Effect of BrU on the transition between wobble Gua-Thy and tautomeric Gua-Thy base-pairs: ab initio molecular orbital calculations

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Abstract. We investigated transition states (TS) between wobble Guanine-Thymine (wG.T) and tautomeric G.T base-pair as well as Br-containing base-pairs by MP2 and density functional theory (DFT) calculations. The obtained TS between wG.T and G*.T (asterisk is an enol-form of base) is different from TS got by the previous DFT calculation. The activation energy (17.9 kcal/mol) evaluated by our calculation is significantly smaller than that (39.21 kcal/mol) obtained by the previous calculation, indicating that our TS is more preferable. In contrast, the obtained TS and activation energy between wG.T and G.T* are similar to those obtained by the previous DFT calculation. We furthermore found that the activation energy between wG.BrU and tautomeric G.BrU is smaller than that between wG-T and tautomeric G-T. This result elucidates that the replacement of CH₃ group of T by Br increases the probability of the transition reaction producing the enol-form G* and T* bases. Because G* prefers to bind to T rather than to C, and T* to G not A, our calculated results reveal that the spontaneous mutation from C to T or from A to G base is accelerated by the introduction of wG-BrU base-pair.

1. Introduction
It was revealed from the studies of the genetic codes that the formation of the canonical Watson-Crick (W-C) base-pairs (i.e. G-C and A-T) for DNA is strictly observed only for the first two base-pairs in the codon-anticodon interactions [1]. At the third codon position, many types of base-pairs including both the canonical W-C and non-canonical W-C base-pairs are involved. Based on this fact, Crick [1] proposed that the base-pair at the 3’ end of the codon can deviate or wobble within arbitrary limits that are consistent with the additional pairing interactions suggested by the code. Since then, specific base-pairings in the codon-anticodon interaction have been investigated for understanding of the basis on which particular base-pairs are selected at the wobble position [2].

High resolution nuclear magnetic resonance (NMR) and ethidium bromide binding studies [3] demonstrated that polymers of deoxynucleotide G and T form ordered double-helical structures at low temperature. In these structures, G and T bases are hydrogen bonded together in a wobble base-pair form, while alternative hydrogen bonding schemes involving the tautomeric form of either G or T are eliminated. In addition, the X-ray diffraction analysis [4] at 2.5 Å resolution for the deoxyoligomer
d(CGCGAATTTGCG) duplex showed that the G-T base-pairs in the duplex adopt a wobble structure, in which T and G bases project into the major and minor grooves, respectively. The wobble G-T (wG-T) base-pair can also be accommodated in the octameric A-form DNA fragment [5] as well as the Z-form DNA with the sequence of d(CGCGTG) [6]. *Ab initio* molecular orbital (MO) calculations [7] demonstrated that the double proton transfer (DPT) in the wG-T base-pair is a potential pathway for the generation of the rare tautomers of guanine. Physical-chemical mechanism of transformation of the wobble DNA base-pairs into the tautomeric base-pairs was suggested by Brovarets and Hovorun [8]. This transition mechanism, involving the tautomeric form of either G or T was investigated by means of *ab initio* MO and DFT methods. The results showed that the structure of the transition state (TS) between wG-T and the tautomeric G-T* base-pairs is planar. On the other hand, the TS between wG-T and enol-form G (G*)-T base-pairs is non-planar, and this TS has larger activation energy than that between wG-T and G-T*, indicating that the transition between wG-T and G*-T is unfavorable in DNA duplex structure energetically and structurally. In addition, Brovarets & Hovorun [9] performed the analogical MO and DFT calculations for the wobble G-BrU (wG-BrU) and G-BrU base-pairs to elucidate the effect of BrU on the transition mechanism. The effect of the other halogenated derivatives of U on the DNA replication errors was also investigated by *ab initio* MO calculations [10].

