Theoretical study on the detailed repair of O6-methyl guanine to guanine by cysteine

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Abstract. The repair process of O6-methylguanine (O6mG) by cysteine (Cys145) has been studied using the quantum mechanical calculations in the presence of solvent water molecules and also using the polarizable continuum model (PCM). The proton transfer from Cys145 to O6mG, directly and mediated by bridged water molecules, has been considered to investigate the role and the sequence of the proton transfer on the repair process. In both models, the energy barrier of the methyl transfer is reduced when the proton is initially transferred from O6mG to Cys145. In addition, the protonation of N3 and N7 sites of O6mG in the acidic environment facilitates the repair process. Results of this study help to understand repair mechanisms of damaged DNA bases better on the basis of proton transfer between that and nucleic acids.

Keywords. O6-alkylguanine-DNA alkyltransferase; DNA repair; DNA damage; Mismatch; methylating agents.

1. Introduction

Living cells are constantly exposed to both exogenous (chemical pollutants and UV radiation) and endogenous (the normal metabolic byproducts, especially in the oxidative deamination process) sources of DNA damaging agents.^{1–6} Methylating agents which lead to a wide range of DNA damage such as aging, chronic inflammatory diseases, mutation, cancer, Alzheimer's and Parkinson's diseases^{7–10} can be located on the endocyclic nitrogen and exocyclic oxygen atoms of DNA bases (N7, N3 and O6 in the purines and O2, O4 and N3 in the pyrimidines).^{11,12}

Though N7-methylguanine (N7mG) is the most vulnerable site, yet O6-methylguanine (O6mG) has been found to be very toxic, mutagenic and carcinogenic base across various methylated DNA bases.^{13–15} O6mG mispairs with thymine during the DNA replication which causes GC-AT transversion mutation and cancer.^{16–20} O6-alkylguanine-DNA alkyltransferase (AGT) or O6-methylguanine-DNA methyltransferase (MGMT) enzyme, repairs O6mG via transferring methyl group from O6 position of guanine to S15 of the cysteine residue (Cys145 in human MGMT) in an irreversible stoichiometric reaction.^{21,22}

Many studies were conducted on the repair of O6mG by the MGMT protein, in which the proton simultaneously transfers with methyl in the absence of solvent molecules.^{23,24} There are also some studies in which one part of MGMT, i.e., Cys145, Arg or Tyr to accompany in the repair processes of O6mG to G.^{23,25,26}

In the present study, a novel path for the repair of O6mG by Cys145 has been suggested and that its energy barrier is lower than those of the mechanisms that was previously suggested by P. K. Shukla *et al.*²³ This study emphasizes the role of the solvent molecules and the proton transfer from Cys145 to O6mG on the repair process of O6mG by Cys145. The solvent effects were investigated by locating some water molecules around the complex and between the components as bridges and also using a continuum medium as solvent. The proton is transferred before methyl and also simultaneously with it. The process has also been studied in the acidic environment using protonation of the N7 and N3 sites. Results of this work give helpful insights to the repair mechanisms of the damaged DNA bases on the basis of proton transfer between the DNA bases and nucleic acids.

2. Computational

The effects of hydrogen bonds between Tyr114, Lys165, and Ser159 and the N3, N1 and O6 atoms on the repair process of O6mG have previously been

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investigated by Jena *et al.*²⁷ In the present study, the effects of hydrogen bonds, whether mediated by H₂O molecules or not, between Cys145 and O6mG, solvent molecules surrounding the components, and the protonation of N3 and N7 sites of O6mG on the repair process have been considered.

