International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 6 Issue 4 Ver. I ǁ 2018 ǁ PP. 54-65

The Effect Of Proton And Water Molecule On Watson Crick Hydrogen Bonds And The Formation Of Cytosine(C)-Thymine(T) Base Pairs.

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SUMMARY: *Density functional studies are performed to understand the effect of proton and water molecule at certain sites of Watson Crick AT and GC. The effect of proton on structure and stability of AT and GC pair is observed and formation of several types of H bonds is found . Weak hydrogen bonding between AT and GC with water molecule is found. Protonation at certain sites of base pairs dislocate some hydrogen bonds and new types of hydrogen bonds are formed. Further existence of CT mismatches in DNA is analysed from the metastable CT pairs. Some metastable CT pairs are found favorable, which in fact may be important for explaining CT mismatch in DNA.*
KEYWORDS: *AT, GC, Mismatches, CT, DNA, Density functional*.

Date of Submission: 24-03-2018 Date of acceptance: 09-04-2018

I. Introduction

The hydrogen bonding in DNA and formation of other multiplet helices such as triple and tetrad helices are related to physiochemical responses of the biological molecules in the cell(1-5). The single stranded oligonucleotide may recognize another double stranded oligonucleotide to form triple helix which also leads to the formation of mismatch base pairs in oligonucleotides. Sometimes the Watson Crick hydrogen bonds may be dissociated at certain pH(5-10). The four different nucleobases are involved in the formation of two Watson Crick type base pairs, AT and GC, which are arranged in the double helical DNA. On the other hand these nucleobases can pair up in a different manner to form mismatch base pair. As we know the acidic and basic properties of adenine, guanine, cytosine and thymine are not equal, and the guanine nucleobase is found to be the most basic nucleobase among the four nucleobases. The ions and water molecules are usually accumulated around DNA(2-6). Moreover conformation of DNA and other structured water molecules are stabilized through hydrogen bonds. There may be reorganization of the normal hydrogen bonds in the WC base pairs due to the disruption of these outer hydrogen bonds. In fact the double helical DNA can adopt different forms, such as A-DNA, Z-DNA and G-DNA(1-10). On the other hand the evolution of mismatch base pair and its relationship with emergence of various diseases has been demonstrated in many studies. All these findings are dependent on the conformational variability of normal base pair in B-DNA in different biological environments. Significant progress on the experimental studies of DNA structures in solution has been made in the recent years(12-17). Such experimental techniques can characterize many DNA structures but precise explanation on the existence of different forms has not been reported. The evolution of mismatches under different situations should also be addressed. So the gas phase studies of nucleobase pairing under certain conditions may be useful to demonstrate the structure and energetic of transformation of different forms of base pairs.

The computational techniques can be applied as supplementary study to explain the experimental findings as well as to understand the quantitative values of nucleobase interactions. The success of accurate *ab initio* methods in the studies of nucleic acid base pairs has been shown in many literatures(10-20). So the *ab initio* methods may be used in studying the effect of proton and water molecule on the hydrogen bonds in Watson Crick(WC) base pair(21-23).

The present study focuses on the gas phase calculation of proton interaction with donor sites of base pairs. It should be noted that there are various donor sites in the WC AT and GC base pairs. These donor sites may be distinguished as WC site and non WC sites. The interactions of H^+ and water molecule with these sites have been taken up for calculations. Thus interactions of H^+ and water molecule around AT and GC base pairs have been taken up in the present study.

In the most cases the stabilization of WC hydrogen bonds may be addressed with respect to the types of hydrogen bonds(1-6). Likewise in other biomolecules such as protein-ligand interaction the formation of the

hydrogen bonds within the active sites are apparently responsible for the stabilization of ligand. So the importance of hydrogen bonds in biomolecule has been addressed in many studies(11-15). Again the protein-DNA interaction occurs through hydrogen bonds. In this respect any change in the protein structure in turn affect the WC hydrogen bonds thereby may result the non WC types of hydrogen bonds. That is the variation of protein structure can pass the effect to the hydrogen bonding in proteins and DNA(18-20). Because of so many complex hydrogen bonding in protein molecule, it is rather difficult to estimate hydrogen bond strength between amino acid residues. So, structure and stability of biomolecules are related to hydrogen bonds. So the study has been focused on the types of hydrogen bonds and the effect of ions and water molecule by taking WC hydrogen bonds in AT and GC.

Sometimes the cleavage of DNA due to dissociation of hydrogen bonds between two counter nucleobases is relevant to the generation of mismatch pairs in DNA. There are several factors for generating such mismatches, but the formation of tautomer nucleobases and further association to form mismatch pairs have been emphasized. On the otherhand guanine, cytosine, thymine and adenine can exist in several tautomeric forms(14-17).

