Journal of Physical and Theoretical Chemistry
of Islamic Azad University of Iran, 6 (2) 87-95: Summer 2009
(J.Phys.Theor.Chem. IAU Iran)
ISSN: 1735-2126

NMR and Solvent Effect Study on the Active Site of Oxidized Azurin

K.Shahanipour[1], T.Nejad Satari[2], F.Mollaamin[4] and M.Monajjemi[3,*]

[1] Ph.D. student, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[3] Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran
[4] Department of Chemistry, Qom Branch, Islamic Azad University, Qom, Iran

ABSTRACT

We have evaluated the NMR shielding tensors for active site of oxidized azurin .Azurin is classified to a type I copper protein with ET functionality. We have computed NMR shielding tensors at B3LYP and HF levels by using 6-31G basis set in the gas phase and in different solvents such as water, DMSO, Nitromethane, methanol, ethanol, acetone ,dicholoroethane. These solvents represent a wide range of solvent properties from the point of view of polarity as well as hydrogen bonding interactions.The NMR shielding tensors were calculated using the GIAO and CSGT methods. Our results reveal that NMR chemical shielding parameters are strongly affected by inducing different solvent media Regarding to our plotted graphs of σ_{iso} , σ_{aniso}, $\Delta\sigma$, η , δ versus ε, the largest σ_{iso} values obtained in ethanol and water for Cu atom whereas the smallest one belonged to DMSO. It is interesting to note that the opposite trend have been observed for asymmetry parameters (η).

Also, calculations at the HF in CSGT and GIAO methods have shown that molecular geometry and shielding properties are better than the other methods , B3LYP in GIAO and CSGT methods.

Keywords: Azurin; NMR parameters; Blue copper protein; Solvent effect

INTRODUCTION

The metalloproteins have attracted considerable interest among many researchers because of their various functionalities, such as electron transfer (ET), redox reaction, oxygen transportation, and activation. Blue copper protein is one of metalloproteins, which can be classified into three types on the basis of spectroscopic properties.

Type I copper protein has an absorption peak near 592-625 nm; type II copper protein has a similar peak to that of the general Cu(II) complex. Type III copper protein exhibits antiferromagnetism and has an absorption peak near 300 nm.

Azurin is classified to a type I copper protein with ET functionality. The active site of azurin consists of one copper ion and five ligand residues: Gly 45, His46, Cys112, His117, and Met121.The copperion is strongly coordinate-bonding to His46, Cys112, andHis117, and bonds weakly to other residues[1].

The Cu site has a distorted trigonal geometry that is intermediate between that preferred by Cu(I) complexes (tetrahedral) and that preferred by Cu(II)complexes (tetragonal). Thus, the change in geometry with redox is limited, and the energy required for this structural rearrangement is small [2].These observations

*Corresponding author: m_monajjemi@yahoo.com

led to the induced rack theory [3] and the entatic state theory[4], based on the hypotheses that the protein matrix forces the Cu(II) site into a catalytically poised geometry similar to the Cu(I) geometry.These thearies, which trace the peculiar properties of the cupredoxins and their electron transfer capability back to the strained geametry of their active site, have been challenged recently[5,6], and the issue has been addressed through different theoretical methnds.

Fig.1.Overview of the second structure of azurin. The active site consists of the copper ion and five ligand residues Gly45,His46, Cys112, His117, and Met121[1].

Ryde et al.[5,6] perfarmed quantum-mechanical(QM) aptimizatinn in vacuo nf the geometry nf the nxidized and reduced Cu sites, using the B3LYP bybrid density functional (DFT) method[6,7] .Surprisingly, the results nf the calculations nn the copper site were in clear enntrast with the rack and entatic state hypotheses. The in vacuo optimized geometry nf the isolated Cu(II) site was highly similar tn the experimental genmetry of the site in the protein structure. This suggested that the geometry of the Cu (II) site in cupredoxins is not strained by the prntein matrix [8, 9].

Swart investigated the electranic structure of the active site of azurin by density functional theory (DFT) and carried out MD simulation using the results.

Sugiyama et al.[1] have also carried out MD simulatian of oxidized and reduced azurins and have analyzed their backbone dynamics

Nuclear magnetic resonance (NMR) spectroscopy is a valuable technique for obtaining chemical information. This is because the spectra are very sensitive to changes in the molecular structure. This name sensitivity makes NMR a difficult case for molecular modeling [10-12].

NMR spectroscopy is a powerful tool for study the structure dynamics and interaction of biological molecule such as protein and nucleic acids in solution [13-16].