In order to search for more stable and planar TS structures for these transitions, we investigated in detail the transition mechanism between wG-T and the G-T base-pairs involving the enol-form of either G or T by using the *ab initio* MP2 method. This method takes into account correlation of electrons, which is important for determining stable structures and evaluating activation energies of reactions more accurately. In addition, to reveal the effect of the BrU substitution on the transition mechanism, we performed the same MO calculations for the wobble and the tautomeric G-BrU base-pairs and evaluated the activation energies for the transitions between these base-pairs. From the comparison of the results obtained for both the G-T and G-BrU base-pairs, we attempted to demonstrate that introduction of Br atom to the 5th position of T increases the probability of the tautomerization reaction, finally leading to spontaneous mutations of DNA/RNA bases.

### 2. Details of ab initio MO calculations

We first optimized the structures of the wG-T, G*-T and G-T* base-pairs by using the MP2/6-31G(d,p) method of the *ab initio* MO program Gaussian09 (G09) [11]. The structures of the TSs between these base-pairs were searched for by the STQN method [12] of G09, and the obtained TS structures were confirmed to have a single imaginary frequency by the vibrational analysis of G09. Afterwards, the reaction path of proton transfer between bases was followed by performing an intrinsic reaction coordinate (IRC) calculation [13], in order to make sure that the obtained TS structure really is TS structure connecting the reactant and the product structures. The potential energy surface for the transitions between the G-T base-pairs was evaluated by including the zero-point vibrational energy (ZPE), and the free energies at 298.15 K and 1 atmosphere of pressure were also evaluated by MP2/6-31G(d,p). The same procedure was adapted for the analysis of the transition mechanism between the G-BrU base-pairs.

In addition, to confirm the validity of the above procedure based on MP2/6-31G(d,p), we preformed the same calculations employing the meta-hybrid DFT method based on generalized gradient approximation. The M06 functional [14] and the 6-311++G(d,p) basis-set were employed, because the M06 functional has good performance for calculating activation energies and thermochemical kinetics.

### 3. Results and discussion

#### 3.1. Optimized structures for wG-T, G-T*, G*-T and their TS structures

We first optimized the structures of wG-T, G-T* and G*-T in gas phase by the MP2/6-31G(d,p) and M06/6-311+G(d,p) methods. The structures optimized, the hydrogen bond lengths between bases and total energies are shown in Figure 1. The structures optimized by the MP2 and M06 methods are

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**Figure 1:**

- Optimized structures of wG-T, G-T*, G*-T base-pairs and their TS structures.

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| Structure          | MP2/6-31G(d,p) | M06/6-311+G(d,p) |
|--------------------|----------------|------------------|
| wG-T               |                |                  |
| G-T*               |                |                  |
| G*-T               |                |                  |
| TS (wG-T -> G-T*)  |                |                  |
planar and almost the same to each other. In addition, the distance between the nitrogen atoms connecting to the sugar parts of DNA duplex is 8.83 (wG-T), 8.92 (G-T*) and 8.98 Å (G*-T), respectively, in the MP2/6-31G(d,p) calculations. These distances are near to those for the Watson-Crick base-pairs of the B-form DNA duplex: 9.04 (G-C) and 9.08 Å (A-T). Therefore, the base-pairs shown in Figures 1a, 1b and 1c are expected to fit into the B-form DNA duplex. In addition, these base-pairs are planar to be stacked to each other in the DNA duplex.

Figure 1. Structures and total energies of (a) wG-T, (b) G-T*, (c) G*-T, (d) TS between wG-T and G-T*, (e) TS between wG-T and G*-T, and (f) TS between G-T* and G*-T optimized by MP2/6-31G(d,p). The distances of hydrogen bonds are shown. The structures optimized by M06/6-311++G(d,p) are almost the same, and the distances of hydrogen bonds are shown in parentheses.
To confirm these structures are energy minimum structures, we performed vibrational analysis using MP2/6-31G(d,p). These structures have no imaginary frequency and six nearly zero frequencies related to the rotation and the translation of the whole structure. In Table 1, the total energy including zero point vibrational energy (ZPE) and free energy are listed. Among the three structures, G*-T is the most stable. Although the energy values obtained by the MP2 and the M06 methods are remarkably different to each other, the relative energies are not so different. The size of ZPE is about 150 kcal/mol, and the entropic effect included in free energy is about 30 kcal/mol. Therefore, their effect should be considered in the comparison of relative stability among the base-pairs.

