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PERSPECTIVES

Investigation of solvent effect and NMR shielding tensors of p53 tumor-suppressor gene in drug design

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Abstract: The p53 tumor-suppressor gene encodes a nuclear phosphoprotein with cancer-inhibiting properties. The most probable cancerous mutations occur as point mutations in exons 5 up to 8 of p53, as a base pair substitution that encompasses CUA and GAT sequences. As DNA drug design represents a direct genetic treatment of cancer, in the research reported computational drug design was carried out to explore, at the Hartree–Fock level, effects of solvents on the thermochemical properties and nuclear magnetic resonance (NMR) shielding tensors of some atoms of CUA involved in the hydrogen-bonding network. The observed NMR shielding variations of the solutes caused by solvent change seemed significant and were attributed to solvent polarity, and solute–solvent and solvent–solute hydrogen-bonding interactions. The results provide a reliable insight into the nature of mutation processes. However, to improve our knowledge of the hydration pattern more rigorous computations of the hydrated complexes are needed.

Keywords: p53, CUA, mutation, ab initio method, NMR shielding

Introduction

The p53 tumor-suppressor gene encodes a nuclear phosphoprotein with cancer-inhibiting properties. However, the development of human cancer often involves inactivation of this suppressor function through various mechanisms including gene deletions and point mutation. The most probable cancerous mutations occur as point mutations in exons 5 up to 8 of p53, as a base pair substitution that encompasses CUA and GAT sequences. Including uracil and adenine, the positions where the mutations occur are called the 'hot spots' of mutations.[1,2] The hydrogen-bonded complexes generated by solute are the main reason for these changes. Hydrogen bonds play a key role in maintaining the structure and specificity of biological systems.[3–6] Further studies have focused on the acidity and basicity of uracil. The proton affinities and the deprotonation enthalpies of nucleobases have been also studied, in particular their relationship with the interaction with one water molecule.[7–10] The important point of the study reported here is that experimental investigation of nucleic acid base pairing is difficult. However, gas phase association energies have been reported for some systems and in nonpolar solvents.[12,13] Due to the limited experimental NMR data, the extent to which a simple dielectric medium model affects the dominating solute–solvent interactions of CUA sequence in different solvents remains unknown.[14,15] This lack of experimental NMR data motivated us to calculate NMR shielding tensors of nitrogen, oxygen, and phosphorous atoms involved in the hydrogen-bonding network of a CUA model and

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to investigate the solvent-induced effect on these parameters. Due to the importance of hydrogen-bonding interactions in biological systems, the main theoretical attention has been focused on NMR parameters of nitrogen, oxygen, and phosphorous nuclei involved in the hydrogen-bonding network of CUA.

In order to identify the most probable nucleobases for mutation among CUA, all energy values as well as relative energies (ΔE) of the studied systems were calculated in vacuum at the level of RHF/6–31G theory and a logical trend was obtained in different solvent media.

Computational details

In the present work, we optimized the CUA codon (Figure 1) with 3 basis sets Sto-3g, 3–21g, 6–31g in the gas phase with the Gaussian 03 package[19] by the Hartree–Fock (HF) method. The calculations including the intermolecular interactions give semiquantitative information on the effects of hydrogen bonding on the principal values of chemical shift tensors. We studied the influence of acetone, dimethyl sulfoxide (DMSO), ethanol, methanol, and water on chemical shielding tensors. There are different methods of salvation. One family of models for systems in solution is referred to as the self-consistent reaction field (SCRF) method. The simplest SCRF model is the Onsager reaction field model. For the simulation of a polar environment this model was used as implemented in Gaussian 03. In general, the following quantities are often used to describe NMR shielding tensors, namely, the isotropic, anisotropic shielding, and the asymmetry parameters:

a) The isotropic value σ_{iso} of the shielding tensor which can be defined as:[16,18]

$$\sigma_{iso} = \frac{1}{3}(\sigma_{11} + \sigma_{22} + \sigma_{33}) \quad (1)$$

b) The anisotropy parameter ($\Delta\sigma$) defined as:

$$\Delta\sigma = \sigma_{33} - \frac{1}{2}(\sigma_{11} + \sigma_{22}) \quad (2)$$

and

c) The asymmetry parameter (η) which is given by:[18]

$$\eta = \frac{|\sigma_{22} - \sigma_{11}|}{|\sigma_{33} - \sigma_{iso}|} \quad (3)$$

The polarized continuum model is the most frequently used method employed to study solvent effects. However, the capability of the method for describing the effect of the formation of hydrogen bonds between the solvent and the solute is always controversial.[19]