Herein, the repair process of O6mG has been considered using six models: (1) only the O6mG and Cys145 components are considered along the process, (2) two water molecules bridged between main components and the proton transfer is approximately synchronized with the methyl transfer (3), two water molecules bridged between main components and the proton transfer precedes the methyl transfer (4) nine water molecules located around the components in order to consider the effect of water solvent on the repair process, (5) protonated O6mG (at N7 site) is repaired by Cys145, and (6) protonated O6mG (at N3 & N7 sites) is repaired by Cys145. All geometries were optimized by the Gaussian09²⁷ program package using the hybrid density functional B3LYP in conjunction with the 6-311++G** basis set.^{28,29}

The solvent aqueous media effects on the reaction energy were also treated using the polarizable continuum model (PCM).³⁰ Single-point calculations were carried out using the B3LYP and MP2 methods in conjunction with the AUG-cc-pVDZ and 6-311++G** basis set respectively, in both gas phase and aqueous media.^{31,32} All stationary points verified by vibrational frequency analysis was carried out at the B3LYP/6-311++G** level of theory. All vibrational frequencies are positive for reactant and product complexes, and only one imaginary frequency is observed for each transition state. The net atomic charges were calculated using the ChelpG scheme on the structures optimized at the above mentioned level.³³

To determine the special characters of the structures in stationary points along the repair process, population analysis has been performed by the natural bond orbital (NBO)³⁴ method on the wave functions obtained at the HF/6-311++G** level of theory by NBO3.1³⁵ program.

3. Results and Discussion

The cis and trans-O6mG conformers are different in the orientation of methyl group. In addition, cis-O6mG is more stable than trans-O6mG.^{23,25} There are two conformers for Cys145;^{23,36} herein, stable conformer were selected in the calculations.

3.1 Effect of proton transfer on the repair process of O6mG in the presence of cysteine: Model 1

The cysteine thiolate anion facilitates the repair of O6mG.²³ The negative charge on Cys145 helps separate the methyl group and facilitates the repair process. It has been previously proposed²³ that the proton is transferred from S15 site of Cys145 to the histidine through a bridged water molecule and so the negative charge is located on Cys145. The next steps of repair are the transfer of one proton from Cys145 or environment to O6mG and methyl transfer from O6mG to Cys145. Is the proton transfer from Cys145 to O6mG prior to the methyl transfer or simultaneous with it? In order to answer this question, two pathways were considered in the repair mechanism: (1) the proton is transferred before the methyl migration, and (2) the proton and the methyl transfer take place at the same time. Herein, the binary complex of O6mG and Cys145 is stabilized by two strong hydrogen bonds, O26H27...N1 (1.84 Å) and N2H2...O25 (1.90 Å). The second path has previously been considered by Shukla *et al.*²³ The most important stationary points including the reactant complex (RC), two transition states (TS1 and TS2), one intermediate (INT) and the product complex (PC) have been considered in the first pathway. The gas phase structures optimized at the B3LYP/6-311++G** level of theory are shown in figure 1.

The transfer of H27 from Cys145 to O6mG leads to INT via TS₁(1). Subsequently, PC(1) is made from INT via TS₂(1), in which the methyl group is transferred from O6mG to Cys145. The proton transfer from S15 site of Cys145 to histidine has previously been considered²³ through a bridged water molecule before methyl transfer. In fact, the negative charge on S15 site of Cys145 facilitates the methyl transfer and the repair process.

As can be seen from geometrical parameters, the proton transfer weakens the O6-C10 bond and pushes the C10 atom to a point on the direct path between O6 and S15, and therefore decreases the energy barrier in the latter step. The barrier ΔE^1 corrected for zero-point energy (ZPE) and the Gibbs free energy ΔG^1 at different levels of theory obtained in the gas phase and aqueous media are presented in table 1. The $\Delta E_{p,f}^1$ and $\Delta G_{p,f}^1$ are equal to 4.74 and 2.87 kcal mol⁻¹ for the first step and $\Delta E_{m,f}^1$ and $\Delta G_{m,f}^1$ are equal to 28.18 and 29.92 kcal mol⁻¹ for the second step in the gas phase at the B3LYP/6-311++G** level of theory (table 1). The repair of O6mG to guanine was considered in the presence of Cys145 via S_N2 mechanism, in which $\Delta E_{m,f}^1$ obtained 34.2 kcal mol⁻¹ at the B3LYP/6-311++G** level of theory in the gas phase.²³ Thus, the

Figure 1. Repair of O6mG to guanine by Cys145. The structures of reactant **RC(1)**, transition states **TS1(1)** and **TS2(1)**, and product **PC(1)** complexes optimized at the B3LYP/6-311++G** level of theory. The Gibbs free energies (in kcal mol⁻¹) and the net ChelpG charges are shown in the figure.