In the previous DFT study [8], the structures of wG-T, G-T* and G*-T base-pairs were optimized by the B3LYP/6-311++G(d,p) method. The optimized structures and hydrogen-bond lengths between bases obtained by our MP2/6-31G(d,p) and M06/6-311++G(d,p) calculations are shown in Figures 1a, 1b and 1c. These bond lengths are identical to those obtained by the DFT method in the previous work [8] within the error of 0.02 Å. Therefore, the comparison of our present and the previous DFT results shows a good enough consent between them. Hereafter, we will describe mainly the results of MP2/6-31G(d,p) calculations.

To obtain TS structure between wG-T and G-T* base-pairs, we performed the QST2 calculation using the initial structures shown in Figures 1a and 1b and the MP2/6-31G(d,p) or the M06/6-311++G(d,p) method. Figure 1d shows the obtained TS structure, which has only one imaginary (-170 cm⁻¹) frequency, as listed in Table 2. In addition, the IRC analysis starting with the TS structure obtained the reactant and the product structures similar to wG-T and G-T* shown in Figures 1a and 1b. Consequently, the TS structure shown in Figure 1d is confirmed to be a real TS structure connecting between wG-T and G-T* base-pairs. This TS structure is identical to that obtained by the previous B3LYP/6-311++G(d,p) calculation [8] within an error of 0.06 Å in hydrogen bonds.

### Table 1. Total energy (TE) (kcal/mol), total energy with zero-point vibrational energy (ZPE), and total free energy (FE) at 298.15 K and 1 atmosphere of pressure for the optimized structures of wG-T, G-T*, G*-T base-pairs, and the transition states between them evaluated by (a) MP2/6-31G(d,p) and (b) M06/6-311++G(d,p) methods.

|                  | wG-T   | TS(wG-T→G-T*) | G-T*   | TS(G-T*→G*-T) | G*-T   | TS(wG-T→G*-T) |
|------------------|--------|---------------|--------|---------------|--------|---------------|
| **TE**           | -623698.5 | -623679.2     | -623698.4 | -623693.7     | -623700.8 | -623679.2     |
| (Relative energy)| 0.0    | 19.3          | 0.1    | 4.8           | -2.3   | 19.3          |
| **TE + ZPE**     | -623550.0 | -623532.0     | -623550.0 | -623548.7     | -623552.1 | -623532.0     |
| (Relative energy)| 0.0    | 18.0          | 0.0    | 1.3           | -2.1   | 18.0          |
| **FE**           | -623580.2 | -623560.7     | -623578.8 | -623576.9     | -623581.2 | -623560.7     |
| (Relative energy)| 0.0    | 19.4          | 1.3    | 3.3           | -1.0   | 19.4          |

|                  | wG-T   | TS(wG-T→G-T*) | G-T*   | TS(G-T*→G*-T) | G*-T   | TS(wG-T→G*-T) |
|------------------|--------|---------------|--------|---------------|--------|---------------|
| **TE**           | -625243.1 | -625226.4     | -625243.6 | -625237.7     | -625244.7 | -625226.4     |
| (Relative energy)| 0.0    | 16.6          | -0.6   | 5.3           | -1.6   | 16.6          |
| **TE + ZPE**     | -625097.5 | -625081.4     | -625097.7 | -625095.2     | -625098.7 | -625081.4     |
| (Relative energy)| 0.0    | 16.1          | -0.2   | 2.3           | -1.2   | 16.1          |
| **FE**           | -625127.8 | -625109.9     | -625126.7 | -625125.2     | -625128.9 | -625109.8     |
| (Relative energy)| 0.0    | 17.9          | 1.0    | 2.6           | -1.1   | 17.9          |
Table 2. Vibrational frequencies (cm⁻¹) of the lowest 10 modes for the TS structures between wG-T, G-T* and G*-T base-pairs evaluated by MP2/6-31G(d,p).

| Mode | wG-T → G-T* | wG-T → G*-T | G-T* → G*-T |
|------|-------------|-------------|-------------|
| 1    | 170.8       | 170.8       | 900.0       |
| 2    | 21.2        | 21.2        | 32.4        |
| 3    | 28.6        | 28.5        | 37.7        |
| 4    | 76.0        | 76.0        | 81.8        |
| 5    | 100.8       | 100.8       | 110.5       |
| 6    | 107.0       | 107.0       | 115.4       |
| 7    | 133.8       | 133.8       | 135.1       |
| 8    | 146.8       | 146.8       | 139.4       |
| 9    | 171.0       | 171.0       | 155.0       |
| 10   | 173.2       | 173.2       | 184.2       |