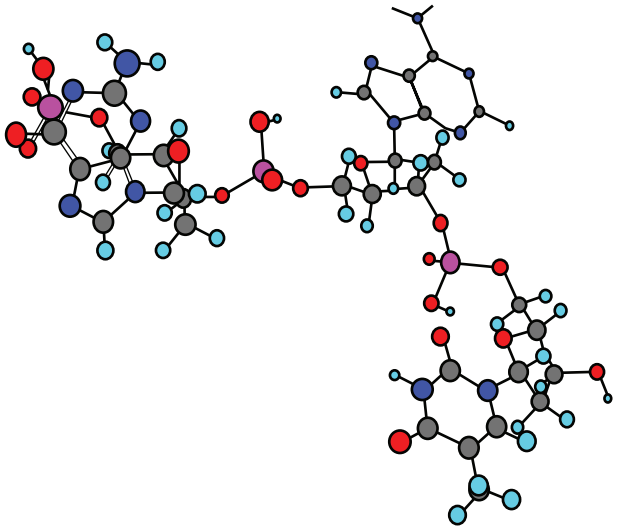

Figure 1 Molecular structure of CUA.

Results

The treatment of large biological systems in aqueous solution using ab initio methods is extremely expensive. However, analysis of NMR parameters is essential for understanding the role they play in biological processes. The calculated NMR shielding tensors of nitrogen, oxygen, and phosphorous atoms of CUA are listed in Table 1. At this stage, the interaction with water was found to be a fundamental tool for deriving further information about these systems. In this study, we determined the existing interactions by means of theoretical calculations of energy values as well as several thermochemical parameters.

The theoretical values of σ_{iso}, $\Delta\sigma$, and η of oxygen, nitrogen, and phosphorous atoms of CUA in different solvents are shown in Table 1. On the basis of the obtained results, it can be understood that NMR shielding values of the CUA model often yielded maximum dielectric constant values of 78.39, 32.65, and 46.8. So, it can be concluded that hydrogen bonding is the most important reason for this behavior that causes deshielding. For nitrogen atoms in the CUA structure, the highest isotropic shielding values have been obtained in water and ethanol as protic solvents whereas the lowest values have been obtained in DMSO as a protic solvent. However, for both N_{25} and N_6 atoms, the differences in these values are insignificant. More interestingly, in the case of N_{25} atoms involved in uracil, the differences between maximum and minimum values of asymmetry

Table 1 Nuclear magnetic resonance parameters of nitrogen, oxygen, and phosphorus atoms involved in hydrogen-bonding network of CUA codon in different solvent media at the level of RHF/6–31G theory

ε	σ_{iso} (ppm)	Δσ (ppm)	η	ε	σ_{iso} (ppm)	Δσ (ppm)	η
O_7				**N_{25}**			
Gas phase	95.2372	382.5681	0.096	Gas phase	146.4689	64.0938	2.2914
Acetone	95.1978	382.9628	0.096	Acetone	146.4689	64.1044	2.2916
Ethanol	95.5318	382.5528	0.0917	Ethanol	146.4596	64.072	2.2924
Methanol	94.6308	381.5632	0.1025	Methanol	146.4689	64.212	2.2894
DMSO	94.6309	381.1584	0.9527	DMSO	146.4389	64.1338	2.292
Water	94.5627	382.8416	0.0945	Water	146.4988	64.2024	2.2869
O_{27}				**N_{42}**			
Gas phase	–43.0922	624.5832	0.3759	Gas phase	44.5425	366.4983	0.4001
Acetone	–43.0358	624.5032	1.4153	Acetone	44.5327	366.5345	0.4002
Ethanol	–43.0222	624.496	0.3759	Ethanol	44.5605	366.6549	0.3999
Methanol	–43.022	624.5865	0.3753	Methanol	44.5082	366.4463	0.4003
DMSO	–43.0091	624.5032	0.376	DMSO	44.4513	366.3341	0.4007
Water	–43.0842	624.724	0.3759	Water	44.6374	366.6083	0.4003
N_4				**N_{51}**			
Gas phase	77.9384	271.2676	0.5245	Gas phase	210.4776	61.7195	1.744
Acetone	77.9529	271.2605	1.1627	Acetone	210.5002	61.735	1.7427
Ethanol	77.8721	271.1569	0.525	Ethanol	210.4967	61.7738	1.7404
Methanol	77.9814	271.6405	0.5255	Methanol	210.4882	61.7793	1.7318
DMSO	77.7726	271.5487	0.5261	DMSO	210.6101	61.5098	1.7554
Water	78.119	271.2953	0.5249	Water	210.7024	61.7024	1.7432
N_6				**P_{18}**			
Gas phase	209.1206	82.9855	1.0885	Gas phase	438.8783	188.3539	0.1427
Acetone	209.1806	82.9668	1.089	Acetone	438.8768	188.326	0.1433
Ethanol	209.1346	82.9979	1.0886	Ethanol	439.1655	189.0943	0.1433
Methanol	209.1914	82.8613	1.0878	Methanol	438.842	189.2536	0.138
DMSO	209.0845	83.0695	1.0881	DMSO	439.7603	189.2342	0.146
Water	209.0927	83.0543	1.0906	Water	438.7755	186.6593	0.1559
N_8				**P_{38}**			
Gas phase	151.3728	134.6544	0.769	Gas phase	439.7697	239.0349	0.0563
Acetone	151.3603	134.6779	0.7698	Acetone	439.7765	239.0907	0
Ethanol	151.3278	134.6122	0.7701	Ethanol	439.813	239.0854	0.0607
Methanol	151.3391	134.1849	0.7698	Methanol	439.8232	239.0563	0.0543
DMSO	151.3391	134.5631	0.7717	DMSO	439.6389	238.319	0.5694
Water	151.1883	133.7583	0.7739	Water	440.0129	240.6869	0.0564
P_{61}							
Gas phase	433.032	169.7455	0.052				
Acetone	433.0188	169.7421	0.0524				
Ethanol	434.0836	176.2191	0.7884				
Methanol	160.4634	160.4634	0.409				
DMSO	151.8018	151.8018	0.366				
Water	152.5027	152.5027	0.6888				

