

The Stability of Anionic States of Thymine-Glycine Dimers with Excess Electron

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Abstract: It has been demonstrated that low energy electrons (LEEs) can induce serious DNA damages including bases loss and even single and double strand breaks. Experiments also showed that LEE induced DNA damages will be reduced with the presence of amino acids. For understanding of the protection of amino acids to DNA, the stability of 6 kinds of thymine and glycine (T-g) dimers with planar configurations with an excess electron were studied with density functional theory (DFT) method. The results show that, when the excess electron is vertically attached, all the dimers become more active with higher energy. After re-optimization, 4 kinds (66.7%) of T-g dimers become more stable than the corresponding neutral states. For the most stable anionic dimer noted as [34-A]⁻, the excess electron is localized on the thymine, while one proton transfers from glycine to thymine. The proton transformation decreases the activities and prevents further reactions of the excess electron. For other three dimers, there is no chemical topology change. The glycine attracts the excess electron with hydrogen-bonding to the thymine.

Keywords: Low energy electrons (LEEs), DNA damage, thymine-glycine dimers, stability.

1 Introduction

It is well known that DNA of living organisms can be damaged by ionizing radiations. As a result, serious genetic disorder or even lethal diseases might take place [von Sonntag (1987); Fabrikant, Eden, Mason et al. (2017); Dumont, Zheng, Hunting et al. (2010); Burrows and Muller (1998); Pimblott, Laverne, Mozumder, et al. (1996)]. Experiments show that DNA damages are mainly produced by active secondary species along the radiation track [Alizadeh and Sanche (2012); Kohanoff, McAllister, Tribello et al. (2017)]. The low energy electrons (LEEs) with energy below 20 eV are the most abundant of secondary species *in vivo* [Pimblott and LaVerne (2007)]. It has been verified that, low energy electrons (LEEs) might break chemical bonds and give rise to radicals and ions [Dumont, Zheng, Hunting et al. (2010)], by forming either an electronic excited state or a transient anion. Radiation damages to DNA can be classified into base

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and sugar modification, base release, single strand breaks, and clustered lesions [von Sonntag (1987); Dumont, Zheng, Hunting et al. (2010); Kohanoff, McAllister, Tribello et al. (2017)].

The attachments of LEEs to DNA bases are considered as the primary cause of DNA alteration, which ultimately lead to base release for direct electron capture and strand breakage for indirect effects such as electron transferring from the bases to the phosphate groups in the backbone of DNA [Kohanoff, McAllister, Tribello et al. (2017)]. In physiological environment, the role played by histones in chromatin to the LEE induced DNA damages has been considered [Kohanoff, McAllister, Tribello et al. (2017); Gu, Smyth and Kohanoff (2014)]. Experiments show that when chromatin are exposed to radiation, the amount of double strand breaks are increased as a result of removing of the proteins contained in it [Solomun and Skalický (2008)]. It is believed that residues of a DNA-binding biomolecules, serve mainly as scavengers for radicals and might repair the radical or transient ions of DNA bases [Marques, Rubio, Varsano et al. (2009)]. The occurrence of such reactions has already been observed by the flash-quench technique, pulse radiolysis and other experimental studies [Kohanoff, McAllister, Tribello et al. (2017)]. The role of glycine and arginine in preventing of strand breaks induced by LEEs and cold plasma radiations has also been studied. In aqueous solvent of DNA, when the ratio between amino acid to nucleotide is increased from 0 to 2, the single strand breaks will decrease by 70% [Jena, Mishra and Suhai (2009)]. In addition to the physical shielding to DNA, N.R. Jena and coworkers showed that cytosine and thymine can help guanine radicals and anions retrieve to normal state [Jena, Mishra and Suhai (2009)]. But it is still not clear whether amino acids protect DNA bases through reaction with LEE directly or afterward repairation.

