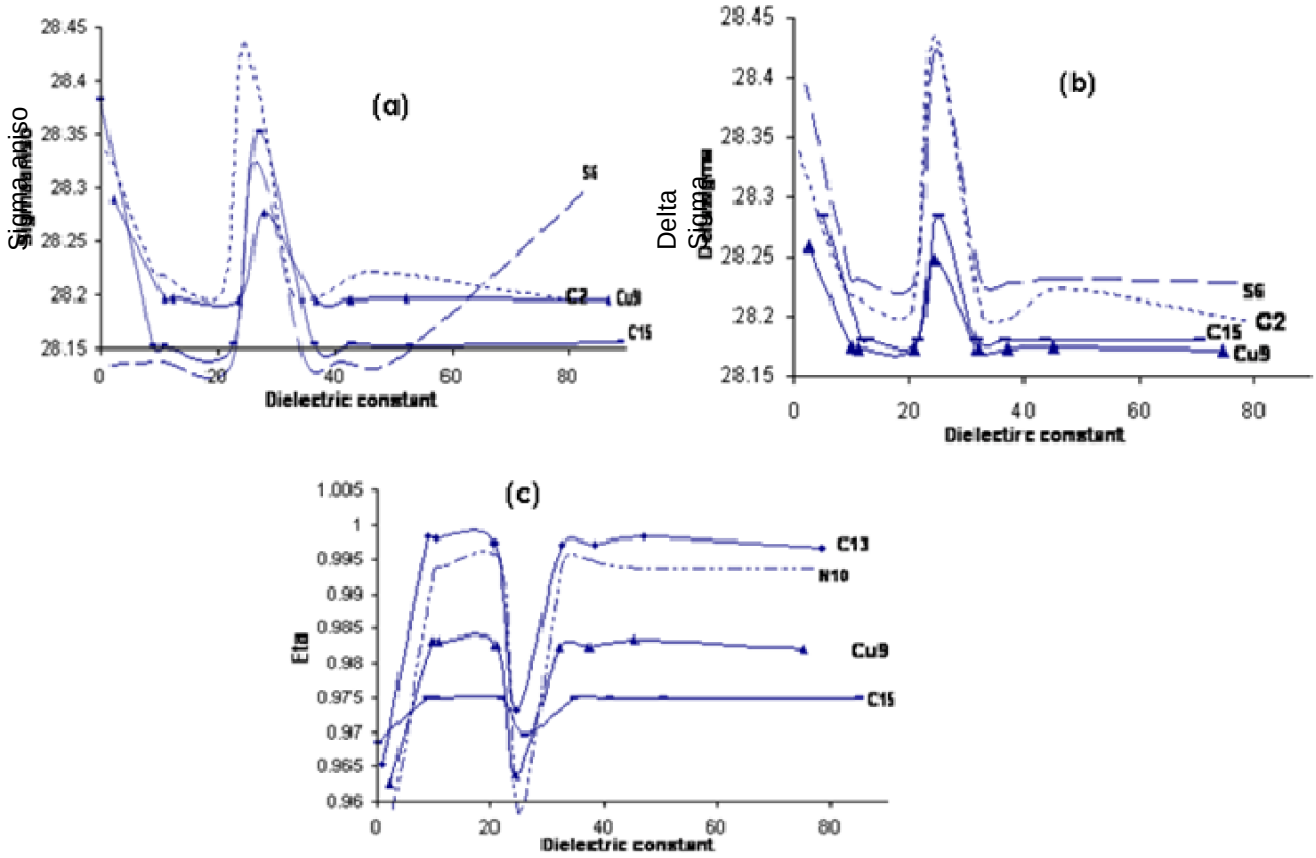

Figure 3. The graphs of (a) anisotropic shielding values(aniso), (b) indirect shielding( ), (c) asymmetry parameters ( ) of propose atoms of active site azurin in different solvent media at the level of HF/6-31G theory in CSGT method.

constant. We see that, as the dielectric constant changes from dichloromethane (= 8.93) to polar water (= 80) at 25, 27, 32 and 37°C, the stabilization energy decreases at B3LYP levels (Figure 2). Table 1 and Figure 2 show that the free energies of interactions (*G*) of active site in solution are more negative than in the gas phase, that is, interactions in solution are stronger than in the gas phase.