Abbreviation: DMSO, dimethyl sulfoxide.

parameter (η) seem insignificant and had a trivial effect on this parameter. For P_{38} the maximum values of σ_{iso} were obtained in protic solvents such as water and ethanol while the minimum values were observed in DMSO. Conversely, for P_{18} involved in uracil the opposite trend was observed. For O_{17} atom of uracil, the obtained negative values of σ_{iso} may indicate that in protic solvents including water and methanol the charge density around nuclei tended to be deshielded.

According to the table of σ_{iso} versus dielectric constants of different solvents, it can be seen that in most of the ethanol nuclei considered ($\varepsilon = 24.55$) the expected trend of variation will change. Also, in the gas phase, it can be seen that the lowest value of σ_{iso} for O_7 and P_{18} corresponds to uracil. In the case of CUA sequences, the most negative value was observed for σ_{iso} for O_{27}. Moreover, the graph of δ_{iso} of all the nitrogen atoms versus dielectric constant revealed that the deshielded points were observed at $\varepsilon = 46.8$ and the

Table 2 The Hartree–Fock calculations of thermochemical parameters of CUA in different solvent media at 3 different temperatures

Solvent	Temperature (K)	CUA			
		ΔE (Kcal/mol)	ΔH (Kcal/mol)	ΔG (Kcal/mol)	ΔS (Kcal/mol)
Ethanol	300	−521251.772	−521251.1792	−521281.712	0.10241
	310	−568221.988	−568221.3953	−568254.997	0.112696
	313	−568204.944	−568204.3517	−568236.969	0.109398
Methanol	300	−568176.722	−568176.1299	−568208.983	0.110191
	310	−568222.749	−568222.157	−568254.549	0.108644
	313	−568206.857	−568206.2644	−1136477.01	0.108135
DMSO	300	−568214.593	−4315146.578	−568246.974	0.110191
	310	−568204.944	−568225.6008	−568258.816	0.111404
	313	−568212.218	−568211.6257	−568248.142	0.122478
Water	300	−567956.406	−567955.8134	−567955.698	0.10485
	310	−568209.43	−568208.8377	−568240.096	0.104843
	313	−568226.875	−568226.2822	−568258.442	0.107867

Abbreviation: DMSO, dimethyl sulfoxide.

more shielded regions were observed at ε = 78.39 and ε = 32.63.

Discussion

To the best of our knowledge, there have been numerous reports about the analysis of thermochemical parameters of isolated uracil and its hydrated model.[23–25] However, there are no experimental data on the relative energies or enthalpies of these systems.[26]

The current study focuses on the variations in thermochemical parameters due to effects of temperature in different solvents. Let us focus first on the uracil part of the CUA model, as a hot spot in mutation. Certainly, from the thermochemical parameters in solvent media, at different temperatures, we can gain further information and about the stability of uracil structure as a mutation hot spot, and then obtain useful results about solvent and temperature effects on the point mutation of CUA. All the relative thermochemical parameters were calculated. According to the thermochemical parameters reported in Table 2, the most positive entropy value of uracil was yielded in water at 313 K due to its high stability and then showed its lower tendency for mutation. Also, the most negative value of enthalpy and the most negative value of ΔG was obtained in water at 313 K. In general, based on analysis of our obtained thermochemical data, the lowest stability of uracil was observed in ethanol at 300 K.