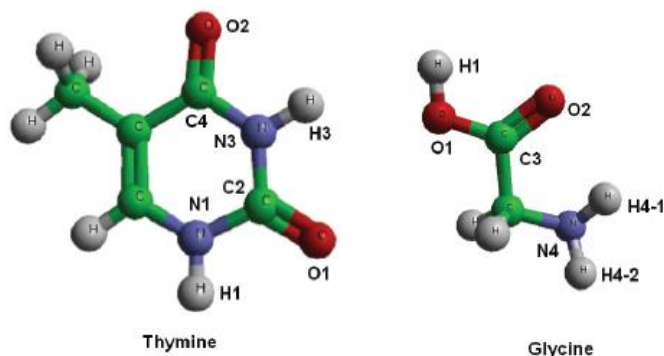


Figure 1: (color online) The diagram of thymine and glycine molecules with nomination of concerned atoms

It is known that electron and proton transferring are most important for the delicate interactions between DNA and protein [Solomun and Skalický (2008)]. As shown in Fig. 1, glycine is the simplest amino acid. It is not only proton donor (H₁ in the carboxyl group, H₄₋₁ and H₄₋₂ in the amino group) but also proton acceptor (O₂ in the carboxyl group). Glycine has great chance to interact with most of the bases in both DNA and RNA, during DNA duplication and protein fabrication. Hence, glycine is suitable for

theoretical study on how protein affects the attachment of LEEs to nucleobases. In all nucleobases of DNA, thymine has the highest electron affinity [Aflatooni, Gallup and Burrow (1998)]. Hence, thymine will bear the brunt of the LEEs under ionizing radiation. In this work, thymine and glycine are taken as examples, to study the mechanism of the direct protection of amino acid to DNA from LEEs with simulations based on density functional theory (DFT). The energy and electron affinity of the thymine-glycine dimers with different configurations are studied in detail, to reveal possible details for protecting of amino acids against DNA damages.

The model and theoretical scheme of our calculations are presented in Section 2. The simulation results and discussions on the amino acid protection to DNA bases are presented in Section 3. Finally, Section 4 concludes the paper and gives our prospects on further studies.

2 Models and simulation methods

2.1 Thymine and glycine dimer models

The molecular structures of thymine and canonical glycine with atom nominations are shown in Fig. 1. As to thymine, the protons of H₁ and H₃, which connect with nitrogen atoms of the hex-atomic ring, have great chances to transfer, while the oxygens of O₁ and O₂ are potential hydrogen accepters. The thymine and glycine dimer might be generated through single point, bidentate and multi-point interactions.

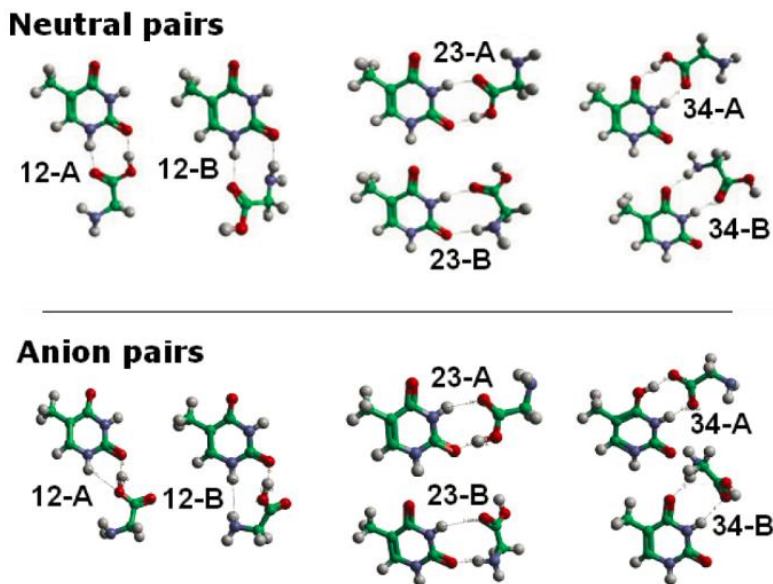


Figure 2: (color online) Optimized structures of the 6 thymine and glycine dimers of both neutral and anionic states. See the text for detailed nominations

As shown in Fig. 2, we designed 6 dimers with different double hydrogen bonding forms, according to the main sensitive hydrogen-bonding sites discussed above. In their names, the numbers indicate the positions of the hex-atomic ring of thymine connected with

protons. Taking 12-A and 12-B as examples, 12 means the hydrogen-bonded atoms of thymine are connected to N₁ and C₂ individually. **A** means that both the hydrogen bonds are connected to the carboxyl group of glycine, while **B** means that the hydrogen donor sites locate at the amino group of glycine. The initial structure of these configurations were constructed with basic intuition using the molecule structure editor ATEN.

2.2 Simulation methods

Based on the Born Oppenheimer approximation, electrons keeps adiabatically in their ground state without electron-nucleus coupling, while nucleus are treated as classical mass points, obeying Newton's law [Li and Sevilla (2007)]. In density functional theory (DFT) method the electronic energy can be determined as a functional of electron density. The many body Schrödinger equations of electrons can be solved with Kohn-Shame single electron method with a proper approximation of the exchange-correlation term.