$\Delta E^1_{m,f}$ value for the repair of O6mG in the presence of Cys145 reduces by 6.02 kcal mol⁻¹ along the two-step mechanism.

As can be seen in table 1, Δ values of proton transfer change by 1.28–4.74 kcal mol⁻¹ when basis set changes from 6-311++G** to AUG-cc-pVDZ and by 3.56–4.74 kcal mol⁻¹ when method changes from B3LYP to MP2. These ranges are 28.18–28.87 kcal mol⁻¹ and 28.18–28.22 kcal mol⁻¹ for methyl transfer, respectively. Thus, negligible changes are observed by the change in the level of computation in both Δ values.

The $\Delta E^1_{m,f}$ and $\Delta G^1_{m,f}$ values calculated at different levels of theory are given in table 1. In the gas phase, the energy barrier increases by ~2.41% by changing the basis set from 6-311++G** to

AUG-cc-pVDZ using the B3LYP method and increases by ~0.14% by changing the method from B3LYP to MP2 using the 6-311++G** basis set. In going from the gas phase to aqueous media, the value of $\Delta E^1_{m,f}$ is reduced by 4.37, 4.84 and 1.59 kcal mol⁻¹ at the B3LYP/6-311++G**, B3LYP/AUG-cc-pVDZ and MP2/6-311++G** levels of theory, respectively, and almost the same is also found to be true for the corresponding values $\Delta G^1_{m,f}$ (table 1).

The effects of proton transfer from Cys145 to histidine ($\Delta E = 9.2$ kcal mol⁻¹) and the tyrosine... N3 hydrogen bond on the repair process of O6mG ($\Delta E_{\text{methyl}} = 18.2$ and 21.2 kcal mol⁻¹, the latter were obtained in the presence of solvent) were considered by Georgieva *et al.*³⁷ In the present work, the effects

Table 1. The barrier energies (ΔE and ΔG in kcal mol⁻¹) involved in the repair of O6mG with Cys145 at different levels of theory for model 1.

	B3LYP 6-311++G**	B3LYP AUG-cc-pVDZ	MP2 6-311++G**
$\Delta E_{p,f}^1$	4.74(4.18)	1.28(1.159)	3.56(3.26)
$\Delta E_{p,b}^1$	1.82(2.17)	1.97(1.13)	2.22(2.19)
$\Delta G_{p,f}^1$	2.87(1.63)	1.97(1.13)	4.58(3.43)
$\Delta G_{p,b}^1$	1.42(0.75)	2.04(0.08)	2.29(1.14)
$\Delta E_{m,f}^1$	28.18(23.81)	28.87(24.01)	28.22(26.63)
$\Delta E_{m,b}^1$	49.62(25.48)	51.17(22.06)	57.45(29.71)
$\Delta G_{m,f}^1$	29.92(24.40)	30.37(25.30)	29.72(27.46)
$\Delta G_{m,b}^1$	50.46(22.58)	49.56(21.96)	56.58(29.61)

Energies obtained in aqueous media are given in the parentheses. (p: proton transfer, m: methyl transfer, f: forward and b: backward)

of intermolecular hydrogen bond between Cys145 and N1, and thus, the proton transfer from Cys145 to O6mG on the repair process has been considered both in the absence (present section) and in the presence of solvent H₂O molecules (next section). The energy barrier for TS₁, which corresponds to the proton transfer, is equal to 4.74 kcal mol⁻¹ in model 1.