Solvent effects on nuclear magnetic resonance parameters

In this section we report and analyze the solvent effects on our NMR shielding tensors of ^{13}C, ^{15}N, ^{64}Cu, ^{32}S-NMR shielding of active site of oxidized azurin obtain at the HF and B3LYP levels. In our current research, we have presented the results of our extensive studies of solvent induced effects on the of ^{13}C, ^{15}N,^{64}Cu, ^{32}S-NMR shielding of active site of oxidized azurin site in a wide range of solvents encompassing a broad spectrum of polarity and hydrogen-bonding properties. According to our theoretical data, it is apparent that the solvent effects seems quite significant in either their diverse biological or physicochemical behavior. At first glance, the shielding variation seems to follow the polarity of solvent in the sense of enhanced deshielding with the increasing polarity, and this point is discussed in detail in conjunction with equations (Monajjemi et al., 2008). The ^{13}C, ^{15}N, ^{64}Cu, ^{32}S-NMR parameters of active site of oxidized azurin in various solvent media are given in Table 2. Also, several graph of NMR parameters of mentioned nuclei versus dielectric constant have been displayed in Figures 3 - 5. As expected, the NMR shielding tensors of ^{13}C, ^{15}N, ^{64}Cu, ^{32}S nuclei are drastically affected by what it is bonded to and the type of bond to its neighbor. Our obtained results yielded strong evidence that intermolecular effects such as electron transfer interactions play very important role in determining the ^{13}C, ^{15}N, ^{64}Cu, ^{32}S-NMR chemical shielding tensors of active site of oxidized azurin and some systematic trends appeared from the analysis of the calculated values.

Electron transfer between azurin and its proposed natural partners cytochrome C551 and nitrite reductase, as well as to some other redox proteins, was experimentally demonstrated. The need for specific but not too specific binding due to the fact that ET occurs