As we know the effect of solvent molecules on proteins plays an important role in the chemical behavior of them and the effects span a considerable range and are governed primarily by solvent polarity. So in our current research, we have theoretically studied the effects of DMSO ,nitroinethane , methanal ,ethanol, acetone, dicholoroethane , water and gas phase on the chemical shielding paramcters nf ^{13}C, ^{15}N , ^{17}O ,^{64}Cu, ^{32}S nuclei involving in active site and its structural stability. The Gauge Including Atomic Orbitals (GIAO) and Continuous Set of Gauge Transfnrmations (CSGT) approaches within the SCF-Hartree-Fock and B3LYP apprnximatian have been used in nrder tn investigate the influence different solvent media on the magnetic shielding tensors through Hartree-Fock and B3LYP approximation using 6-31G basis set.This basis set seems prnper, because the CPU-consuming calculations using the HF method generally inhibit its applicatinn for NMR calculations of binlngical macromolecules with large basis sets.

COMPUTATIONAL DETAILS

In the present wnrk, extensive quantum extensive quantum mechanical calculation of electronic structure nf the active site nf oxidized azurin (Fig.1) and solvent effects nn ^{13}C, ^{15}N , ^{17}O ,^{64}Cu, ^{32}S -NMR parameters have been performed in different solvent media and in two available methods using GAUSSIAN 98 program.

We have studied the influence nf OMSO, nitromethane, methanol, ethanol, acetone, dichnlnrnethane, water and gas phase on

chemical shielding tensors.There are different methods of salvation. One family of models for systems in solution is referenced to as Self-Consistent Reaction Field (SCRF) method. The simplest SCRF model is the onsager reaction field model. For the simulatioo of a polar environment, this model was used as implemented in GAUSSIAN 98.In this model the solvent is consider as a uniform dielectric with a given dielectric constant.

At first, we have modeled the active site of azurin with chem. Offiee package and then optimized at the Hartree-Foek and B3LYP levels of theory with 6-31G basis set.

In view of the fact that the results of our earlier works [17-18] proved that addition of a greater number of polarization functions and extension of the basis set did not lead to a significant improvement of the results, the choice of the middle basis set 6-31G was in our opinion justified.

After fully nptimization 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) and have been reported in table 1(a,b,c). For more investigatinn of solvent effect, the graphs of obtained NMR parameters versus dielectric constant have been evaluated.

RESULTS AND DISCUSSION

In figs. 2-4, chemical shift anisotropy asymmetry (η), isotropy (σ iso). anisotropy (σ anisu), and Δσ and chemical shift tensor (δ) are observed for 13C, 15N, 17O,64Cu. 32S nuclei in active site of oxidized azurin with respect to dielectric constants.

