

## Molecular dynamic study and the effect of solvent dielectric constant on structure of heme-containing protein Cytochrome c in different media

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The solvent dielectric constant is considered an important factor in determining the properties of the heme-containing protein cytochrome c in solution. Therefore, we investigate solvents effects on the relative energies, dipole moment values, and structural properties of water, methanol, and ethanol surrounding heme c (HEC), by using molecular dynamics simulation. In this study, we substitute the Iron atom of HEC with other metal atoms such as Cobalt, Nickel, Copper and Zinc and polar solvent effect has been performed using Onsager model. Calculations were carried out with the GAUSSIAN 98 program based on density functional theory (DFT) at B3LYP/6-31G\* level. We have compared gas phase and solvent calculations.

**Key words:** density functional theory, gaussian 98, heme c (HEC), molecular dynamic, Onsager model.

### INTRODUCTION

Cytochrome c is a small (MW: 12400 Da), water-soluble, electron-transporting protein that includes heme group (2, 21, 14) and its structure contains a polypeptide chain of 104 amino acids that are covalently bounded to the heme (16). Tetra pyrrole ring structure of HEC (heme type-c) porphyrin contains a Fe (III/II) unit which it is matched to four pyrrole nitrogens (20). The iron atom of the HEC center changes between an oxidized ferric (+3) and a reduced ferrous (+2) state, which this peripheral membrane protein acting as a one electron transporting from the coenzyme QH<sub>2</sub>-cytochrome c reductase complex to the cytochrome c oxidase complex during respiration. As well as acting as a mitochondrial electron carrier, cytochrome c has a key position in apoptosis, (1&21) that is frequently liberated from mitochondria in the early steps of apoptosis

(13). Cytochrome c with peroxidase reaction acts to trigger the liberation of pro-apoptotic proteins into the cytosol (19). It is able to activating the caspase cascade only in oxidized form (17). After apoptotic stimulation happens, released cytochrome c from mitochondria links with procaspase-9 (47 KDa)/Apaf-1. Procaspase-9/Apaf-1 modifies pro-enzyme to its active form. This occurrence also triggers caspase-3 activation and finally causes to apoptosis (5). Heme proteins are important molecules (metallo proteins) demonstrating many different roles such as electron carrying, Redox activities and oxygen keeping in several biological processes. Design of innovative HEC and determination binding proteins can be carried out by the analysis of structural properties of heme binding surroundings (15, 7). The chemical structure of HEC has been illustrated in Figure 1. In curative

tactics of diseases, the effective drug delivery has an important role in drug delivery system. Despite the biological affinity probes on nanovector by aiming the receptors on cell membrane increase successfully the delivery of drug, the binding of nanovector on purpose cell and the focus of nanovector in extracellular situation in fact are related to each other. Finally, the drug delivery efficiency would be progressed with knowing the target cell by the nanovector and suitable interaction between probes and their receptors (6). The study of structural and biological properties and functional mechanism of cytochrome c can be employed to get the biocompatible nanoparticles with biologic environment. As this intracellular component involves in mitochondrial electron carrying, Cytochrome c has been considered the suitable entrant for the nanovector applications (3). Considering the cytochrome c is known in immune system, our hypothesis is based on immune system might not cause hypersensitive

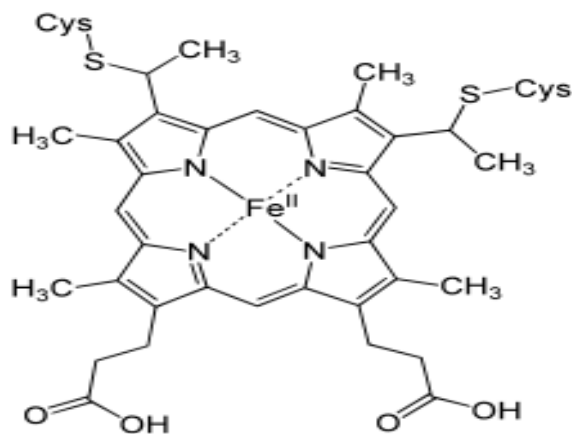


Figure 1. Chemical structures of heme c.

