Hydroxyl Radical Reactivity with Cytosine and Thymine: A Computational Study

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Abstract

Addition of hydroxyl radical (OH•) at the different sites of cytosine and thymine were studied at the different levels of theory of the density functional theory (DFT). All the molecular geometries were optimized at B3LYP, B3PW91, WB97XD, and MPW1PW91 methods with 6-31G (d,p) basis set. The binding energies at the different sites of these two DNA bases were calculated at all the mentioned levels of theory in gas phase and also in different solvents of dielectric constant varying from lower to higher values. The dependence of binding energies in water, methanol, ethanol, acetone, toluene and benzene reveals that binding energies are not much affected by salvation.

1. Introduction

Reactive oxygen species (ROS) generated in biological systems causes several types of modified 2’-deoxyribonucleic acid (DNA) bases [1]. These modified DNA bases causes an alteration in a base pairing which results in mutation and causes different diseases such as inflammation, aging and cancer [2,3]. Hydroxyl radical reacts efficiently with all the DNA bases than the other reactive oxygen species [4]. Guanine has the lowest ionization potential among the DNA bases so it is the primary target for a reaction [5]. In the reaction of guanine and reactive oxygen species, mutagenic product 8-oxoguanine is formed which mispair with adenine causes mutation [6].

It has been experimentally observed that OH radical react with cytosine and thymine and produce various stable products such as 5,6-thymine glycol, 5-hydroxy- cytosine, 5-hydroxy-5-methyl-hydantoin, cytosine glycol etc. [7]. In another experimental study, it has been observed that the C5 site of cytosine is more reactive than the other sites of the molecule about the OH radical addition [8-10]. The geometries, relative energies, and hyperfine coupling constants of dehydrogenated, hydrogenated products of thymine and cytosine have been performed theoretically using DFT [11].

In present study, the binding energy of OH radical adducts at different sites of cytosine and thymine and also their behavior in different solvents have been investigated. In previous theoretical studies using DFT [12], barrier energies and released energies at the different sites of cytosine and thymine were reported. To the best of my knowledge, these results are not reported earlier.
2. Computational Details

Molecular geometries of the adduct of hydroxyl radical at the different sites of cytosine and thymine were fully optimized at the B3LYP, B3PW91, MPW91PW91 and WB97XD with the 6-31G (d,p) basis set of DFT in the gas phase. In case of cytosine, an adduct of OH radical at the N3, C4, C5 and C6 sites while in case of thymine, at C5 and C6 sites were fully optimized and the binding energies were calculated at mentioned levels of theory in the gas phase. To study the bulk solvent effect on binding energies, single point energy calculations were performed employing a polarizable continuum model (PCM) [13]. In optimization of the OH radical, multiplicity is taken doublet due to the odd electron species while for the cytosine or thymine, multiplicity is singlet due to the even electrons. The adduct geometries multiplicity are taken doublet due to the odd electrons.

For solvation effect, single point energies were calculated in water ($\varepsilon$=78.36), methanol ($\varepsilon$=32.61), ethanol ($\varepsilon$=25.3), acetone ($\varepsilon$=20.7), toluene ($\varepsilon$=2.38) and benzene ($\varepsilon$=2.27) using the geometry optimized at B3LYP/6-31G(d,p) level in the gas phase. Total energy (kcal/mol) is used for geometry and binding energies calculations. All the calculations were performed using the Windows version of the Gaussian09 (G09W) suite of programs [14]. The Gauss View program (version 5.0) was used to visualize the molecular structures [15].

3. Results and Discussion

3.1 Addition of hydroxyl radical at cytosine and thymine

The chemical structures of thymine and cytosine and their parameters are given in Figs. (a,b). Addition of OH radical at the N3, C4, C5 and C6 sites of cytosine (Figs. c to f) and C5 and C6 sites of thymine(Figs. g,h) have been have studied at all above mentioned level of theory. The binding energy ($\Delta E$) is calculated for all these sited using the equation (1)

$$\Delta E = E(C \text{ or } T + \text{OH}^\cdot) - (E(C \text{ or } T) + E(\text{OH}^\cdot))$$ (1)

Where $E(C \text{ or } T + \text{OH}^\cdot)$ is the total energy of adduct of cytosine or thymine with OH radical, $E(C \text{ or } T)$ is the energy of cytosine or thymine and $E(\text{OH}^\cdot)$ is the energy OH radical.

Binding energies at the N3 site is positive value except at one level WB97XD/6-31G(d,p), at which value is small negative. This shows that the N3 site is not reactive site and the reaction will be endothermic. At the C4, C5 and C6 sites, the binding energies are negative value shows that reaction will be exothermic at these sites. At C4 cite, the binding energy is less negative than the C5 and C6, reveals that the C4 cite is less preferable than the other two sites. At the C5 and C6 sites, binding energies are less comparable to each other and the larger negative values than the N3 and C4 sites at the mentioned level of theory. Thus C5 and C6 sites are the most preferred sites for the addition reactions. The order of binding energies was found N3 $>$ C4 $>$ C5 $\sim$ C6 at all mentioned level of theory. In most cases, binding energies at C5 site are a little bit larger.
than the C6 site. Experimentally observed stable products were found at C5 and C6 sites [7,10], which means that these sites are the most preferred for the reaction.

In case of thymine, binding energies at C6 site were found more negative values than the C5 site (Fig.1, Table 2) at all mentioned level of theory. This reveals that the C6 site is the most stable site for addition. In previous study [12], the C6 site was also found barrierless for the OH radical and small barrier energy for perhydroxyl radical using the different levels of DFT. In case of thymine also, experimentally observed stable products were found at the C5 and C6 sites [7], which justifies the results obtained from theoretically.