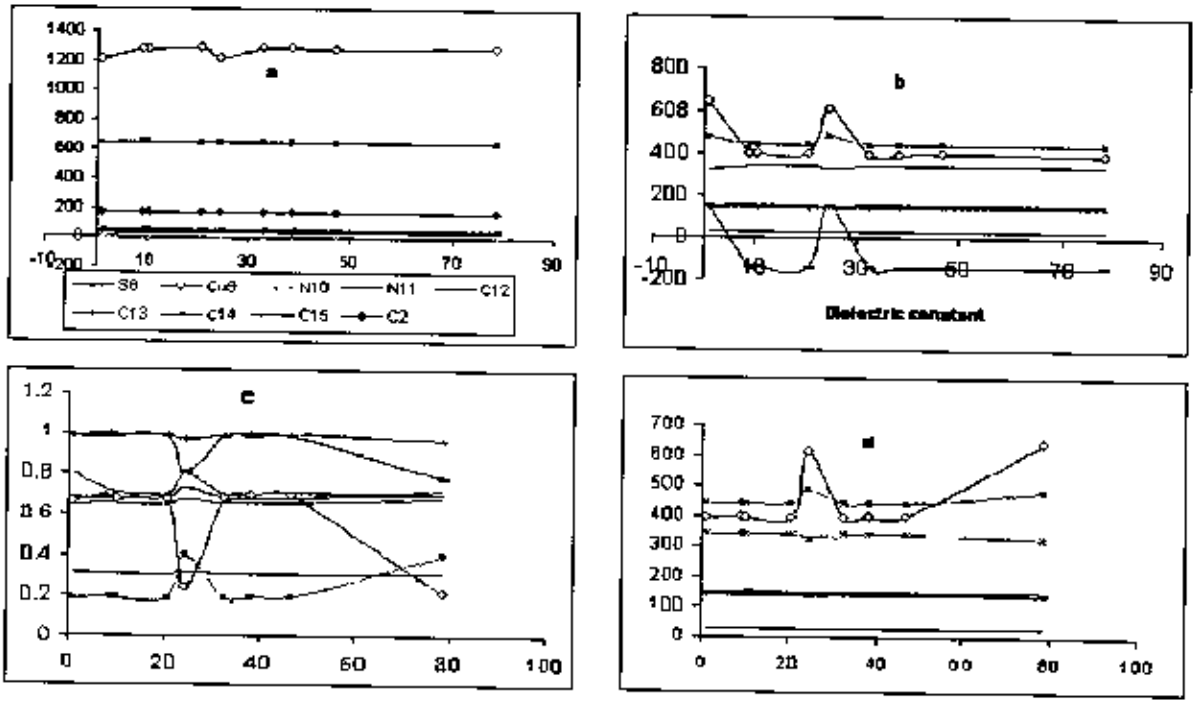

Fig. 2. The graphs nf **a)** isntropic shielding values (σiso), **b)** indirect shielding (Δσ), **c)**asymmetry parameters(η), **d)** anisotropic shielding value(σaniso), of prapase atoms nf active site azurin in different solvent media at the level uf HF/6-31G theory in CSGT methnd.

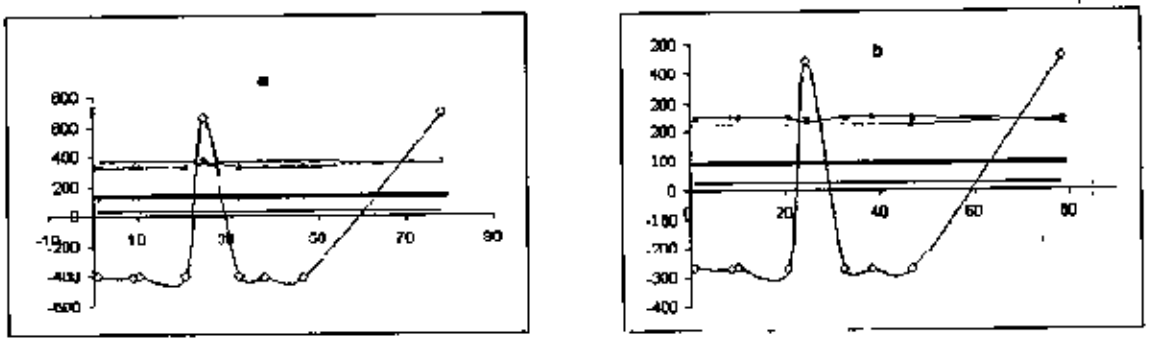

Fig. 3. The graphs of a) indirect shielding (dσ), b) chemical shift tensor(δ), of propose atoms of active site azurin in different solvent media at the level of HF/6-31G theory in GIAO method

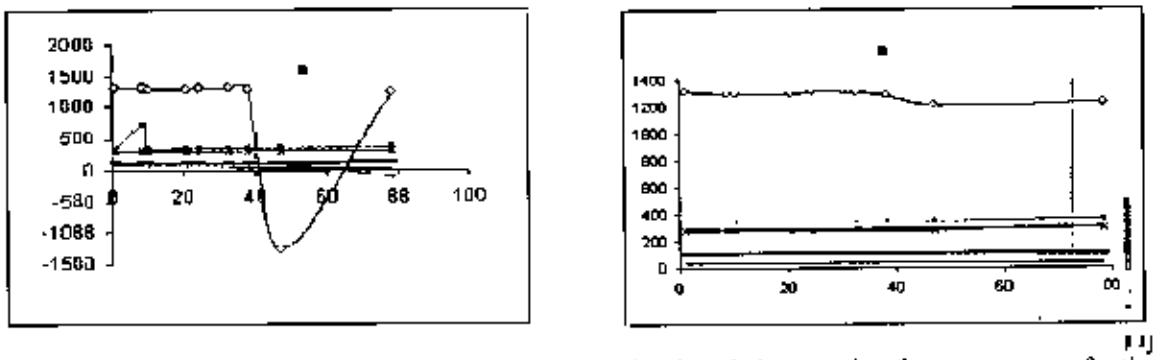

Fig. 4. The graphs of a) isotropic shielding values (σiso), b)chemical shift tensor(δ), of propose atoms of active site azurin in different solvent media at the level of B3LYP/6-31G theory in CSGT method.

In the basis of the above diagrams, table 1(a,b,c) list values of the σ iso, σ aniso, Δσ, δ, η values for ^{13}C, ^{15}N , ^{17}O , ^{64}Cu, ^{32}S nuclei in different dielectric constants

As expected, the NMR shielding tensors of ^{13}C, ^{15}N , ^{17}O ,^{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 , ^{17}O ,^{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.