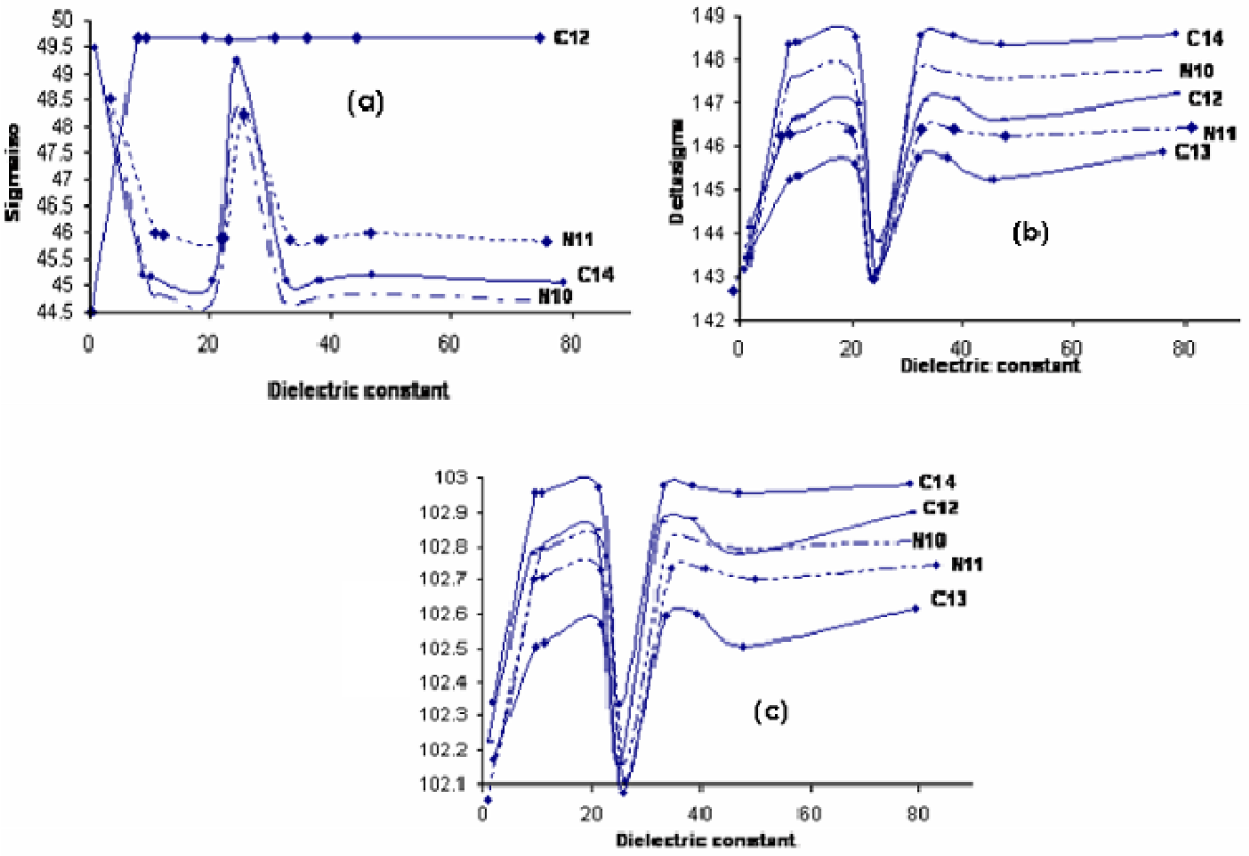

Figure 4. The graphs of (a) isotropic shielding values(iso), (b) indirect shielding ( ), (c)chemical shift tensor( ) of propose atoms of active site azurin in different solvent media at the level of HF/6-31G theory CSGT method.

between azurin and various partners has also been addressed (Zhuravleva et al., 2004). On the basis of both geometrical positions of ^{13}C, ^{15}N, ^{64}Cu, ^{32}S existing in active site of oxidized azurin and computed results, for nuclei involved in electron transfer the obtained NMR parameters are not the same as those computed for other atoms. For Cu_9 atom which behaves as electron donor, the iso component showed the largest intermolecular effects and it shows positive shielding values that are, the electron transfer interaction produced a deshielding in this position.

Comparison of iso, aniso, and values of Cu_9 atom with another shielding values in Table 2 and also analysis of graphs of iso, aniso, and ’s versus dielectric constant exhibited in Figures 3 - 5 revealed that, the largest values observed for ethanol and water, whereas the smallest belongs to DMSO. It is interesting to note that on the contrary, the opposite trend has been observed for asymmetry parameter ( ). This logical behavior may be readily understood in accord with biological conceptions.

The metal ion in the electron-transfer copper proteins such as the type copper proteins has been proposed to exist in an entatic state. These studies as well as earlier reports suggest that the metal-ligand interactions in the blue copper proteins indeed play an important role in imparting extra stability to the metal binding site of the protein (Sujak et al., 2007) . The holoprotein was more stable than the apo-protein, indicating that the metal ion plays an important role in stabilization of the protein. As expected, after Cu_9, S_6 shows positive shielding values. Cys 112 is among the ligand residues the one that more strongly hybridizes with the Cu orbitals. The covalency of the copper - ligand bonds is very anisotropic and it was suggested that this should favor hole super exchange pathways that couple to the Cu through the Cys112 ligand (Schleyer, 1998). The cysteine ligand decreases the reorganization energy. This decrease is caused by the transfer of charge from the negative charged thiolate group to Cu , which makes the oxidized and reduced structures quite similar (Ryde et al., 2001). As it can be seen in Table 2, an important piece of information can be derived from the analysis of the satisfactory correlation between the NMR parameters and dielectric constants of different solvents. So reported results in the presence of certain solvent molecules indicate that there is a significant cooperative effect that can strongly affect these parameters. For both N_{10} and N_{11} atoms which are