In practice, the PBE (Perdew-Burke-Ernzerhof) [Heyd and Scuseria (2004)] form of exchange correlation functional and trip- ζ 6-311++G** basis [Kohanoff (2006)] are utilized. In addition, the GTH (Goedecker-Teter-Hutter) pseudo-potential [Hutter, Parrinello and Lippert (1999)] for core electrons is adopted. We employed the *ab initio* quantum module Quickstep (QS) of CP2K code [Vandevondele, Krack, Mohamed et al. (2005)], which is a free fast and accurate DFT implementation using a mixed Gaussian and augmented plane waves (GAPW) [Hutter, Parrinello and Lippert (1999)], to optimize the ground states of the thymine-glycine pairs. After the optimization of the dimers with pure DFT, one excess electron is vertically attached to each system. The states with vertically attached electron and re-optimized anions are studied and compared in detail. In the optimization of the anion, the orbital transferring effects [Kohanoff, McAllister, Tribello et al. (2007)] are also taken into consideration. In addition, the related states of isolated thymine and glycine were calculated also.

3 Results and discussions

3.1 Structure and energy changes

Fig. 2 shows the optimized structures of the neutral and anionic states of thymine-glycine dimers in gas phase. The lengths of the hydrogen-bonds are shown in Tab. 1. For each neutral state, the planer configuration of thymine and the chain of glycine is kept well after optimization. It can be seen that, in neutral states, all the hydrogen bonds of **A** dimers and the H_T-O_g-**B** are shorter than 2Å. As an exception, H_g-O_T-**B** is a little longer than 2 Å. In fact, there are two hydrogen atoms in the NH₂⁻ group of glycine. They have equal possibility to be involved in the H_g-O_T-**B** bond. As a result, the H-O bond with thymine is weaker and longer than normal hydrogen bond.

With the excess electron, the dimers experience evident structure changes after the re-optimization. In Fig. 2 the perspective of thymine keeps the same for both neutral and anion states for clarity. Thus the configuration changes can be detected from the stereo view of the glycine. Firstly, the excess electron makes the glycine chain bended out of plane against the neutral state. Secondly, the distances between the two atoms of hydrogen-bonding pairs are violently changed as shown in Tab. 1. Compared with neutral states, the distances between the atomic pairs of one hydrogen bond in neutral states are

all increased evidently, while those of the other hydrogen bond are decreased. It should be noticed that, for 12-**A** and 12-**B**, the distance between H_{1T} and O_{2g} is enlarged to be 4.169 Å and 3.702 Å individually. They are beyond the normal length of hydrogen bond. Here, we cannot judge whether the stability of these anionic dimers is increased or decreased through the variations of the hydrogen bonds. The dimer stability can be analyzed further from the point of view of energy.

Table 1: The Hydrogen-bond length (in Å) of the thymine and glycine dimers in both neutral (neu) and anionic (e⁻) states

| Dimer state | A: H _T -O _g | A: H _g -O _T | B: H _T -O _g | B: H _g -O _T |
|------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| 12neu | 1.769 | 1.634 | 1.868 | 2.030 |
| 23neu | 1.837 | 1.662 | 1.919 | 2.042 |
| 34neu | 1.816 | 1.637 | 1.893 | 2.033 |
| 12 ^{e-} | 4.069 | 1.419 | 3.794 | 1.434 |
| 23 ^{e-} | 2.149 | 1.442 | 2.750 | 2.005 |
| 34 ^{e-} | 1.494 | 1.754 | 2.397 | 1.911 |

The comparison of the total energy of the dimers are shown in Fig. 3. For neutral pairs, the 12-A dimer is most stable with the lowest energy. We set it as the relative zero point for convenience. The **A** patterns are more stable than the corresponding **B** patterns. This means that in gas phase carboxyl group is more active and has higher affinity for DNA bases than amino group. After the excess electron is attached, the stability order of these dimers changes evidently. The 34-A becomes the most stable one with a relative total energy of -1.1 eV with a very short hydrogen bond of 1.494 Å.