On the basis of both geometrical positions of ^{13}C, ^{15}N , ^{17}O ,^{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 σiso component showed the largest intermolecular effects and it shows positive shielding values ,i.e, the electron transfer interaction produced a deshielding in this position.

Comparison of σiso, σaniso, Δσ and δ values of Cu_9 atom with another shielding values in table 1 and also analysis of graphs of σiso, σaniso, Δσ and δ 's versus dielectric constant exhibited in figs 2-4 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 have been observed for asymmetry parameter (η). 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 I 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[19].

Table 1.NMR parameters of C ,N,O, Cu, S nuclei inuolving in active site azurin in different solvent media at the levels of RHF/6-31G and B3LYP/6-31G theory in GIAO and CSGT methods(a),(b),(c)
(n)

Solvent	Method	Atoms	GIAO					CSGT				
			σiso	σaniso	Δσ	η	ô	σiso	σaniso	Δσ	η	ô
78.39	HF	C2	187.18	34 14	34.14	0.5276	22,763	173 85	28.197	28 197	8.6812	18.748
			169 76	37.91	37 91	0 5377	25 277	157 68	32.883	32.883	0 627	21.869
		S6	740.4	332 09	332 09	0 3817	221 397	651 4	442 155	442 16	0 185	294.77
			566.54	344.81	344.81	0 68	229 876	498,75	432 355	432.36	8.5289	288 237
		Cu9	1758 5	260 43	-405 19	0 285	-270 13	1292 3	394,373	394,38	0 666	262.915
			168 64	1312.34	1312 34	0 9158	874 895	413 49	1151 17	-1058 5	0.694	-905.666
		N10	8.25	361.85	361.84	0 351	241 229	14 294	332.85	332 85	0 29	221.9
			21.45	275.11	275.11	0.1024	183 305	11 892	256 303	256,3	0.1327	170.868
		N11	8.23	369 04	369 83	0 3577	246 022	13 62	341,554	341.55	0 3059	227 702
			24 56	298 67	298 67	8 184	199 112	11 988	275 877	275 88	0,1984	183,918
		C12	55.32	146.41	146.41	0 6943	97 603	38 938	154 349	154 35	0.6529	102.899
			47.46	122.03	122.03	0 557	81 35	33 438	129 223	129.22	0 472	86 149
		C13	62.89	127.57	127 56	8.9525	85 043	34 052	140.947	140 95	0 9967	93.965
			65 7	90 95	90 95	0 9493	60 635	39 791	103,257	-103 3	0 9992	-68 865
		C14	61,83	138,47	138.47	0.7404	92 315	35 035	148.58	148 58	0.7003	99,054
			59.19	189.55	109.55	0 6586	73 035	43 766	119 154	119.15	0.5946	79.436
		C15	62.68	128 79	129	0,9713	85 86	34 133	141 648	-142 58	0 9864	-95 055
			58 01	182 07	182 07	0 8136	68 047	33 508	111.963	111.96	0 8837	74,642
46.8	HF	C2	187 23	34 17	34.17	0.5228	22 778	173 88	28 222	28,224	0.6815	18 815
			169.85	37.81	37 81	0 5315	25 207	157 75	32.721	32.721	0 6207	21.814
		S6	739,9	332.83	332 83	0.382	221 88	650 75	443.025	443 83	0.1899	295 35
			565.8	341.47	716.01	0 678	227 647	497 87	428 762	428 76	0.5285	285.84
		Cu9	1744 4	262.51	-413 12	0 27	-275 41	1282 9	400 404	400 4	0 6963	266.936
			169.81	1288.84	1298.84	0 925	865 89	-411 19	1139 48	-1351	0 6868	-900 67
		N18	8 59	361.62	361 62	0.3496	241,079	-13 806	332.385	332,38	0 29	221.59
			21.75	276.03	276 03	0 1022	184 019	12.081	257 174	257 17	0.1275	171.45
		N11	8 83	368.08	368 89	8.3539	245 388	-13.03	340 546	340.55	0 3019	227.03
			24 5	299.14	299 14	0.1865	199 42	14.864	275.946	275.95	0 1991	183.963
		C12	55 41	146.29	146 29	0.6991	97.527	39 024	154 166	154,17	8.658	102 778
			47 4	122.19	122 19	0 556	81 46	33.346	129 318	129 32	0 47	86 212
		C13	62.92	127.33	127 33	8.9548	84 885	34 124	140 641	140.64	0.9983	93 761
			65 51	91.01	91 01	8 9506	60.676	39 603	103.264	-103 41	0 9972	-68 938
		C14	62 05	138.21	138 21	0 7535	92.142	45 196	148 341	148 34	0 7044	98 894
			58.24	188.48	109.48	8.66	72 985	43 793	119.883	119 98	0.5955	79.389
		C15	62.73	128 77	128 77	0.9695	85 848	34.203	141.523	-142 27	8.9895	-94.846
			58.02	102.18	102 18	0 8124	68 118	33 51	111.959	111 96	0 8835	74 639
38.215		C2	187 19	34 15	34.15	0 5277	22.764	173.86	28.2	28 2	0 6813	18 8
			169.77	37.89	37.9	0.5334	25 263	157.67	32.796	32 796	0 6229	21.864
		S6	740.31	332.18	332 18	0.38188	221.451	651.3	442.237	442 24	0 186	294.825
			585.82	343.04	343.04	0 677	228.695	498 12	438 164	430 16	0 528	286.776
		Cu9	1749 8	260 97	-406.52	0 2838	-271 02	1291	395.214	395.22	0 672	263 476
			172.16	1294.77	1294.77	0.9235	863 18	-410 35	1137.09	-1346	0.689	-897.343
		N10	8.29	361.83	361 83	0 3509	241 22	-14 247	332.787	332.79	0 291	221.858