With respect to the ChelpG charges calculated on O6mG (0.074 e) and Cys145 (-0.074 e), the components are expected to be neutral in the **RC**(1) point. In **TS**₁(1), in which corresponds to the proton transfer, the charges calculated on O6mG and Cys145 are equal to 0.18 and -0.70 e, respectively. The charges calculated on the mentioned components are equal to 0.82 and -0.82 e when proton transfer is completed in **INT**. Thus, after proton transfer, O6mG has a higher tendency to transfer the methyl group and reduces the positive charge density. The progress in the methyl transfer changes the charges on the components to 0.11 and -0.55 e, respectively, in **TS**₂. Finally, the methyl group attaches to Cys145 and completes the repair process in **PC**(1).

In order to explore other features of stationary points on the repair process, the NBO analysis has also been carried out on the wave functions obtained at the HF/6-311++G** level of theory. The weakening of the O26-H27 bond is accompanied by the increase in the occupation number of the σ_{O26H27}^* orbital and the decrease in the occupation number of σ_{O26H27} orbital in **RC**(1), and is continued until the O26-H27 bond cleavage is completed in the **INT** structure.

The sum of the donor-acceptor energies ($\sum E^2$) were calculated for the most important interactions. When

the σ_{C26C27}^* orbital acts as acceptor, the $\sum E^2$ value for the cleavage of the O26-H27 bond in **TS**₁(1) is 32.41 kcal mol⁻¹. On the other hand, when the σ_{O6C10}^* is an acceptor, the $\sum E^2$ value for the cleavage of the O6-C10 bond in **TS**₂(1) is 36.06 kcal mol⁻¹.

Similarly, the weakening of O6-C10 bond is accompanied by the increase in the occupation number of the σ_{O6C10}^* orbital and the decrease in the occupation number of the σ_{O6C10} orbital in **TS**₂(1), and is continued until the O6-C10 bond cleavage is completed in the **PC** structure and methyl group locates on S15 site of Cys145. The most important interactions which affect the weakening of the O26-H27 and O6-C10 bonds are $lp_{N1} \rightarrow \sigma_{O26H27}^*$ and $lp_{S15} \rightarrow \sigma_{O6C10}^*$, respectively.

With respect to the energy gap between HOMO and LUMO, which are equal to 6.76 and 5.35 eV for **RC** and **INT** in the one step and two steps pathways, **INT** is more reactive than **RC** with a lower energy barrier in the gas phase.

3.2 The repair process in the presence of water molecules: Models 2 and 3

The effects of bridged and surrounding water molecules on the proton transfer from Cys145 (O26 site) to O6mG (N1 site) and on other aspects of repair process have been investigated in this section. In model (2), only two water molecules bridged between O6mG and Cys145 via four hydrogen bonds O26H27...O28, O28H29...N1, O31H30...O25 and N2H2...O31 (figure 2). The H-bond lengths lie in the range of 1.58–2.07 Å. Herein, the proton transfer from Cys145 to O6mG can be mediated by a water molecule. H27 is transferred from Cys145 to a water molecule and H29 is simultaneously transferred from a water molecule to the N1 site of O6mG. In the next step, the methyl group is transferred to Cys145 and the repair process is completed. Without proton transfer, the repair mechanism that involves a single transition state can be considered S_N2. The energy barrier of methyl transfer is 37.51 kcal mol⁻¹ which is 9.33 kcal mol⁻¹ higher than that in the model (1). Thus, the bridged water molecules can increase the energy barrier of the repair process.

The $\Delta E_{m,f}^2$ and $\Delta G_{m,f}^2$ values obtained at different levels are given in table 2. $\Delta E_{m,f}^2$ is 37.51 kcal mol⁻¹ in the gas phase at the B3LYP/6-311++G** level of theory. In the gas phase, the energy barrier increases by ~1.90% in changing the basis set from 6-311++G** to AUG-cc-pVDZ using the B3LYP method, while this value increases by ~0.58% by changing the method from B3LYP to MP2 using the 6-311++G** basis set.