We furthermore investigated the change in structure during the transition according to the path obtained by the IRC analysis. In the wG-T base-pair, G and T bases are displaced each other and have only two hydrogen bonds. To obtain the G-T* structure having three hydrogen bonds, some alterations of hydrogen bonds are necessary. As a first step of the transition, the proton bound to the nitrogen atom of T in wG-T is transferred to the G side, making a weak hydrogen bond between the oxygen atoms of G and T bases. At the same time, both G and T rotate to the anti-clock direction on the plane shown in Figure 1a. As a result, two weak hydrogen bonds between the nitrogen atoms of G and T and between the NH2 group of G and the oxygen atom of T are made as shown in Figure 1d. In this structure, three protons exist on the G side, so that G is a proton donor, while T is a proton acceptor. Moreover, the two oxygen atoms of G and T come closer to each other and finally the proton is transferred from G to T, leading to the G-T* structure shown in Figure 1b. Therefore, it is elucidated that the proton between the oxygen atom of G and the nitrogen atom of T in wG-T (Figure 1a) moves frequently between G and T bases during the transition from wG-T to G-T*. On the other hand, the other proton between G and T in wG-T remains at the G base to bind strongly to the nitrogen atom of G. This fact is likely to be related with the result that there is no direct path between wG-T and G*-T, as will be shown below.

As for the transition structure between wG-T and G*-T, the previous B3LYP calculation [8] predicted the non-planar TS structure. To elucidate whether more stable and planar TS structure exists or not, we performed the QST2 calculation using the initial structures of wG-T and G*-T shown in Figures 1a and 1c. The TS structure obtained (Figure 1e) is planar and almost identical to the TS structure between wG-T and G-T*. This result is greatly different from the TS structure obtained by the previous DFT study [8]. We consider that this result may come from the close similarity of G-T* and G*-T structures (Figures 1b and 1c) in the comparison with the wG-T structure (Figure 1a). In fact, the positions of two protons contributing to the hydrogen bonds between the G and T bases are slightly different (about 0.1 Å), while the positions of the other atoms are the same to each other. In the QST2 calculation, only the structures of the reactant and the product are used for proposing the TS structure. Accordingly, the QST2 results for the transition states between wG-T and G-T* and between wG-T and G*-T are almost the same each other.

In order to search for the other TS structures between wG-T and G*-T, we furthermore performed QST3 calculations using the structures of wG-T and G*-T as well as the candidate TS structure. In fact, we constructed the six types of the candidate structures for TS based on the structure (Figure 1d) of the TS between wG-T and G*-T obtained by the QST2 calculation. To obtain the structure similar to G*-T, three protons contributing to the hydrogen bonds were shifted. By using these initial structures, we
performed the six types of QST3 calculations, three of them obtained the converged structure. However, the obtained TS structures are almost the same to the TS structure between wG-T and G-T*. This result is completely different from that obtained by the previous B3LYP/6-311++G(d,p) calculation [8], which predicted that the activation energy from wG-T to G*-T to be 39.21 kcal/mol is much larger than that (17.51 kcal/mol) for the wG-T to G-T* transition. In contrast, our QST2 and QST3 calculations based on MP2/6-31G(d,p) and M06/6-311++G(d,p) revealed that the activation energies for the transitions of wG-T → G-T* and wG-T → G*-T are the same to be 19.4 (MP2) and 17.9 kcal/mol (M06). Therefore, in the view point of the activation energy, our obtained TS structure for the transition between wG-T and G*-T is more preferable than the previous DFT study [8]. In addition, our predicted TS structures are planar to be fitted into the DNA duplex, while the TS structure obtained by the previous study is non-planar. It is thus concluded that our predicted TS structure is more realistic in the view points both of energy and structure.