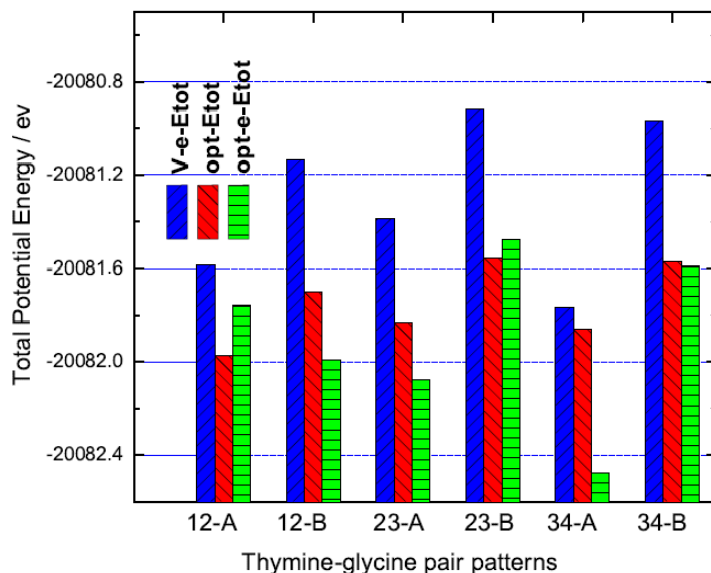


Figure 3: (color online) Total potential energy of different Thymine-Glycine pairs with

(black and blue) and without (red) one excess negative electron

3.2 Electron affinity

The electron affinity (EA), defined as the change of energy when an electron is added to a neutral species, is helpful to understand the stability of the localization of the excess electron in these dimers. In specific, the Vertical Electron Affinity (VEA) is defined as the difference in energy between the neutral and anion at the equilibrium geometry of the neutral state: $VEA = E_{neu} - E_{no-opt}^{-e}$, while the Adiabatic Electron Affinity (AEA) is defined as the difference in energy between the neutral and anion species with individually optimized geometries: $AEA = E_{neu} - E_{opted}^{-e}$. VEA indicates how much energy is needed for an electron to be attached, while AEA shows how the attached electron is localized in the system. To study the energy needed to inject an electron into the π^* orbital of DNA bases, Aflatooni et al. [Aflatooni, Gallup and Burrow (1998)] calculated the VEA of the bases in gas phase. The results show that it is possible for electrons with energies ranging from 0.4 to 0.6 eV to attach effectively to every DNA base. When the AEA is positive, the energy of the anion is less than the neutral one and thus the dimer is more favorite for free excess electrons.

Table 2: The VEA and AEA (in eV) of the thymine and glycine monomers and their dimers with different configurations

| System | Thymine | glycine | 12-A | 12-B | 23-A | 23-B | 34-A | 34-B |
|--------|---------|---------|--------|--------|--------|--------|--------|--------|
| VEA | -0.212 | -0.512 | -0.390 | -0.568 | -0.447 | -0.638 | -0.095 | -0.601 |
| AEA | -0.044 | -0.494 | -0.214 | 0.291 | 0.242 | -0.080 | 0.613 | 0.022 |

Tab. 2 shows the VEA and AEA of thymine, glycine and their dimers. The VEA and AEA of thymine is higher than that of glycine. The VEA and AEA of thymine and glycine are all negative. In addition, thymine has higher electron affinity than glycine. Thus LEEs prefer to locate at isolated thymine than isolated glycine. With optimization, the structure of anionic thymine and glycine will change to more stable states with energy decreased by 0.148 eV and 0.018 eV. The VEA of the dimers are all decreased than the monomer of thymine. This means that the energy needed for the injected electrons to be attracted by nuclear bases is increased. This might be one reason for the protection of DNA base from the damages of very low energy electrons. For the dimers of 12-B, 23-A, 34-A and 34-B, the AEAs are positive. The AEA of 23-B is almost zero. The adiabatic electron affinity of dimers are increased than the monomers. This indicates that these re-optimized dimers are more preferred by the excess electron than the isolated thymine. As an exception, the AEA of 12-A dimer is -0.214 eV. This means that 12-A^{-e} is the most unstable state. It is corresponding with the violent changes in hydrogen-bond length of 12-A dimer as shown in Tab. 1.