Here the hydrogen bonding ability of these tautomers rather than normal hydrogen bonds in WC base pairs is a matter of concern to explain how the stable mismatches are available in DNA through different hydrogen bonds(14-18). As we know that several tautomers of C and T may pair up to form CT mismatches, once the normal AT and GC base pairs are disrupted. We are not concerned about the pairing of normal forms of C and T through various hydrogen bonds. It is likely that tautomerization of C or T could be the important pathway on subsequent disruption of normal H-bond. So several CT mismatches may be generated in the process, where the chemical reactivity of hydrogen bonding sites of C or T tautomers may be involved(19). Hence theoretical studies on several metastable CT mismatches through different hydrogen bonds other than WC GC and AT have been taken up.

II. Methodology

The energies of protonated base pairs are calculated with B3LYP/6-31+G(d,p) calculations. The interaction energies of Watson crick base pairs with proton and water molecule are computed from the following equations.

$\Delta E1 = E_{NH+} - E_N$

$\Delta E2 = E_{NW} - E_N - E_W$

All the structures involved in the calculations are optimized with 6-31G+d(d,p) basis sets adopted in the Gaussian program code[25].

III. Results And Discussion

The interaction of H^+ with the non Watson Crick(NWC) atomic sites in turn produces elongation of certain WC hydrogen bonds. There observed changes in the geometries of base pairs after protonation at certain sites(Figures 1(a-d) and 2(a-i)). There are possibilities of transforming WC either to NWC base pair or mismatch pairs. The protonation energies at the several sites are shown in Tables 1 and 2, and the corresponding structures are shown in Figures 1 and 2. The variation of hydrogen bond lengths of WC base pair after protonation at certain atomic sites are shown in Tables 3 and 4. As indicated in Table 4**,** the H^+ interaction with atomic sites of NWC sites of AT base pair do not show much changes in the structure except the hydrogen bond lengths. Obviously there should not be exactly equal WC hydrogen bond distances of GC after interaction with H^+. Distortion of hydrogen bond in some structures is observed and the dihedral angles are tabulated in Table 3. The H^+ can interact with the sites N3, N1 and N7 of G present in NWC region of GC(Figure 2). The changes in the hydrogen bonding distances and the corresponding effect on the three hydrogen bonds of WC GC are significant. Depending on the site of interaction with H^+, the effect on WC hydrogen bonds can be noted from the elongation of hydrogen bonds. Thus the H^+ is capable of exerting strong effect on the exposed NWC donor sites of GC base pair. It is evident that the N7 of guanine has a tighter interaction with H^+ than the other sites of cytosine resulting change in the WC GC hydrogen bonds(Table 1). Thus, the WC hydrogen bonds have a different balance of H^+ interaction with atomic site between the NWC GC and WC GC.

Similarly, the interaction of H^+ with AT base pair at the NWC sites has been calculated and the interaction energies are shown in Table 2**.** In the optimized structures of protonated AT base pair, the two WC hydrogen bonds are changed significantly whereas the other geometrical parameters are not drastically changed. However, in certain structures distorted H bonds are found(Figure 1). The values of dihedral angles vary from 44.91º to 64.13º. The H^+ interacts favorably with N1 of AT and N7 of GC(Tables 2 and 1).

Similar observations are found for the H^+ interaction with NWC sites of AT base pair. The differences in the WC hydrogen bond lengths due to the effect of H^+ on the donor sites of NWC AT are observed and some of the hydrogen are non planar(Figure 1, Table 4). The observation explains the variation of hydrogen bonding

ability around AT and GC sequences of DNA. In the sense, the hydrogen bonding at NWC sites can in turn influence the WC hydrogen bonds of AT and GC.

Two WC hydrogen bonds of AT and the three hydrogen bonds of GC can also exchange proton under certain condition such as the increase of solution pH. So the H^+ is capable of exerting strong effect on the WC hydrogen bonds, say O atom of WC GC may interact with H^+ resulting destabilization of existing hydrogen bonds. This situation may thereby responsible to form mismatch pairs. The various base pairs due to the interaction of H^+ with donor sites of WC GC regions are shown in Figures 2(a-i). The H^+ interaction energies for GC base pairs are given in Table 1. Obviously the formation of such mismatch base pair will not be more favorable pathway than the WC base pairing. But it may happen under certain circumstances since the effect of H^+ within WC hydrogen bonding sites may undergo proton exchange between the incoming H^+ and H bond. At an instant the formation of mismatch base pair is possible**.**