Figure 2. Repair of O6mG to guanine by Cys145 in the presence of two bridged water molecules when proton is transferred through water molecules simultaneously with methyl group. The structures of reactant **RC(2)**, transition state **TS(2)** and product **PC(2)** complexes optimized at the B3LYP/6-311++G** level of theory. The Gibbs free energies (in kcal mol⁻¹) and the net ChelpG charges are shown in the figure.

In going from the gas phase to aqueous media, the value of $\Delta E_{m,f}^2$ is reduced by 2.71, 4.65 and 0.21 kcal mol⁻¹ at the B3LYP/6-31++G**, B3LYP/AUG-cc-pVDZ and MP2/6-311++G** levels of theory, respectively. Similar results are also obtained for the $\Delta G_{m,f}^2$ values (table 2).

The CHelpG charges calculated on O6mG, Cys145 and the water molecules of **RC(2)** are approximately equal to zero and the system is neutral. The charge on O6mG, Cys145, the water molecules, and the methyl group of **TS(2)** is equal to -0.36, 0.04, -0.06 and 0.38 e, respectively. Thus, the electron density is more localized on O6mG in **TS(2)**. After transition state, the proton is transferred through a bridged water molecule and it reduces the negative charge of guanine.

With respect to the NBO analysis, the most important interaction in which the σ^*_{O26H27} orbital acts as acceptor

Table 2. The barrier energies (ΔE and ΔG in kcal mol⁻¹) involved in the reaction of O6mG with Cys145 at different levels of theory for models **2** and **3**.

	B3LYP 6-311++G**	B3LYP AUG-cc-pVDZ	MP2 6-311++G**
$\Delta E_{m,f}^2$	37.51(34.80)	38.23(33.58)	37.29(37.45)
$\Delta E_{m,b}^2$	23.72(4.12)	35.29(15.03)	27.19(16.12)
$\Delta G_{m,f}^2$	36.70(37.88)	36.37(37.65)	38.37(41.50)
$\Delta G_{m,b}^2$	19.17(18.27)	17.01(17.48)	25.91(20.10)
$\Delta E_{m,f}^3$	25.71(24.70)	24.74(21.77)	22.16(20.10)
$\Delta E_{m,b}^3$	19.89(20.31)	18.18(16.27)	23.40(21.64)
$\Delta G_{m,f}^3$	25.74(24.37)	24.72(21.64)	25.14(20.10)
$\Delta G_{m,b}^3$	21.86(20.07)	20.42(21.14)	25.64(29.23)

Energies obtained in aqueous media are given in the parentheses. (m: methyl transfer, f: forward and b: backward)

is $\text{lp}_{\text{O}28} \rightarrow \sigma^*_{\text{O}26\text{H}27}$. This interaction, which increases the occupation number of the $\sigma^*_{\text{O}26\text{H}27}$ orbital, in **TS(2)** is stronger than **RC(2)**. The energy of mentioned donor-acceptor interaction which helps the cleavage of O26-H27 bond and shifts the H27 atom towards the O28 site is equal to $47.33 \text{ kcal mol}^{-1}$ in **TS(2)**. On the other hand, the N1 lone pair of O6mG helps the complex to weaken the O28-H29 bond. The $\text{lp}_{\text{N}1} \rightarrow \sigma^*_{\text{O}28\text{H}29}$ interaction energy increases from **RC(2)** to **TS(2)** and becomes $17.66 \text{ kcal mol}^{-1}$ in **TS(2)**. Finally, H27 attaches to the water molecule and H29 attaches to the N1 site of O6mG. This model has a lower $\sum E^2$ value (for interactions with $\sigma^*_{\text{O}6\text{C}10}$) and therefore a higher energy barrier ($\sim 10.37 \text{ kcal mol}^{-1}$) relative to the previous model.

The energy barrier is reduced in the presence of water molecules when the proton transfer occurs before methyl migration. Herein, the energy barrier of methyl transfer is equal to $25.71 \text{ kcal mol}^{-1}$ (figure 3) and

increases to $37.51 \text{ kcal mol}^{-1}$ when the proton transfer is not considered in a separate step.