### Computational Method

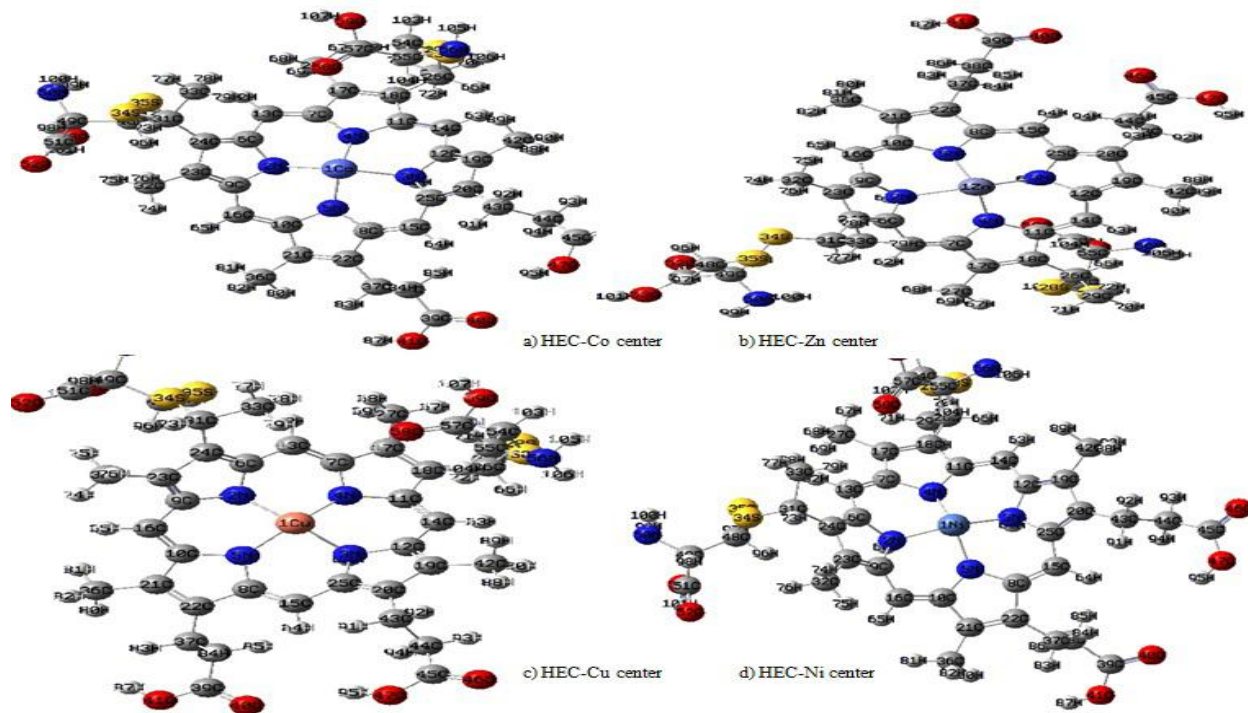
The term “ab initio” is given to computations that are derived directly from theoretical principles, with no inclusion of experimental data. The most common type of ab initio calculation is a Hartree Fock calculation (HF), in which the primary approximation is called the central field approximation. In general, ab initio calculations provide good qualitative results and can give increasingly accurate quantitative results as the molecules in question become smaller (10).

reactions to cytochrome c. Therefore, it can be a positive aspect in selection of this protein as a nanovector. In this research, the structural analogy of cytochrome c to the nanovector encourages us to evaluate the reliability of B3LYP theory with 6-31G\* basis set in predicting the structural properties of this compound. We have use of the ab initio calculations to finding minimum energy conformation of the protein in gas phase. We have modified the central atom of HEC (Fe) to CO, Ni, Cu and Zn. We have optimized the geometries of the protein in an assortment of solvents via the self consistent reaction field (SCRf) method. This method is based on Onsager reaction field theory of electrostatic salvation at the B3LYP level of theory and compared our consequences with those obtained for the gas phase also; permittivity result of solvents on the stability of this structure was explored and discussed. The calculations were performed using the 6-31G\*, basis set .