Proton Interaction with some sites of AT:

As far as we know that the protonation energies at various donor sites of AT are not equal and the values are computed with B3LYP/6-31+G (d,p). The formation of protonated structures at AT is computed choosing the donor sites A1 and A3. Similarly, the protonation at T1 and T2 are also studied. In the protonated T1, we observed three hydrogen bonds, but the pyrimidine ring of thymine appears to be broken. But the protonation at T2 does not produce much effect on the WC hydrogen bonds. There are enormous changes in the WC AT due to protonation. The structures of these protonated AT, shown in Figures 1(a-d), can be examined focusing on the hydrogen bonds. The additional -CH---O is observed in some structures and the bond lengths are shown in Table 4. The formation of -CH---O may not be ignored. Likewise, we have computed protonation energies at AT. But the effect of H^+ at A3 site results in drastic changes in the AT base pairings. Only one H-bond N---H---O is found in this base pair after protonation(Table 4, Figure 1(a)).

The T1 and T2 base pair are stabilized with three hydrogen bonds and the interaction energies are large negative values compared to other protonated pairs(-238.002 kcal/mol and -243.126 kcal/mol) which are shown in Table 2. The types of hydrogen bonds in this pairs are H...O=N-, -N-H...N-, and -O-H…O-(Figures 1(a-d)).

Proton Interaction with some sites of GC:

The sites G7, G3 and G1 are chosen for computing protonation in WC GC. The protonation energies at G7 and G3 do not show much variation in hydrogen bond pattern of GC. The three hydrogen bonds O---H-N, N-H—N and N-H—O are present in the protonated structures. Protonation at G1, G2 and G6 produces significant changes in the hydrogen bonding region(Figure 2). There are two distorted hydrogen bonds in G1 protonation, but only one distorted hydrogen bond is formed upon G2 protonation. Although G6 site is present in WC hydrogen bonding region, we still observe WC types of hydrogen bonds. Similarly, C2 and C4 are the donor sites for interaction with H^+. The C2 and C3 sites are bonded at the WC GC region, the effect of H^+ at these sites drastically change the hydrogen bonding pattern within WC region. Figure 2(g) show distorted hydrogen bond due to interaction of H^+ at C2. The protonation energies and hydrogen bond lengths are shown in Table 3. It appears that some of the WC sites and NWC sites are susceptible to proton. Moreover the types of bonding, -NH---O- and -O---H-O are very important to the formation of effective hydrogen bonds. Some of the hydrogen bonds may appear due to close contact of the sites as a result of other hydrogen bonds. However, the types of donor-acceptor in the hydrogen bonding sites are also very important for understanding the formation of stable protonated base pairs.

Interaction of WC AT and WC GC with water

As we know that a number of water molecules and ions are present around WC AT and WC GC base pairs. Some of the sites of base pairs may form hydrogen bonds with water molecule. Figures 3(a-c) display the hydrogen bond formation at A1, A3, T1 and T2. We therefore suggest that there must be some rearrangement of hydrogen bonds due to the outer (NWC site) hydrogen bond. Table 5 shows the hydrogen bonding ability at certain sites at distances ~2, the values ranges from -6.386 kcal/mol to -10.360 kcal/mol. Again we observed H_2O molecule bonded as -N---O-N- with H of H_2O, orienting towards N of A and O of T. Moreover the WC hydrogen bonds are displaced drastically in some of protonated AT, but hydrogen bonds between water donor sites of these NWC sites do not contribute much effect on WC hydrogen bonds. The observed N---H-OH bond with water at the outer site does not affect much in the WC hydrogen bond, -N-H---O- bonds. The interaction energies are also very small and the values are given in Table 5 and hydrogen bond distances are shown in Table 5.

The interactions of H_2O molecule with various sites of C of GC are also explored. The formation of hydrogen bonds at C1, C2 and C4 with H_2O are possible but interaction energies are small (Table 6). As far as the structural changes at the WC hydrogen bonding region, C1 and C4 do not produce much changes but C3 interactions produce strong effect resulting the change in the orientation of guanine nucleobase in GC.

Similarly H_2O interaction through hydrogen bonds is identified at G1, G2, G3, G6 and G7. We have found not much change in the WC hydrogen bonding and the values of interaction energies are shown in Table 6. Although, hydrogen bonding takes place at long distances with these atomic sites, the WC hydrogen bond still exists(Table 6).

Formation of metastable CT mismatches

So there are possibilities of forming mismatches after interruption of WC GC and AT. We have analysed the hydrogen bonding in a rare CT mismatch. Several tautomer pairs of C and T have been taken up and the structures of these mismatches are analysed from the pattern of the hydrogen bonds. Figures 5(a-g) shows the CT mismatches constructed from C and T tautomers(Figures 5(a-g)).