The $\Delta E^3_{\text{m,f}}$ value calculated at the B3LYP/6-311++G** level and the results of single-point energy calculations at the B3LYP/AUG-cc-pVDZ and MP2/6-311++G** levels are also given in table 2. The energy barrier increases by $\sim 3.85\%$ in changing the basis set from 6-311++G** to AUG-cc-pVDZ using the B3LYP method, while that value increases by $\sim 14.83\%$ in changing the method from B3LYP to MP2 using the 6-311++G** basis set in the gas phase (table 2). In going from the gas phase to aqueous media, the value of $\Delta E^3_{\text{m,f}}$ reduces by 1.01, 2.97 and $2.06 \text{ kcal mol}^{-1}$ at the B3LYP/6-311++G**, B3LYP/AUG-cc-pVDZ and MP2/6-311++G** levels of theory, respectively. Similar results are also obtained for the $\Delta G^3_{\text{m,f}}$ values (table 2).

The charges calculated on O6mG, Cys145 and two water molecules are equal to 0.87, -0.76 and -0.11,

Figure 3. Repair of O6mG to guanine by Cys145 in the presence of two water molecules when proton is transferred through water molecules before methyl transfer. The structures of reactant **RC(3)**, transition state **TS(3)**, and product **PC(3)** complexes optimized at the B3LYP/6-311++G** level of theory. The Gibbs free energies (in kcal mol^{-1}) and the net ChelpG charges are shown in the figure.

respectively, in **RC(3)** in the model (3). The positive charge on O6mG can speed up methyl migration to Cys145. In **TS(3)**, the calculated charges on the guanine, Cys145, the methyl group and the two water molecules are 0.07, -0.44 , 0.44 and -0.06 e, respectively. All moieties are approximately neutral in **PC(3)**.

The most important interaction with σ^*_{O6C10} is $\text{lp}_{\text{S15}} \rightarrow \sigma^*_{\text{O6C10}}$ which increases the occupancy of the σ^*_{O6C10} orbital. The E^2 value of $\text{lp}_{\text{S15}} \rightarrow \sigma^*_{\text{O6C10}}$ interaction increases from **RC(3)** ($0.34 \text{ kcal mol}^{-1}$) to **TS(3)** ($34.22 \text{ kcal mol}^{-1}$). The $\sum E^2$ value for interactions with σ^*_{O6C10} is $39.29 \text{ kcal mol}^{-1}$ in **TS(3)**. In comparison with the previous models, higher $\sum E^2$ value reduces the energy barrier in this model. The energy gap between the HOMO and LUMO in **RC(2)** and **RC(3)** is

equal to 6.74 and 4.88 eV , in the gas phase. This result is in agreement with the greater reactivity of **RC(3)**.

In addition to bridged water molecules, seven water molecules were located around the components in model (4) (figure 4). Herein, it is assumed that the proton transfer occurs before the methyl transfer. The $\Delta E^4_{\text{m,f}}$ value calculated at various levels of theory are given in table 3. The values of $\Delta E^4_{\text{m,f}}$ and $\Delta G^4_{\text{m,f}}$ are equal to 26.26 and $24.59 \text{ kcal mol}^{-1}$, respectively. The water molecules located between the components reduce the energy barrier, but the water molecules arranged around the components do not affect the barrier.

The $\Delta E^4_{\text{m,f}}$ and $\Delta G^4_{\text{m,f}}$ values obtained at different levels are given in table 3. In the gas phase, the energy barrier increases by $\sim 9.04\%$ in changing the basis set

Figure 4. Repair of O6mG to guanine by Cys145 in the presence of solvent water molecules. The structures of reactant **RC(4)**, transition states **TS(4)** and product **PC(4)** complexes optimized at the B3LYP/6-311++G** level of theory. The Gibbs free energies (in kcal mol^{-1}) and the net ChelpG charges are shown in the figure.

Table 3. The barrier energies (ΔE and ΔG in kcal mol⁻¹) involved in the repair of O6mG with Cys145 at different levels of theory for model **4**.