To confirm that the TS structure (Figure 1e) for the wG-T → G*-T transition can connect the wG-T and G*-T structures or not, we performed the IRC analysis. The analysis obtained the structures of wG.T and G.T*, indicating that there is no direct transition between wG-T and G*-T base-pairs. As mentioned above, the proton bonded to the nitrogen atom of G in wG-T (Figure 1a) remains to strongly bind to the same atom during the transition from wG-T to the TS of wG-T → G*-T, while the other proton contributing to the hydrogen bonds between G and T bases moves easily between the bases. This means that G in wG-T can not release the proton bonded to the nitrogen to tautomerize into G*. Consequently, wG-T can not be transferred directly into the tautomeric G*-T.

Finally, to elucidate the transition mechanism between G-T* and G*-T base-pairs, we performed QST3 calculation based on MP2/6-31G(d,p) and M06/6-311++G(d,p) methods. The obtained TS structure and its vibrational property are shown in Figure 1f and Table 2, respectively. This structure has one imaginary (-900 cm⁻¹) frequency to be confirmed a TS structure. In addition, by the IRC analysis starting with the TS structure, we confirmed that the TS structure connects the structures of G-T* and G*-T. It should be noted that during the transition, there are antiparallel migrations of two protons between the oxygen atoms of each base and between the nitrogen atoms of each base.

As mentioned above, it is not an easy task to search for correct TS structures. However, finding of the correct TS structure between a reactant and its product is critically important for predicting the kinetic constant of the chemical and biochemical reactions.

### 3.2. Optimized structures for G-BrU, G-BrU*, G*-BrU and their TS structures

By the same procedure as for the wG-T base-pair, we optimized the structures of wG-BrU, G-BrU* and G*-BrU base-pairs in gas phase. The structures and the hydrogen bond distances optimized by the MP2/6-31G(d,p) or M06/6-311++G(d,p) method are shown in Figures 2a, 2b and 2c. The effect of substitution of CH₃ group of T by Br is only at most 0.02 Å displacement of protons contributing to the hydrogen bonds. The total energies and free energies for wG-BrU, G-BrU* and G*-BrU are listed in Table 3. G*-BrU is the most stable among the base-pairs. In the previous DFT study [9], the structures of wG-BrU, G-BrU* and G*-BrU base-pairs were optimized by the B3LYP/6-311++G(d,p) method. The lengths of hydrogen bonds between G and BrU bases are identical to those obtained by our present MP2 and M06 calculations within the difference of 0.02 Å. Therefore, the structures of these G-BrU base-pairs obtained by both the MP2 and M06 methods are confirmed to be comparable to the previous B3LYP results [9].
Figure 2. Structures and total energies of (a) wG–BrU, (b) G–BrU*, (c) G*-BrU, (d) TS between wG–BrU and G–BrU*, (e) TS between wG–BrU and G*-BrU, and (f) TS between G–BrU* and G*-BrU optimized by MP2/6-31G(d,p). The distances of hydrogen bonds are shown. The structures optimized by M06/6-311++G(d,p) are almost the same, and the distances of hydrogen bonds are shown in parentheses.
Table 3. Total energy (TE) (kcal/mol), total energy with zero-point vibrational energy (ZPE), and total free energy (FE) at 298.15 K and 1 atmosphere of pressure for the optimized structures of wG-BrU, G-BrU*, G*-BrU base-pairs, and the transition states between them evaluated by (a) MP2/6-31G(d,p) and (b) M06/6-311++G(d,p) methods.

(a) MP2/6-31G(d,p)

| TS          | wG-BrU   | (wG-BrU→G-BrU*) | G-BrU*   | TS          | (G-BrU*→G*-BrU) | G*-BrU   | TS          | (wG-BrU→G*-BrU) |
|-------------|----------|-----------------|----------|-------------|----------------|----------|-------------|----------------|
| TE          | -2211433.8 | -2211417.1      | -2211433.2 | -2211429.7 | -2211435.6      | -2211417.1 |
| (Relative energy) | 0.0      | 16.7            | 0.6       | 4.1         | -1.8           | 16.7     |
| TE + ZPE    | -2211309.8 | -2211294.2      | -2211309.4 | -2211308.9 | -2211311.5      | -2211294.2 |
| (Relative energy) | 0.0      | 15.6            | 0.4       | 0.9         | -1.7           | 15.6     |
| FE          | -2211340.7 | -2211323.7      | -2211339.0 | -2211337.9 | -2211341.2      | -2211323.7 |
| (Relative energy) | 0.0      | 17.0            | 1.7       | 2.9         | -0.5           | 17.0     |