At first, we have modeled the HEC with each atoms (Fe, Co, Ni, Cu, Zn) separately with HyperChem 7.0 program package (4) then all calculations were performed using the Gaussian 98 program (Frisch et al., 1998). There are three steps in carrying out any quantum mechanical calculation in Gaussian; First, prepare each molecules with an appropriate starting geometry. Second, choose a calculation method and its associated options. Third, choose the type of calculation with the relevant options. The Quantum Mechanics (QM) calculations of electronic structure of the active site of heme center and solvent effects on it were carried out in different solvent media with the Gaussian 98 program based on the B3LYP/6-31G\* level. A general characteristic amongst the heme protein is the geometry around the Fe center. In the Gaussian program, a simple approximation is used in which the volume of the solute is used to compute the radius of a cavity which forms the hypothetical surface of the molecule (18, 12). We have compared the gas phase and different solvent media such as water, methanol, and ethanol. We investigated polar solvents effects on HEC within the Onsager self-consistent reaction field (SCRf) model using a Hartree-Fock method and the substitution effects on the

stability of HEC in various solvents(8,9,11). The effect of the permittivity of solvents on the

stability of this structure, molecular energies and dipole moments was searched and discussed.



**Figure 2.** Optimized structure of HEC with different centers.

## Results and Discussion

In this work, we studied the structural properties of water, methanol and ethanol surrounding HEC using molecular dynamics simulations. The optimized structures of protein with different central atoms are shown in Figure 2. We used different atoms to determine energy and other type of geometrical parameters on the particular HEC. Because of the differences among atoms, the energy of a molecule calculated using four different atoms will not be the same. Therefore, it is not reasonable to compare the energy of one molecule calculated with a particular atom with the energy of another molecule calculated using a different atom. In this study, differences in atoms were illustrated by comparing the energy of calculated by using force field MM<sup>+</sup>. The solute dipole moment induces a dipole moment in the opposite direction in

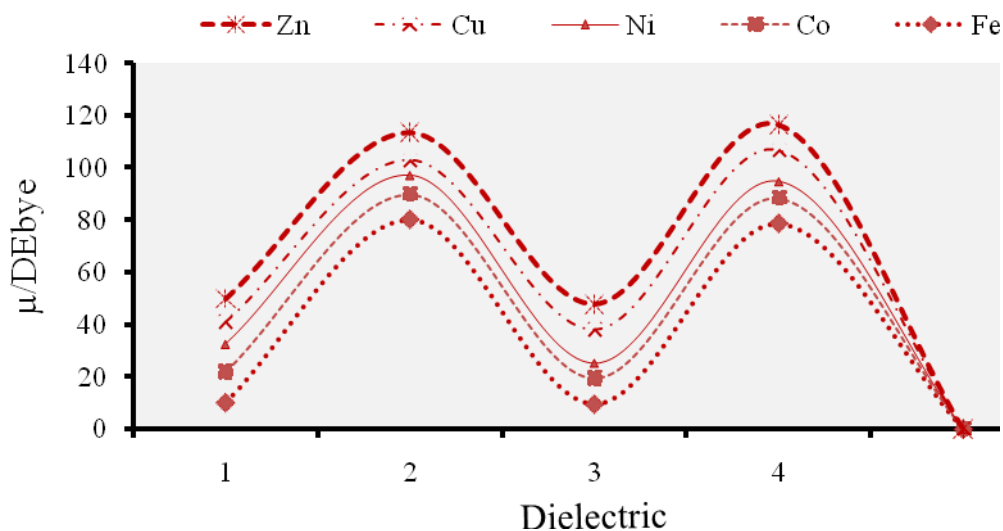
**Table 1.** Theoretical dipole moment values in different solvents at same central atoms.

Medium	Central atoms				
	Fe	Co	Ni	Cu	Zn
	<b>Dipole moment</b>				
Gas phase	10.1482	11.7222	10.6857	8.4542	8.8497
Water	80.3283	9.6017	7.2500	5.9537	10.4737
Methanol	9.5704	9.8682	5.8793	12.7850	9.7852
Ethanol	78.8042	9.6660	6.3238	12.0550	9.7537