	B3LYP 6-311++G**	B3LYP AUG-cc-pVDZ	MP2 6-311++G**
$\Delta E_{m,f}^4$	26.26	23.99	27.32
$\Delta E_{m,b}^4$	42.00	35.14	46.25
$\Delta G_{m,f}^4$	24.59	24.46	27.79
$\Delta G_{m,b}^4$	32.28	29.54	40.65

(m: methyl transfer, f: forward and b: backward)

from 6-311++G** to AUG-cc-pVDZ using the B3LYP method while that increases by $\sim 3.9\%$ in changing the method from B3LYP to MP2 using the 6-311++G**

basis set (table 3). The $\Delta E_{m,f}^4$ value increases by $\sim 2.1\%$ and the $\Delta G_{m,f}^4$ value decreases by 15.5% in changing the model from (3) to (4).

3.3 The effect of acidic environment: Models 5 and 6

The energy barrier decreases to 30.57 and 17.85 kcal mol⁻¹ by protonation of the N7 site of O6mG in the model(5) (figure 5) and protonation of both the N7 and N3 sites of O6mG in the model(6) (figure 6), respectively. **RC(5)** and **RC(6)** have the same O6-C10 bond lengths (~ 1.46 Å). The most important geometrical parameters of **TS (5)**, i.e., O6-C10 and S15-C10 bond lengths, are equal to 1.96 and 2.56 Å. However, these bond lengths are equal to 2.05 and 5.87 Å in **TS (6)**.

Figure 5. Repair of O6mG to guanine by Cys145; the protonated site is shown by a hollow circle.

Therefore, the O6-C10 bond length increases by protonation of O6mG and the repair process is facilitated in the acidic environment. The O6-C10 bond length indicates that the methyl transfer in model (6) is faster than model (5).

The $\Delta E_{m,f}^5$ and $\Delta G_{m,f}^5$ values obtained at different levels of theory in the gas phase and aqueous media are presented in table 4. The $\Delta E_{m,f}^5$ and $\Delta G_{m,f}^5$ values are equal to 30.57 and 31.19 kcal mol⁻¹ in the gas phase at the B3LYP/6-311++G** level of theory (table 4). As can be seen, protonation of the N7 site decreases the energy barrier. In going from the gas phase to aqueous media, the value of $\Delta E_{m,f}^5$ is reduced by 1.16, 0.98 and 1.31 kcal mol⁻¹ at the B3LYP/6-31++G**, B3LYP/AUG-cc-pVDZ and MP2/6-311++G** levels of theory. Similar results are also obtained for the $\Delta G_{m,f}^5$ values (table 4).

The ChelpG charges calculated on the O6mG and Cys145 components are equal to 1.03 and -0.03 e,

in **RC(5)**. Herein, O6mG has a higher tendency to transfer the methyl group to Cys145 and reduces the positive charge density. The methyl transfer from O6mG to Cys145 reduces the positive charge on guanine in **TS(5)**. The charges calculated on the guanine, Cys145 and the methyl group are equal to 0.43, 0.37 and 0.19 e, respectively. Finally, the methyl group attaches to Cys145 and completes the repair process in **PC(5)**.

The $\Delta E_{m,f}^6$ and $\Delta G_{m,f}^6$ values obtained at different levels of theory in the gas phase and aqueous media are presented in table 4. The $\Delta E_{m,f}^6$ and $\Delta G_{m,f}^6$ values are equal to 17.85 and 16.57 kcal mol⁻¹ in the gas phase at the B3LYP/6-311++G** level of theory (table 4). As can be seen, protonation of the N3 site of O6mG induces a more decrease in the energy barrier. In going from the gas phase to aqueous media, the value of $\Delta E_{m,f}^6$ reduces by 1.38, 0.49 and 1.60 kcal mol⁻¹ at the B3LYP/6-31++G**, B3LYP/AUG-cc-pVDZ and

Figure 6. Repair of O6mG to guanine by Cys145; two protonated sites are shown by hollow circles.