Solvent effects on the relative structural stabilities of hot spots

According to the graph of relative energy values of CUA versus dielectric constant, a dramatic decrease was observed, and the relative energy value of CUA reached its lowest point at ε = 24.55 (Figure 2). Because polar solvents are molecules with a dipole moment that forms a hydrogen bond, the stability of the CUA system was logically found in ethanol. Meanwhile, along with the increasing trend of the dielectric

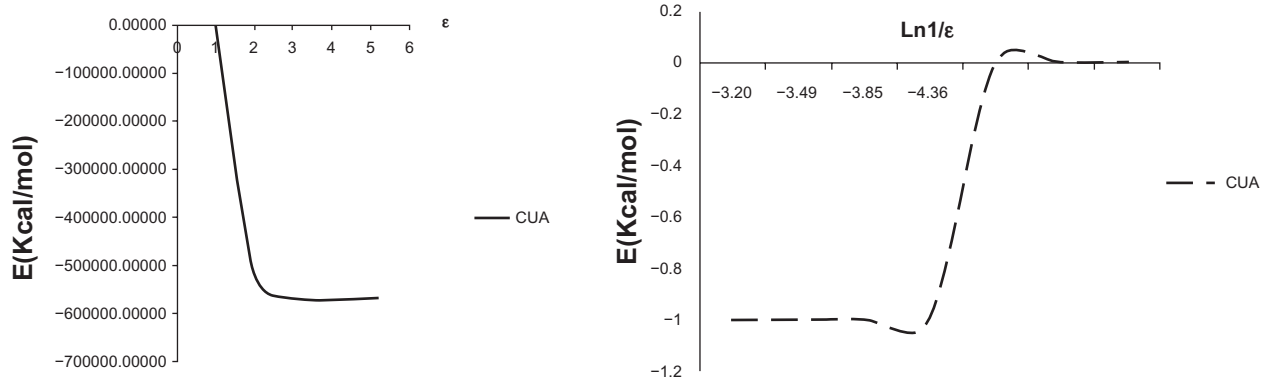

Figure 2 Relative energies ($E_{relatives}$) of CUA sequence versus ε and Ln (1/ε) in different solvent media.

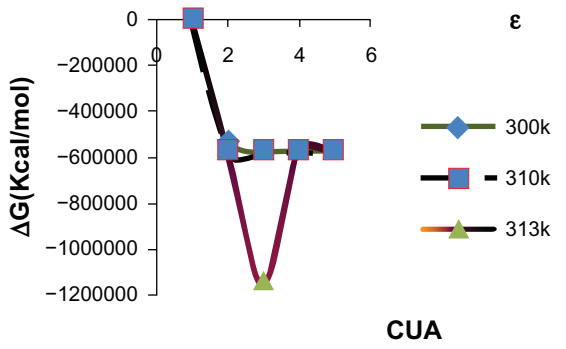

Figure 3 Temperature dependence of relative Gibbs free energies ($\Delta G_{relatives}$) of CUA sequence in different solvent media.

constant the increase of energy values has been observed after the optimal point .

Indeed, one of the key roles of a solvent is to avoid the initial rise in energy and a solvent can also stabilize biological systems.

On the other hand, Figure 2 shows a linear relationship of energy values of CUA versus ln (1/ε), which revealed the contribution of electrostatic interaction with the solvent-induced effect. However, based on the graph of energy values of CUA versus ln (1/ε) a linear relationship has been found which revealed the contribution of electrostatic interaction of the solvent-induced effect rather than the hydrophobic contribution of solvent effect.

Hydrophobic interaction is associated with the energy required to move apart solvent molecules to make space for the solute, which is greater in water and smaller in nonhydrogen-bonding systems. The thermochemical functions of CUA at three different temperatures and with five solvents are shown in Table 2. The energy graphs of CUA and also the graph of Gibbs hydration energies versus dielectric constants are shown in Figures 2 and 3, respectively.

Conclusion

The results described in this article cover extensive developments in reproducing and predicting a wide variety of theoretical physicochemical and structural parameters of a modeled CUA sequence involved in the p53 tumor-suppressor gene. These findings open the way to determine local geometries and also reveal more confidence in using ab initio methods to probe target-drug interactions as a useful application of quantum chemical technology to determine structure–stability correlations of specified sequences.

Based on the energy calculation of CUA it was observed that the relative energies (ΔE) of CUA in solution were smaller than in the gas phase, which is due to interactions in solution that were larger than in the gas phase. Moreover, the lowest ΔE value was found at the lowest dielectric constant and the maximum value was in water with a high dielectric constant and high polarity. Consequently, it can be concluded that the electrostatic and hydrophobic effects as well as dipole effects are important factors in solvation.

Disclosure

The authors disclose no conflicts of interest.

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