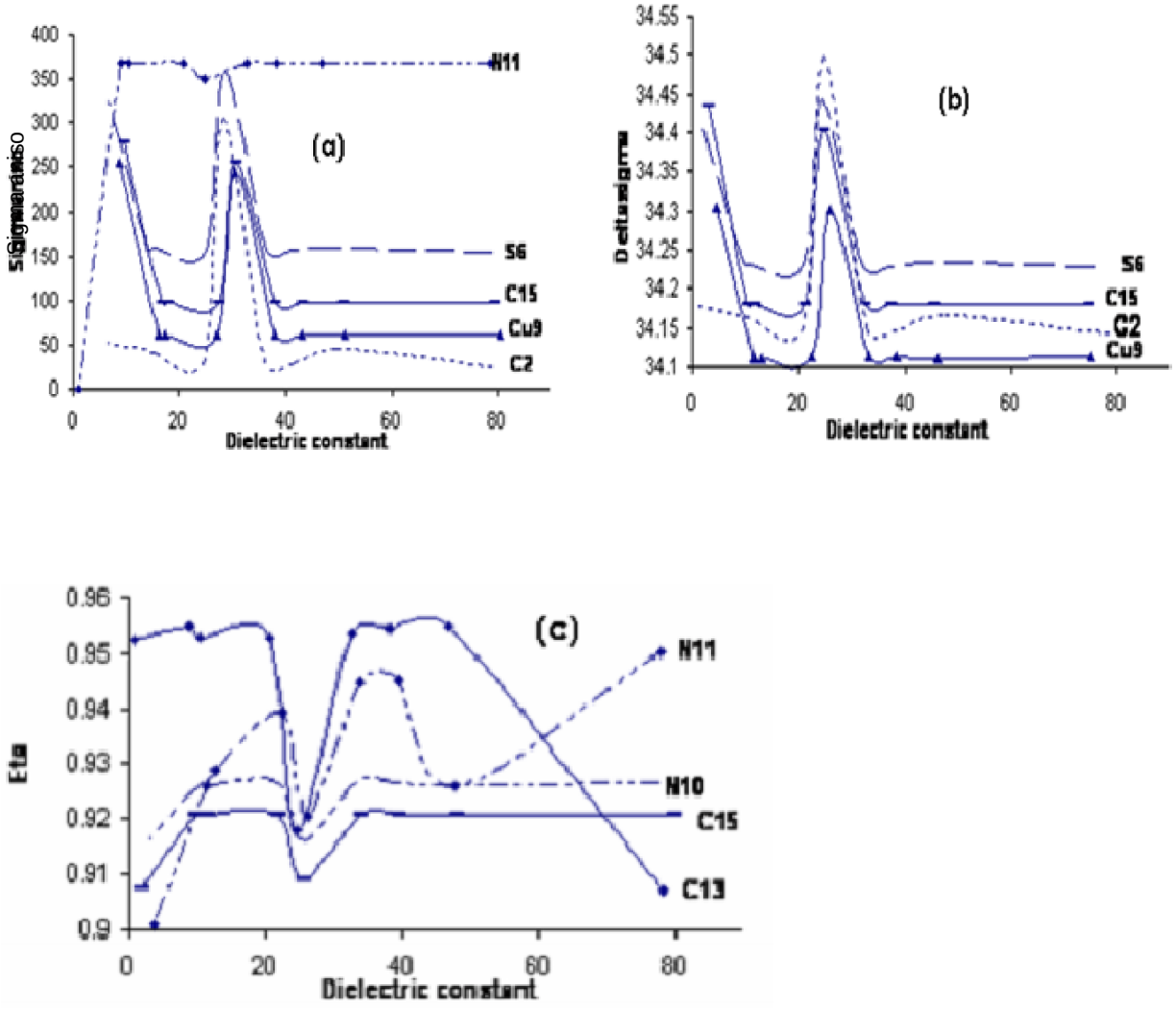

Figure 5. The graphs of (a) anisotropic shielding values(aniso), (b)indirect shielding ( ),(c)asymmetry parameters ( ), of propose atoms of active site azurin in different solvent media at the level of HF/6-31G theory in GIAO method.