(b) M06/6-311++G(d,p)

| TS          | wG-BrU   | (wG-BrU→G-BrU*) | G-BrU*   | TS          | (G-BrU*→G*-BrU) | G*-BrU   | TS          | (wG-BrU→G*-BrU) |
|-------------|----------|-----------------|----------|-------------|----------------|----------|-------------|----------------|
| TE          | -2215424.7 | -2215410.2      | -2215425.1 | -2215420.2 | -2215425.8      | -2215410.2 |
| (Relative energy) | 0.0      | 14.5            | -0.4     | 4.5         | -1.1           | 14.5     |
| TE + ZPE    | -2215302.7 | -2215288.8      | -2215302.9 | -2215301.0 | -2215303.7      | -2215288.8 |
| (Relative energy) | 0.0      | 13.9            | -0.2     | 1.7         | -1.0           | 13.9     |
| FE          | -2215333.7 | -2215318.7      | -2215332.9 | -2215330.3 | -2215332.9      | -2215318.6 |
| (Relative energy) | 0.0      | 15.1            | 0.9      | 3.4         | 0.9            | 15.1     |

The TS structure between wG-BrU and G-BrU* obtained by the QST2 calculations based on MP2 and M06 methods is shown in Figure 2d, which has only one imaginary frequency (-154 cm⁻¹) as listed in Table 4. In addition, the IRC calculation starting with the TS structure obtained the structures of wG-BrU and G-BrU*. Therefore, the structure of Figure 2d is confirmed to be a TS structure between wG-BrU and G-BrU*. This TS structure is identical to that obtained by the previous B3LYP/6-311+G(d,p) calculation [9] within a difference of 0.06 Å in hydrogen bonds. The mechanism of transition between wG-BrU and G-BrU* is almost the same as that between wG-T and G-T*, which was described in detail in the section 3.1.

In the same way, we obtained the TS structure between wG-BrU and G*-BrU. As shown in Figure 2e and Table 4, this TS structure and its vibrational properties are almost the same as those between wG-BrU and G-BrU*. It should be noted that there is no direct path between wG-BrU and G*-BrU as for the transition between wG-T and G*-T. The TS structure between G-BrU* and G*-BrU was obtained by the QST3 calculation. The structure and its vibrational properties are shown in Figure 2f and Table 4, respectively. This structure has only one imaginary frequency (-999.5 cm⁻¹) and was confirmed to be a TS structure between G-BrU* and G*-BrU by the IRC analysis. This TS structure is identical to the TS structure obtained by the previous B3LYP/6-311+G(d,p) calculation [9] within the difference of 0.03 Å in hydrogen bonds. Therefore, it was elucidated that the TS structures between wG-T and tautomeric G-T base-pairs are not affected by the introduction of Br atom into T base.
Table 4. Vibrational frequencies (cm$^{-1}$) of the lowest 10 modes for the TS structures between wG.BrU, G-BrU* and G*-BrU base-pairs evaluated by MP2/6-31G(d,p).

| Mode | wG-BrU → G-BrU* | wG-BrU → G*-BrU | G-BrU* → G*-BrU |
|------|-----------------|-----------------|-----------------|
| 1    | -154.1          | -154.0          | -999.5          |
| 2    | 17.4            | 17.4            | 27.1            |
| 3    | 28.3            | 28.3            | 37.5            |
| 4    | 68.8            | 68.8            | 71.8            |
| 5    | 77.5            | 77.7            | 89.2            |
| 6    | 94.5            | 94.5            | 96.2            |
| 7    | 133.8           | 133.8           | 118.5           |
| 8    | 155.7           | 155.7           | 138.1           |
| 9    | 170.6           | 170.6           | 182.3           |
| 10   | 183.1           | 183.1           | 183.9           |