### Table 1: Binding energies (kcal/mol) of addition of OH• at the different sites of cytosine in gas phase obtained at the different levels of DFT

| Cytosine Site | B3LYP/6-31G(d,p) | B3PW91/6-31G(d,p) | Wb97XD/6-31G(d,p) | MPW1PW91/6-31G(d,p) |
|---------------|-----------------|-------------------|-------------------|---------------------|
| N3            | 2.50            | 1.20              | -0.05             | 1.59                |
| C4            | -8.99           | -9.35             | -8.39             | -9.04               |
| C5            | -23.94          | -24.84            | -30.01            | -24.72              |
| C6            | -25.09          | -25.92            | -27.14            | -25.94              |

### Table 2: Binding energies (kcal/mol) of addition of OH• at the different sites of thymine in gas phase obtained at the different level DFT

| Thymine Site | B3LYP/6-31G(d,p) | B3PW91/6-31G(d,p) | Wb97XD/6-31G(d,p) | MPW1PW91/6-31G(d,p) |
|--------------|-----------------|-------------------|-------------------|---------------------|
| C5           | -25.24          | -26.43            | -28.78            | -26.58              |
| C6           | -31.23          | -32.46            | -37.28            | -32.76              |

#### 3.2 Binding energy in solvents

Binding energy of OH radical at the different sites of cytosine and thymine in different solvents having varying dielectric constant such as water ($\varepsilon=78.36$), methanol ($\varepsilon=32.61$), ethanol ($\varepsilon=25.3$), acetone ($\varepsilon=20.7$), toluene ($\varepsilon=2.38$) and benzene ($\varepsilon=2.27$) have been studied (Table 3, Table 4). For solvation, all the single point energies were calculate in these solvents using the PCM model employing the geometries optimized at B3LYP/6-31G(d,p) level. It is clear from Table 3 and 4 that binding energies are not much varying with the different dielectric constant for the C4 and C5 sites of both cytosine and thymine. For benzene and toluene, binding energies at the N3, C4 and C5 sites of cytosine were found lower (more negative values) than the water thus binding with radical will be more stable for the lower values of dielectric constant ($\varepsilon$). In the case of C6 site, binding energies were less negative values i.e. less stable for the ethanol and acetone while the values are nearly the same in other solvents.
Fig. 1: Optimized geometries of cytosine and thymine (Figs. a, b), OH radical addition at different sites of cytosine (Figs. c, d, e and f) and thymine (Figs. g and h) at the B3LYP/6-31G(d, p) level of theory. Their bond length, bond angles and dihedral angle at the addition sites are also given.

In case of thymine, binding energies are not much varying from lower dielectric constant to higher dielectric constant (Table 4). Binding energy (kcal/mol) along with the
dielectric constant is plotted in Fig. 2. It shows that binding energies are not much affected by varying dielectric constant from water to benzene.

Table 3: Binding energies (kcal/mol) of OH* addition at different sites of cytosine in different solvents obtained using B3LYP method with 6-31G(d,p) basis set

| Cytosine site | Water (ε=78.36) | Methanol (ε=32.61) | Ethanol (ε=25.3) | Acetone (ε=20.7) | Toluene (ε=2.38) | Benzene (ε=2.27) |
|---------------|----------------|-------------------|-----------------|-----------------|----------------|----------------|
| N3            | 11.96          | 11.74             | 12.01           | 11.80           | 7.81           | 7.65           |
| C4            | -2.59          | -2.72             | -2.52           | -2.59           | -5.10          | -5.20          |
| C5            | -20.60         | -20.61            | -19.65          | -19.69          | -21.05         | -21.12         |
| C6            | -23.25         | -23.21            | -22.73          | -22.73          | -23.01         | -23.03         |

Table 4: Binding energies (kcal/mol) of OH* addition at different sites of thymine in different solvents obtained using B3LYP method with 6-31G(d,p) basis set

| Thymine site  | Water (ε=78.36) | Methanol (ε=32.61) | Ethanol (ε=25.3) | Acetone (ε=20.7) | Toluene (ε=2.38) | Benzene (ε=2.27) |
|---------------|----------------|-------------------|-----------------|-----------------|----------------|----------------|
| C5            | -22.21         | -21.93            | -21.96          | -21.98          | -22.84         | -22.88         |
| C6            | -29.20         | -28.71            | -28.42          | -28.44          | -29.08         | -29.12         |

Fig. 2: Variation of binding energy (kcal/mol) with dielectric constants (ε) varying from lower to higher value. Cyt and Thy represent the cytosine and thymine respectively.
4. Conclusions

Addition of OH radical at different sites of cytosine and thymine shows the following results

1. In cytosine, binding energy at C6 site is most stable than the N3, C4 and C5 sites. N3 site has positive binding energy shows an unstable state.
2. In thymine, binding energies for the C5 and C6 were found comparable and the C6 site is the most stable than the C5 site.
3. For the solvation with varying dielectric constant, the binding energies were found nearly the same for C5 and C6 sites of thymine and cytosine. This reveals that stability of OH radical with bases are independent of environment.

Acknowledgment

A.Y. is thankful to U.G.C., New Delhi for Dr. D. S. Kothari postdoctoral fellowship. H.F. is grateful to Prof. B.K. Pandey, P.M.S.D., M.M.M.U.T., Gorakhpur for kind support and help.

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