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...
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 phosphorus 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 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 $\sigma_{\text{iso}}$ of the shielding tensor which can be defined as: $^{16,18}$

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

b) The anisotropy parameter (Δσ) defined as:

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

and

c) The asymmetry parameter (η) which is given by: $^{18}$

$$\eta = \frac{\sigma_{22} - \sigma_{11}}{\sigma_{33} - \sigma_{\text{iso}}}$$  \hspace{1cm} (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.$^{19}$

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

According to the table of $\sigma_{iso}$ 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 $\sigma_{iso}$ for $O_7$ and $P_{18}$ corresponds to uracil. In the case of CUA sequences, the most negative value was observed for $\sigma_{iso}$ for $O_{27}$. Moreover, the graph of $\delta_{iso}$ of all the nitrogen atoms versus dielectric constant revealed that the deshielded points were observed at $\varepsilon = 46.8$ and the

| $\varepsilon$ | $\sigma_{iso}$ (ppm) | $\Delta \sigma$ (ppm) | $\eta$ |
|--------------|----------------------|-----------------------|--------|
| **O$_7$**    |                      |                       |        |
| Gas phase    | 95.2372              | 382.5681              | 0.096  |
| Acetone      | 95.1978              | 382.9628              | 0.096  |
| Ethanol      | 95.5318              | 382.5528              | 0.0917 |
| Methanol     | 94.6308              | 381.5632              | 0.1025 |
| DMSO         | 94.6309              | 381.1584              | 0.9527 |
| Water        | 94.5627              | 382.8416              | 0.0945 |
| **O$_{17}$** |                      |                       |        |
| Gas phase    | –43.0922             | 624.5832              | 0.3759 |
| Acetone      | –43.0358             | 624.5032              | 1.4153 |
| Ethanol      | –43.0222             | 624.4996              | 0.3753 |
| Methanol     | –43.0222             | 624.5865              | 0.3753 |
| DMSO         | –43.0091             | 624.5032              | 0.376  |
| Water        | –43.0842             | 624.724               | 0.3759 |
| **N$_1$**    |                      |                       |        |
| Gas phase    | 77.9384              | 271.2676              | 0.5245 |
| Acetone      | 77.9529              | 271.2605              | 1.1627 |
| Ethanol      | 77.8721              | 271.1569              | 0.525  |
| Methanol     | 77.9814              | 271.6405              | 0.5255 |
| DMSO         | 77.7726              | 271.5487              | 0.5261 |
| Water        | 78.119               | 271.2953              | 0.5249 |
| **N$_4$**    |                      |                       |        |
| Gas phase    | 209.1206             | 82.9855               | 1.0885 |
| Acetone      | 209.1806             | 82.9668               | 1.089  |
| Ethanol      | 209.1346             | 82.9979               | 1.0886 |
| Methanol     | 209.1914             | 82.8613               | 1.0878 |
| DMSO         | 209.0845             | 83.0695               | 1.0881 |
| Water        | 209.0927             | 83.0543               | 1.0906 |
| **N$_6$**    |                      |                       |        |
| Gas phase    | 151.3728             | 134.6544              | 0.769  |
| Acetone      | 151.3603             | 134.6779              | 0.7698 |
| Ethanol      | 151.3278             | 134.6122              | 0.7701 |
| Methanol     | 151.3391             | 134.1849              | 0.7698 |
| DMSO         | 151.3391             | 134.5631              | 0.7717 |
| Water        | 151.1883             | 133.7583              | 0.7739 |
| **P$_{15}$** |                      |                       |        |
| 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.
more shielded regions were observed at $\varepsilon = 78.39$ and $\varepsilon = 32.63$.

**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.\textsuperscript{23–25} However, there are no experimental data on the relative energies or enthalpies of these systems.\textsuperscript{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 $\Delta G$ 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 $\varepsilon = 24.55$ (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

| Solvent  | Temperature (K) | CUA | $\Delta E$ (Kcal/mol) | $\Delta H$ (Kcal/mol) | $\Delta G$ (Kcal/mol) | $\Delta S$ (Kcal/mol) |
|----------|----------------|-----|----------------------|----------------------|----------------------|----------------------|
| Ethanol  | 300            | CUA | $-521251.772$       | $-521251.1792$      | $-521281.712$        | 0.10241              |
|          | 310            |     | $-568221.988$       | $-568221.3953$      | $-568254.997$        | 0.112696             |
|          | 313            |     | $-568204.944$       | $-568204.3517$      | $-568236.969$        | 0.109398             |
| Methanol | 300            | CUA | $-568176.722$       | $-568176.1299$      | $-568208.983$        | 0.110191             |
|          | 310            |     | $-568222.749$       | $-568222.157$       | $-568254.549$        | 0.108644             |
|          | 313            |     | $-568206.857$       | $-568206.2644$      | $-1136477.01$        | 0.108135             |
| DMSO     | 300            | CUA | $-568214.593$       | $-4315146.578$      | $-568246.974$        | 0.110191             |
|          | 310            |     | $-568204.944$       | $-568225.6008$      | $-568258.816$        | 0.111404             |
|          | 313            |     | $-568212.218$       | $-568211.6257$      | $-568248.142$        | 0.122478             |
| Water    | 300            | CUA | $-568209.43$        | $-568208.8377$      | $-568240.096$        | 0.104843             |
|          | 310            |     | $-568226.875$       | $-568226.2822$      | $-568258.442$        | 0.107867             |

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

Abbreviation: DMSO, dimethyl sulfoxide.
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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