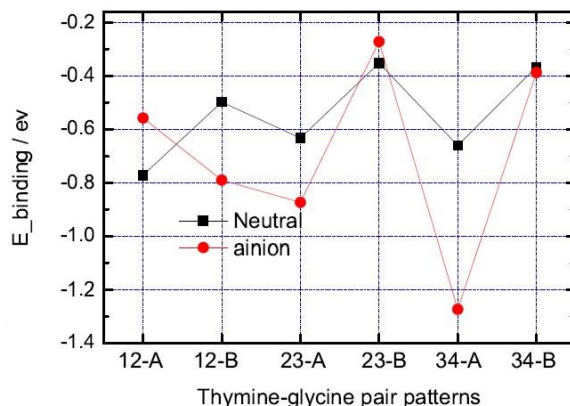


Figure 4: Binding energy of neutral (black block) and anion (red triangle) glycine dimers

The binding energy of the neutral and anion dimers are calculated and presented in Fig. 4. The binding energy of the neutral dimers is defined as $E_b^{neu} = E_{dimer} - (E_{thy} + E_{gly})$ while the binding energy of the anionic dimers is defined as $E_b^{-e} = E_{dimer}^{-e} - (E_{thy}^{-e} + E_{gly}^{-e})$. It can be seen that the neutral state of 12-A and 23-B is more stable with more binding energy than its anion with the excess electron. For other dimers, their anion states are more stable. It should be noticed that the VEA of 34-A is the smallest one. However, its AEA is positive and the highest one. As a result, 34-A is a nontrivial state, which is the most favorable conformation for the electron to be localized in the dimer.

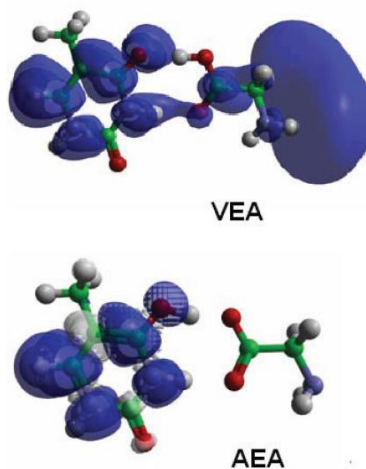


Figure 5: (color online) Spin density distribution of vertical (top) and adiabatic (bottom) attached electron on 34-A pair

To understand the ultra-stability of the dimer of 34-A with excess electron, the spin density of the excess electron, both vertically (VEA) and adiabatically (AEA) attached, are shown in Fig. 5. It can be seen that, the vertically attached electron distributes evenly on both thymine and glycine. This means that the electron is not localized to a specific low energy orbit. After the re-optimization, the electron is settled down mostly on

thymine. The density maximum is increased to be twice of the vertically attached state. We noticed that as shown in Fig. 2 and Fig. 5, the localization of the excess electron is accomplished by a positively charged proton transferring from the carboxyl group of glycine to the hydrogen acceptor O₂ of thymine. As a result, the energy of the anionic dimer is decreased. The proton transferring ring is the chemical protecting mechanism to further DNA damage as studied by the group of Jorge Kohanoff [Gu, Smyth and Kohanoff (2014); McAllister, Smyth, Gu et al. (2015)].

4 Conclusions

It is well known that low energy electrons are important for radiation induced DNA damages including breaks in bases and even strands. Some recent experiments show that at the presence of amino acids, DNA damages will be decreased. In this paper, to trace the molecular mechanisms behind the protection of amino acid to DNA, the interactions between glycine and thymine, with and without an excess electron attached, were studied with high precision density functional theory methods.

After an excess electron is attached, for the 6 dimers of thymine and canonical glycine and with planar configuration, two of them will be a little more unstable by 0.1 eV energy; three of them are more stable with over 0.1 eV in energy; one of them experience little changes (be a little bit more stable). With hydrogen bonding to glycine, the possibility to be more stable for the dimer with excess electron is around 66.7%. For the most stable state of anionic 34-A dimer, the excess electron is localized on thymine with a proton transfer from glycine. As a result, the protection of glycine to thymine with excess electron can be explained with both physical and chemical effects, e.g. with and without proton transferring.

In condensed phase, the proton transferring possibility will be much higher during the combing of protein and DNA (RNA). These associative actions of protons and electrons under ionizing radiation should be studied with large scale *ab initio* simulations, for more realistic environments [Kohanoff, McAllister, Tribello et al. (2017); McAllister, Smyth, Gu et al. (2015)]. Meta-dynamics technology, which has been demonstrated to be efficient for enhance structure sampling [Wang, Huang, Gu et al. (2016); Zheng and Pfaendtner (2015)] might be used in the dynamic simulations on DNA damages in real biological circumstances.

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