Table 4. The barrier energies (ΔE and ΔG in kcal mol⁻¹) involved in the repair of the protonated O6mG (at N7 & N3 site) and Cys145 at different levels of theory for models **5** and **6**.

	B3LYP 6-311++G**	B3LYP AUG-cc-pVDZ	MP2 6-311++G**
$\Delta E_{m,f}^5$	30.57(29.41)	28.34(27.35)	31.72(30.41)
$\Delta E_{m,b}^5$	50.66(48.51)	47.77(46.28)	50.67(49.62)
$\Delta G_{m,f}^5$	31.19(30.61)	29.68(27.51)	34.13(35.73)
$\Delta G_{m,b}^5$	50.24(50.41)	49.93(50.29)	52.83(50.12)
$\Delta E_{m,f}^6$	17.85(16.47)	16.13(15.63)	19.92(18.32)
$\Delta E_{m,b}^6$	29.12(28.62)	28.25(29.37)	36.22(37.63)
$\Delta G_{m,f}^6$	16.57(16.42)	17.52(18.54)	21.32(21.84)
$\Delta G_{m,b}^6$	28.73(27.49)	29.30(29.48)	37.28(37.35)

Energies obtained in aqueous media are given in the parentheses. (m: methyl transfer, f: forward and b: backward)

MP2/6-311++G** levels of theory. Similar results are also obtained for the $\Delta G_{m,f}^6$ values (table 4).

The ChelpG charges calculated on the O6mG and Cys145 components are equal to 1.87 and -0.02 e, respectively, so the positive charge is strongly localized on O6mG in **RC(6)**. In **TS(6)**, the charges calculated on O6mG, Cys145 and the methyl group become 1.30, 0.31 and 0.38 e. With a higher positive charge, O6mG has a higher tendency to transfer the methyl group to Cys145 component. Finally, the methyl group attaches to Cys145 and completes the repair process in **PC(6)**. As a result, the energy barrier reduces significantly by protonation of the N7 and N3 sites of O6mG.

The energy gap between HOMO and LUMO orbital is equal to 5.44 and 4.92 eV in **RC(5)** and **RC(6)**, respectively. It is expected that the **RC(6)** is more reactive than **RC(5)**.

Figure 7. The relative energies in kcal mol⁻¹ obtained for the methyl transfer at the B3LYP/6-311++G(d,p) level of theory. The numbers 1–6 correspond to models 1–6.

The relative energies obtained for the methyl transfer at the B3LYP/6-311++G(d,p) level of theory are shown in figure 7. The relative energies of products do not correspond to the most stable conformers. They correspond to the conformers obtained from TS structures on the scan process. Proton transfer does not take place in some models, and its barrier energy is lower than methyl transfer for other models. Thus, the methyl transfer would correspond to the rate-limiting step.

4. Conclusions

The repair of O6mG has been investigated by the quantum mechanical calculations with six models in the present work. The most important conclusions are:

1. The proton transfer from Cys145 to O6mG, as a separate step, diminishes the energy barrier (model **1**). This step increases the positive charge on O6mG, decreases the stability of damaged base and facilitates the methyl transfer from O6mG to Cys145.
2. The energy barrier increases when two water molecules are bridged between O6mG and Cys145 components and the proton transfer is not considered as a separate step (model **2**). But, the barrier decreases substantially, such that it becomes lower than that of model (**1**) when the proton transfer via bridged water molecule is considered as a separate step (model **3**).
3. Although the change in the barrier (ΔE) is negligible in the presence of nine additional water molecules located around the O6mG and Cys145 components, the ΔG value decreases by about 15% (model **4**).
4. The protonation of the N7 site of O6mG is accompanied by accumulation of positive charge on O6mG helps the methyl transfer from O6mG to Cys145 (model **5**).
5. The protonation of the N3 and N7 sites of O6mG induces more positive charge on O6mG and causes the methyl transfer from O6mG to Cys145. As a result, the energy barrier decreases due to increase in the number of protonated sites (model **6**).

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