fused in imidazole ring, shielding tensors are close to each other but according to our obtained results of Figure 4, as the dielectric constant in passing from the nitromethane to water, the $_{aniso}$ and of N_{11} and N_{10} increase and the decrease in the ethanol which is expected to result in a significant shielding of this nitrogen nuclei at the Hartree-Fock level of theory with CSGT method. Nitrogen ligands give up appreciably lower reorganization energy than water, owing to the lower Cu-N force constant (19). Figure 5 shows that, as the dielectric constant of the solvent increases, the and $_{aniso}$ of C_{15} and C_{13} increase and the of C_{15} and C_{13} decreased in the ethanol at the Hartree-Fock level of theory with GIAO method. Also, calculations at the HF in CSGT method (Figures 3 and 4) and the HF in GIAO method (Figure 5) have shown that molecular geometry and shielding properties are better than the other methods, B3LYP in GIAO and CSGT methods.

Conclusion

This article presents an *ab initio* DFT study on a model active site of oxidized azurin. All the residues directly interacting with the copper ion are considered in the model, including Gly45, which is specific for azurin (Corni et al., 2005). Two fundamental processes in which the BC proteins are involved have been analyzed and studied extensively and from different perspectives: the association of cupredoxins with their partners and their electron transfer. Deriving clues for understanding the details of ET processes in biology is a far more complicated matter to be approached computationally (Ryde et al., 2001). *Ab initio* QM methods are used to focus on the local properties of the region around the Cu site such as reorganization energy or stability. QM method is being increasingly applied and preferred to traditional methods due to their versatility and ability to

describe the same system at different levels of detail. Thus, the solvent (typically water) can be explicitly introduced in the simulations or described using implicit parameterization. The environment not only affects the protein structure but also protein functionality. In computational studies, it is important to take in to account modifications of solvent characteristics such as pH and ionic strength.

On the basis of the results of our calculations, we found that, among various modern quantum mechanical methods, the HF method was the most popular to date. System was optimized by the HF and B3LYP methods. In all cases, the steady-state nature (minimum of the potential energy surface) of the optimized complex has been confirmed through the investigation of theoretical levels. This will allow the most important reactions and the most important species to be identified for the compounds in question.

As a matter of fact, the energies and thermochemical parameters can provide valuable formation about binding stabilities of active site in oxidized azurin with possible electron transfer. The protein environment, which is often aqueous, affects the structure and folding dynamics and, therefore, the functionality of globular proteins. In fact, solvent-protein interactions, together with the interactions between residues in the protein matrix, facilitate the folding process and establishment of intermolecular interactions with other complex system. Furthermore, to be properly folded and fully functional, a protein requires a minimum level of hydration. Of course, the contrary is also true, that is, as far as water affects protein structure, protein can modify the structural and dynamic behavior of Water (Luise et al., 2000). The results reported in this paper indicates that it is possible to measure NMR tensors of various nuclei involving in biological compounds either in gas phase or in the presence of different solvent molecules theoretically. Several conclusions can be made on the basis of the observed results of the present study. Such amount of theoretical data can provide us important insights into the nature of molecular structures in biological systems. Our main findings from the point of view of solvent effects can be summarized as follows:

1. Optimization at the HF level of theory provides a suitable computational model in terms of calculated NMR parameters and relative energies.
2. NMR parameters are very sensitive to small changes in molecular geometry and chemical environment exhibited significant sensitivity to the intramolecular interactions. So, our obtained theoretical results emphasized on the influence of the environment factors.

The largest $_{iso}$ value of mentioned nuclei of active site azurin observed for ethanol and water, whereas the smallest one belongs to DMSO. It is interesting to note that the opposite trend have been observed for asymmetry parameters ( ). This usual behavior may be readily understood in accord with biotechnological conceptions. The calculation of NMR parameters using *ab initio* techniques seems to be a major and a remarkable tool for investigation of how variations of biological systems and provides information on the local environment of selected species and their next nearest neighbors. However, combination of NMR study embedded in solvent medium reveals a logical interpretation of the observed results.

In conclusion, we have shown that theoretical calculations can be used to successfully solve biochemical problems. In similarly with experimental methods, they involve assumptions and interpretation, and they have their limitations, but there are many problems that are best studied by theory. Thus, theoretical methods have become a competitive alternative to experiments for biochemical investigations.

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