3.3. Free energy surfaces for G-T and G-BrU base-pairs

Schematic free energy diagrams of the transition in gas phase between wG-T, G-T* and G*-T base-pairs are shown in Figure 3a. The relative free energies (kcal/mol) evaluated by MP2/6-31G(d,p) are shown, and the M06/6-311++G(d,p) results are shown in parentheses. The activation energy for the wG-T → G-T* transition is evaluated to be 19.4 and 17.9 kcal/mol by the MP2 and M06 methods, respectively. These values are similar to that (17.51 kcal/mol) obtained by the previous B3LYP/6-311++G(d,p) calculation [8]. As for the activation energy for the G-T* → G*-T transition, the B3LYP calculation obtained 0.73 kcal/mol, while our results obtained 2.0 (MP2) and 1.6 kcal/mol (M06), respectively. Therefore, our MP2 and M06 calculations result in the larger activation energy between G-T* and G*-T base-pairs, in comparison with the previous B3LYP calculation. The evaluated activation energy by our calculations is larger than 800 K, so that the transition between G-T* and G*-T seems improbable at a room temperature.

It is noted that the activation energy for the wG-T → G*-T transition is the same as that for the wG-T → G-T* transition in our MP2 and M06 calculations. This fact is a new finding different from the previous B3LYP result [8] and seems reasonable in considering almost the same structures of G-T* and G*-T in the comparison with wG-T. The energy barrier of the double proton transfer (DPT) between the Watson-Crick G-C base pair has been predicted at various MO calculations to be in the range between 15 and 19 kcal/mol [15]. Accordingly, our calculated results indicate that the reactions between wG-T and tautomeric G-T base-pairs are energetically accessible as the DPT between G-C base-pair.

In order to reveal the effect of BrU on the isomerization reaction between wG-T, G-T* and G*-T base-pairs, we investigated the schematic free energy diagrams of the transition in gas phase between wG-BrU, G-BrU* and G*-BrU base-pairs. As indicated in Figure 3b, the activation energy for the wG-BrU → G-BrU* transition is evaluated to be 17.0 and 15.1 kcal/mol by MP2/6-31G(d,p) and M06/6-311++G(d,p), respectively. These values are similar to that (15.35 kcal/mol) obtained by the previous B3LYP/6-311++G(d,p) calculation [9]. Therefore, it was elucidated that the activation energy for the transition between the wG-T and G-T*/G*-T base-pairs is reduced about 2.4-2.8 kcal/mol by the introduction of BrU. The activation energy for the G-BrU* → G*-BrU transition is evaluated to be 1.2 (MP2) and 2.5 kcal/mol (M06), respectively. From the comparison of the results for the G-T and G-BrU base-pairs, we can conclude that the introduction of BrU base into base-pair brings forward the transition reaction from wG-T to the tautomeric G-T* and G*-T. Since G* is rather stable and pairs...
with T instead of C, T is introduced into the site complementary to G at the time of DNA replication. As a result, a mutation from G-C to A-T base-pair is generated at the subsequent duplex formation. The present theoretical study reveals a possible origin for the spontaneous mutation induced by the introduction of BrU into wobble G-T base-pair.

Figure 3. Schematic free energy diagrams of the transition in gas phase between (a) wG-T, G-T* and G*-T base-pairs, and (b) wG-BrU, G-BrU* and G*-BrU base-pairs. The relative free energies (kcal/mol) are evaluated by MP2/6-31G(d,p) at 298.15 K and 1 atmosphere of pressure. The results obtained by M06/6-311++G(d,p) are shown in parentheses.
4. Conclusions
It is experimentally known that 5-bromouracil (BrU) induces spontaneous mutations in DNA sequence. We here investigated the effect of BrU on the transition reactions between wobble G-T (wG-T) and tautomeric G-T base-pairs by \textit{ab initio} molecular orbital and DFT calculations. The evaluated activation energy between wG-BrU and its tautomeric G-BrU base-pairs is smaller than that between wG-T and its tautomeric G-T base-pairs, indicating that the introduction of BrU into base-pair accelerates the transition reaction producing the tautomeric G* and T* bases. Because the tautomerized G* prefers to bind to T rather than to C, the BrU introduction is expected to enhance the spontaneous mutation from C to T base.

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