Table 1. Continue.

(b)

			21 75	276.17	276 17	8.1025	184.115	12 05	257 34	257 34	0.126	171 56
	HF	N11	8.35	368 88	368.88	0 3569	245 92	-13 509	341.39	341.39	0.3051	227.594
	B3LYP		24.24	298.99	298.99	0 1891	199.33	14.704	276 192	276 19	0.1998	184 129
		C12	55.34	146 39	146 39	0 695	97 592	38.952	154.319	154 32	0 653	102.879
			47 36	122.23	122.23	0.5566	81.487	33.341	129.357	129 36	0.4709	86.238
		C13	62 9	127.52	127 52	0 953	85.017	34.067	140.896	140 9	8 997	93.93
			65.44	91.03	91.03	0 9486	60 684	39.55	103 287	-103 35	0 9987	-68 902
		C14	61 87	138.43	138.43	0.7581	92 285	45.858	148.541	148 54	8 7009	99 027
			58 26	109.4	109 48	0.6611	72.987	43.822	119.077	119 08	0.5961	79.385
		C15	62 7	128 78	128 78	0 971	85.856	34.147	141.623	-142 53	0.9873	-95.818
			57.96	102.19	102.23	0 8125	68 128	33 457	112 054	112.05	0 8825	74.703
31.63	HF	C2	187.2	34.15	34.15	8 5279	22.765	173 86	28.201	28.281	8.6616	18.881
	B3LYP		169.74	37 9	37.9	0.5349	25.268	157 66	32 8	32.8	0 6251	21.867
		S6	740.29	332 25	332.25	0 381	221.5	651 28	442.317	442.32	0 186	294 878
			585.78	343.28	343.28	0 673	228.852	498 23	430.587	430.59	0.5249	287 058
		Cu9	1749.6	261.05	-406 89	0.283	-271.26	1290 6	395.729	395.73	8.672	263 819
			174.64	1294.14	1294 14	0.9193	862.76	-407 93	1139	-1341.8	8.697	-894 557
		N10	8.33	381.82	361.82	0.3507	241.215	-14 212	332.769	332 77	8 2913	221.846
			21 61	276.18	276 19	8.1827	184 13	12.112	257.297	257 3	0.1261	171 53
		N11	8 36	368.85	368 85	0 3568	245 902	-13 498	341 353	341.35	0 305	227.569
			24 33	299.58	299 58	0 1889	199 72	14.652	276.22	276.22	0 1982	184 147
		C12	55.35	148 36	146.38	0 6952	97 586	38.959	154 306	154 3	0 6539	102.87
			47.33	122 24	122.25	0.556	81.495	33.328	129 35	129 35	8 4702	86.233
		C13	82.9	127 51	127.51	0 953	85.009	34 069	140 881	140 88	0.9971	93.921
			65.42	81.1	91.1	0 947	60 736	39 546	103.339	103.34	0.9999	68 893
		C14	61 88	138.42	138 41	0.7503	92 28	45 064	148 534	148.53	0.7011	99 023
			59.24	189 43	109.43	0.6632	72 954	43 8	119 028	119 03	0 598	79 352
		C15	62.7	128.78	128.78	0.9711	85 856	34.154	141.616	-142.52	0 9873	-95 01
			57.95	102.32	182.32	0.807	68 211	33.421	112.071	112.07	0.8787	74.714
24.55	HF	C2	184 25	34.5	34.5	0.5652	22.998	171.17	28.435	28.434	0.7339	18.956
	B3LYP		169 7	87.98	37 99	0 539	25.323	157.61	32.865	32.865	0.6277	21.91
		S6	729.6	371.08	371.88	0 502	247.38	645 52	487.887	487.08	8 398	324 725
			566.11	344.66	344.66	6 68	229.77	498.61	431.968	431.97	8.5382	287 979
		Cu9	1857.8	655.76	655.76	0.7365	437 172	12208	612 212	612 21	0 2398	408.142
			178.81	1311.36	1311 36	0.918	874.239	-412.36	1151 19	-1358 5	0.6948	-905 634
		N10	21.85	347.64	347 64	0 279	231 76	-2 035	320 209	320 21	8 248	213.47
			21 69	276.86	276 06	0 1019	184 639	12.027	257 183	257.18	0 1274	171.455
		N11	17.25	358.88	358 88	0.353	233.923	-5 534	325.345	325 35	8.3094	216 897
			24.41	286 63	298 63	0 1864	199 838	14 881	275 98	275 98	8.1978	184.867
		C12	55 49	144 84	144 84	8.7209	96 561	38.859	153.236	153 23	0.6717	102.157
			47.4	122 14	122 14	0 5566	81.427	33 368	129 284	129.28	0 4712	86.189
		C13	61 87	125.85	125 85	0.9182	83 898	32.922	139.49	139 49	6 9732	92.903
			65.53	91.81	91.01	0.9495	60 671	39.648	103 259	-103 33	0 9987	-68.883
		C14	66.96	131.58	131.58	0 8608	87 718	49.221	143 128	143.13	0 8003	95.419
			59 29	109.51	109 51	0.6608	73 008	43.844	119.107	119.11	0.5959	79.405
		C15	55.34	138 75	138.75	0 7238	92.502	28 178	149 955	149.96	0 3082	99.97
			57 98	182.21	162 21	0.8117	68 141	33.473	112 076	112.08	6 8812	74.717
10.7		C2	187.21	34.15	34.15	0.5279	22 768	173.87	28 206	28 206	0 6817	18 804
			169.78	37 91	37.91	0.538	25.275	157.68	32.809	32.809	0 6272	21.873
		S6	748.2	332 37	332.37	0 38169	221.583	651 16	442.472	442 47	0 1869	294 98
			567 34	345 75	345 76	0 677	230.5	499 53	413.166	433.17	0.527	288 777
		Cu9	1748 3	261.53	-408 53	0 2803	-272 36	1288 7	396.964	396.97	0.679	264.643
			170 32	1386.63	1306.83	0 92	871 22	-412 51	1145.55	-1355 8	8.689	-903.88