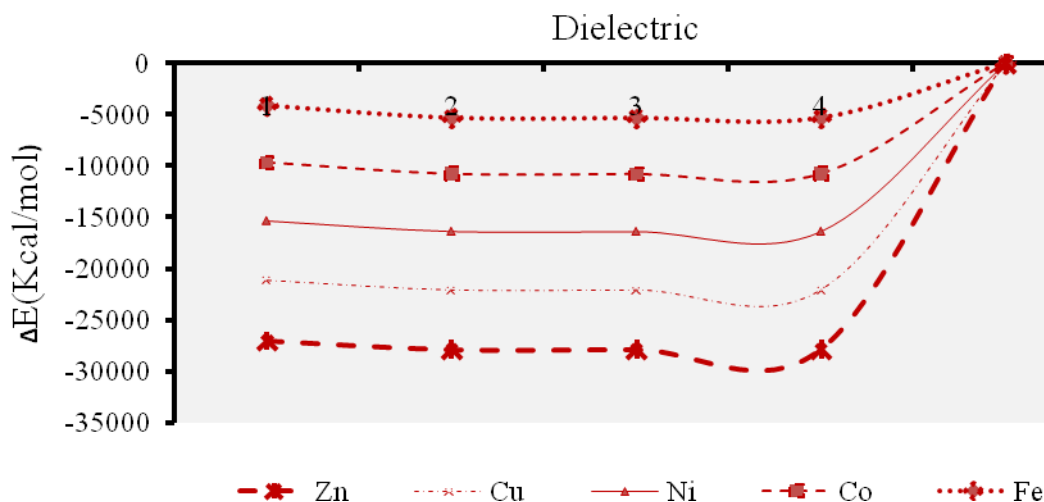
the surrounding medium. Polarization of the medium, in turn, polarizes the charge distribution in the solvent. The dipole moment value of HECs in different solvent media at same central atoms has been reported in Table 1 and Figure 3. One more practical approach consists of calculating the molecular volume as defined through the contour of constant electron density, equating this (non-spherical) molecular volume to the radius of an (ideally spherical) cavity, and adding a constant increment for the closest possible approach of solvent molecules. This latter approach used in Gaussian when the volume keyword was being used, since the influence between a molecule in solution and its medium can describe most simply by using Onsager model. In this model we have assumed that the solute is placed in a spherical cavity inside the solvent. The latter is described as a homogeneous, polarizable medium of dielectric constant. We performed our studies at B3LYP/6-31G\* gas phase geometry and water, methanol and ethanol surrounding HECs. The results obtained from Onsager model calculations illustrate the energy difference between these conformers, which are quite sensitive to the polarity of the surrounding solvent. The solvent effect has been calculated using SCRF model. According to this method, the total energy of solute and solvent, which depends on the dielectric constant  $\epsilon$ , has been listed in Table 2. These energies have been compared to the gas phase at the B3LYP/6-31G\* level of theory, and different solvents and the graph of energy values versus dielectric constant of different solvents have been displayed at considered substitutions in Figure 4.

**Table 2.** Theoretical relative energies in different solvents at same central atoms.

Medium	Dielectric constant	Central atoms				
		Fe	Co	Ni	Cu	Zn
$\Delta E$ (Kcal/mol)						
Gas phase	1	-4122.526	-5533.021	-5658.323	-5790.616	-5929.494
Water	78.39	-5320.390	-5450.214	-5573.689	-5704.271	-5840.993
Methanol	32.63	-5329.985	-5450.246	-5573.713	-5683.146	-5841.020
Ethanol	24.55	-5318.304	-5450.218	-5573.587	-5682.842	-5841.020



**Figure 3.** The dipole moment values in different solvents at same central atoms.



**Figure 4.** The relative energy values in different solvents at same central atoms.

## Conclusion

In this work, we investigated polar solvents effects and different substitutions effects on the stability of HECs in various solvents; Ab initio calculations were carried out with GAUSSIAN 98 program using density functional theory (DFT) at the B3LYP/6-31G\* level of theory. Because of the sulphhydryl groups of cysteine residues of heme group, it is logical that water can be suggested as the most proper solvent for structural properties of HEC due to its strongly hydrophilic property. Also, the orientation of the water molecules at the HEC-water interface can be affected by the orientation of the water dipole moment. In fact, electron transfer in water might occur on time scales faster than required for a complete rotation of the solvent dipole around the solute. The results obtained from Onsager model seemed quite sensitive to the polarity of the surrounding solvent. Moreover, we have compared the energy values obtain from different Fe, Co, Ni, Cu and Zn central atoms and concluded that these atoms were the most proper atoms for studying HEC.

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