Table 1. Continue..

(c)

	HF B3LYP	N10	8.39	361.79	361.79	0.3504	241.195	-14.129	332.686	332.68	0.291	221.791
			21.48	274.81	274.91	0.102	183.273	11.912	256.083	256.08	0.1326	170.722
		N11	8.48	368.68	368.67	0.356	245.784	-13.38	341.163	341.16	0.3041	227.442
			24.49	298.75	298.75	0.1855	199.165	14.957	276.058	276.06	0.1994	184.039
		C12	55.38	146.36	146.36	0.6961	97.57	38.975	154.27	154.27	0.6549	102.847
			47.44	121.97	121.97	0.558	81.312	33.422	129.182	129.18	0.473	86.121
		C13	62.91	127.47	127.47	0.9535	84.977	34.084	140.819	140.82	0.9974	93.88
			65.78	90.98	90.98	0.9482	60.657	30.836	103.276	103.28	0.9999	68.851
		C14	61.92	138.37	138.37	0.7511	92.244	45.095	148.485	148.49	8.7819	98.99
			59.25	109.58	109.58	8.6582	73.057	43.818	119.172	119.17	0.594	79.448
		C15	62.71	128.78	128.78	0.9708	85.851	34.07	141.588	-142.45	0.9878	-94.967
			57.88	182.1	102.08	0.8132	68.066	33.493	112.003	112	0.8829	74.669
10.56	HF B3LYP	C2	187.22	34.18	34.16	0.5279	22.776	173.87	28.218	28.218	0.6815	18.812
			168.73	37.95	37.95	0.5436	25.3	157.63	32.83	32.83	0.6326	21.887
		S6	738.94	332.74	332.73	0.3819	221.82	650.81	442.928	442.93	0.189	295.285
			568.08	350.16	358.16	0.675	233.44	500.12	436.46	436.46	0.528	290.97
		Cu9	1745.2	262.11	-412.26	0.2715	-274.84	1284.2	399.489	399.49	0.6933	266.326
			166.86	1285.58	1285.57	0.9658	857.85	-417.61	1123.84	-1362.1	8.65	-908.081
		N10	8.57	361.65	361.65	0.3496	241.1	-13.923	332.448	332.45	0.2902	221.632
			21.67	275.69	275.69	8.1013	183.796	11.973	256.915	256.91	0.1277	171.277
		N11	8.75	368.22	368.22	0.3543	245.479	-13.114	340.681	340.68	0.3023	227.12
			24.54	298.83	298.83	8.1818	199.219	14.973	275.93	275.93	0.1969	183.953
		C12	55.4	146.31	146.31	8.6984	97.538	39.011	154.191	154.19	8.6573	102.794
			47.39	122.15	122.15	0.556	81.433	33.372	129.31	129.31	0.4703	86.207
		C13	62.92	127.35	127.35	0.9546	84.901	34.015	140.677	140.68	0.9981	93.785
			65.48	91.09	91.09	0.9476	60.73	30.587	103.343	-103.38	0.9993	-68.917
		C14	82.81	138.25	138.25	0.7529	92.166	45.17	148.375	148.38	0.7038	98.917
			59.15	109.37	109.37	0.659	72.91	43.75	118.987	118.99	0.5943	79.321
		C15	82.74	128.77	128.77	8.9699	85.843	34.201	141.529	-142.31	8.989	-94.871
			58.21	101.75	101.71	8.8177	67.832	33.694	111.646	111.65	0.8867	74.431
1	HF B3LYP	C2	184.26	34.18	34.18	0.57	22.785	171.18	28.335	28.339	0.7226	18.89
			178.64	38.12	38.12	0.599	25.413	158.12	33.3	33.3	8.6798	22.2
		S6	728.54	364.7	364.7	0.4999	243.136	644.67	480.694	480.69	0.3965	328.462
			885.61	385.21	365.21	8.473	243.471	540.91	456.891	456.89	0.38	304.594
		Cu8	1649.7	878.57	678.57	0.70187	452.38	1214.1	644.831	644.85	0.2139	429.887
			485.5	1231.35	1231.35	0.287	820.9	-87.266	1152.54	1152.5	0.62	768.356
		N18	20.94	348.04	348.04	0.2799	232.03	-2.06	320.544	320.54	0.248	213.696
			31.67	293.44	293.44	0.179	195.629	16.801	270.447	270.44	8.0537	180.298
		N11	18.33	349.48	349.4	8.3499	232.936	-4.61	323.97	323.97	8.3114	215.98
			25.25	305.08	305.09	0.262	203.393	11.499	281.465	281.47	0.194	187.644
		C12	55.25	144.9	144.9	0.7266	96.599	38.656	153.34	153.34	0.6756	102.226
			58.82	123.87	123.87	0.544	82.578	35.509	130.295	130.29	0.467	86.863
		C13	61.87	126.17	126.17	0.9072	84.111	32.976	139.737	139.74	0.9653	93.158
			69.13	87.58	-90.24	0.941	-60.159	41.887	98.925	-105.14	0.8817	-70.095
		C14	67.21	131.65	131.65	0.8605	87.765	49.464	143.161	143.16	0.8009	95.441
			63.99	110.19	110.19	0.687	73.458	47.499	119.074	119.07	0.6224	79.383
		C15	54.25	148.15	140.15	0.6902	93.433	27.209	151.195	151.2	0.7794	100.797
			60.68	186.38	108.38	0.6803	72.254	34.516	117.446	117.45	8.784	78.298

The metal ion in the electron-transfer copper proteins such as the type I copper proteins has beea 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 impartant role in imparting extra stability ta the metal binding site af the protein[19].

As expected, after Cu_9. S_6 shows positive shielding values. Cys 112 is among the ligand residues the one that more strongly bybridizes with the Cu orbitals . The covalency of the capper-ligand bonds is very anisotropic and it was suggested that this should favur hole super exchange pathways that couple to the Cu through the Cys112 ligand[20].The cysteine ligand decreases the reorganizatian energy. This deercase is caused by the transfer of charge from the negative charged thiolate group to CuII, which makes the oxidized and reduced structures quite similar[21].

For both N_{10} and N_{11} atoms which are fused in imidazole ring, shielding tensors are close to each other but according to our obtained results of fig. 2, as the dielectric constant in passing from the nitromethane to water. the σ_{aniso} and $\Delta\sigma$ af N_{11} and N_{10} inerease and the η deerease 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 methnd.

Nitrogen ligands give up an appreciably lnwer reorganization energy than water, owing to the lower Cu-N fnrce cnnstant[21].

Figure 4 shows that, as the dielectric constant nf the solvent inereases , the $\Delta\sigma$ and σ_{aniso} of C_{15} and C_{13} inerease and the η nf C_{15} and C_{13} decreased in the ethanol at the Hartree-Fock level of theory with GIAO method.

Also, calculatinns at the HF in CSGT method (Fig. 2) and the HF in GIAO method (Fig. 3) have shown that molecular geometry and shielding properties are better than the other methods, B3LYP in GIAO and CSGT metheds(fig. 4).

CONCLUSION

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 malecules theoretically. Several coaclusions can be made on the basis of the observed results af the present study. Such amount af theoretical data can provide us important insights into the nature of malecular structures in bialogical systems.

Our main findings from the point of view of solvent effects can be summarized as follows:

optimization at the HF level of theory provides a suitahle computatinnal model in terms nf calculated NMR parameters and relative energies.

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 σ_{iso} value of mentianed nuclei af active site azurin nbserved for ethanol and water, whereas the smallest one belongs to DMSO. It is interesting to note that the oppasite trend have been observed for asymmetry parameters(η). This usual behavior may be readily understood in aecord with hiotechnnlogical conceptions.

The calculatinn of nuclear magnetic resonance (NMR) parameters using ab initin techniques seems to be a major and a remarkable tool for investigatinn nf how variations of biolngical systems and provides information on the local environment of selected speeies and their next nearest neighbors. Hnwever, combination of NMR study embedded in snlvent medum reveals a logical interpretatinn of the observed results.

In conclusion, we have shnwn that theoretical calculatinns can be used to successfully solve biochemical problems. In similarly with experimental methods, they involve assumptinns and interpretatinn, 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 investigatinns.

REFERENCES

[1] A. Sugiyama, K. Sugimori, T. shuku, T. Nakamura, H. Saito, H. Nagao, H. Kawabe, K. Nishikawa. Int J Quantum Chem.105, 2005 , 588-595.

[2] F. Rienzo.R. Gabdoulline,R. Wade,M. Sola, M. Menziani. CMLS. 61. 2004 . 1123-1142.

[3] B.G. Malmstrom.Eur J Biochemistry 223. 1994. 711-718.

[4] R. J. P. Williams.Eur J Biochem.234. 1995. 363-381.

[5] U Ryde, M H Olsson,K Pierloot.Theoretical and Computational Chem. 9. 2001.1-55.

[6] U Ryde, M H Olsson, B O Roos, A C Borin.Theor Chem Acc.105. 2001.452-462.

[7] M J Frisch, G W Trucks, AB Schlegel , P M Grill, B G Johnson, M A Robb GAUSSIAN 94, Gaussian1994, Pittsburgh , PA.

[8] M. H. M. Olsson , Theoretical studies of blue copper proteins. Ph.D thesis,2000.

[9] U Ryde, M H Olsson, B O Roos, O A Kerpel, K Pierloot.J Biol Inorg Chem. 5. 2000.565-574.

[10] M Muhl.Encycl Comput Chem.3. 1998. 1835.

[11] C J Jameson, H J Osten.Annual Reports on NMR spectroscopy. 17. 1986. 1.

[12] C J Jameson. Annual Reports on NMR spectroscopy.21.1989.51.

[13] B Furtin. C Richter. J H Schwalbe. Chem Biochem.2003.10-36.

[14] S E Bucher. J M Burke. Biochemistry. 33. 1994. 992-999.

[15] T J Warren. P B Moore. J Biomol NMR. 20. 2001.311-323.

[16] T J Warren. P B Moore. J Biomol NMR. 20. 2001.311-323.

[17] S A Mc Callum. A J Pardi . J Mol Biol..325. 2003.843-856.

[18] M Monajjemi.S Ketabi. A Amiri. Russ J P hys Chem. 80. 2006.55-62.

[19] M.Monajjemi, A. Hadadi, , B. Honarparvar, s. Irani, F. Mollaamin, A. TahanThe Egyptian J. of Biochemistry& Molecular Biology.26. 2008. 83-100.

[20] A. Sujak, N. J. M. Sanghamitra, O. Maneg, B. Ludwig, S. Mazumdar. Biophys. J. 93. 2007.2845-2851.

[21] P. V. R, Schleyer, Eacyclopedia of Computational Chemistry (Wiley, Chichester, 1998), Vol. 4. pp. 2547--2574.

[22] U. Ryde, M. Olsson, K. Pierloot.Eriksson L. A, editor, Elsevier, Amsterdam. Theoretical and Computational Chemistry.9 . 2001. 1-5.