We investigate the types of hydrogen bonds in investigating the CT mismatch formation(Figures 6-7, 1NJZ.pdb)(19). Normally CT pairing could take place after destabilization of WC GC and AT. So the types of hydrogen bonds in several metastable C-T may be analysed. Once the metastable CT pair is formed, it may transfer to stable CT pair. So the types of hydrogen bonds involved in metastable CT pairing are analysed. The CT pairs are stabilized by N-H...O, N-H...N, C-H…N and O...H-N types of hydrogen bonds(Figures 5(a-g)). The most stable pair *cis*C1-*cis*T2 is stabilized by these three H-bonds whereas in some metastable pairs the presence of one or two hydrogen bonds of these types is found. Some of the CT pairs are distorted, but most of the stable CT pairs found from the contribution of these three bonds.

From the analysis of protonation on several basic sites WC GC and AT, we have noted significant changes in the WC hydrogen bonds. It means that there may be strategies to form any types of hydrogen bonds other than WC types. In this case the existence of CT is taken as an example, which has been probed from the types of hydrogen bonds in the metastable structures. The formation of metastable structures are very important for further investigation of stable mismatches because destabilization of WC GC and AT can lead to either stable tautomers of nucleobases or may pair up to form these metastable structures before transforming to stable mismatches.

In turn the type of H-bond, -N---H-N- is dominant in CT base pairs that may contribute stabilization of *cis*C1-*cis*T2 and other pairs(Table 7). It has been observed that -O---H-O- type of hydrogen bonding might be less effective than -N---H-N- type hydrogen bonding which can be indirectly assessed from the interaction energies through these bonding. Instead -O---H-O- bonding is bent whereas -N---H-N- bonding is found perfectly planar(Table 8). Hence the conditions of mispairing between C and T may be focused from the alteration of acidic or basic properties of several sites where a H-atom should mimic the -N---H-N- or -O---H-O- bonding region. Moreover structural alignment of C and T at an instant is also relevant for generating various CT mispairs.

Considering the cytosine and thymine, the mispairing sites to form hydrogen bonds between these two nucleobases are fewer compared to other purine bases(1NJZ .pdb). The structure was downloaded from protein data bank. Hence limited number of CT mispairs has been available in literature because of the conformations required for CT mispairs to generate several hydrogen bonding pattern is less, hence little information is available about CT mispairs. However it is essential to understand the structure and stability of this uncommon CT mispairs from the types of hydrogen bonds.

IV. Conclusion

The trends in the proton and H_2O interaction with several sites of WC AT and GC could analyse the types of hydrogen bonds responsible for the stabilisation of GC and AT. However, the interaction with H^+ is more effective then the hydrogen bond formation in water molecule. Investigations led to the following conclusions

1) Some of the sites in A and T are very sensitive to proton which leads to the formation of protonated species. The effect of H^+ from the other site of A and T is quite significant in few AT and GC.We have found such changes in A1T, AT1, G1C, G2C, GC1,GC3 and GC4.
2) The hydrogen bonded structures of WC AT and GC with water are not so favorable like that of protonated structures, the decomposition of hydrogen bond after interaction with H_2O molecule is rare except in GC3.
3) The types of H-bonds contributed for the stability of GC (protonated and H_2O interaction) are H-O---H-NH, N-H—N and N-H---O. Similarly, the contributed H-bonds are N-H---O and N---H-N. The hydrogen bonds are very important for the stabilization of base pair.
4) The formation of mismatch is possible depending on the orientation of hydrogen bonding sites of C and T. Hence some mismatches are found in DNA sequences other than WC base pairs.

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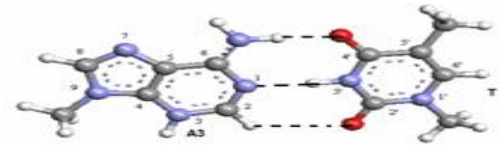
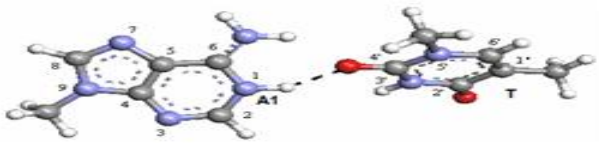
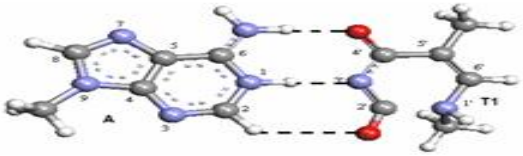


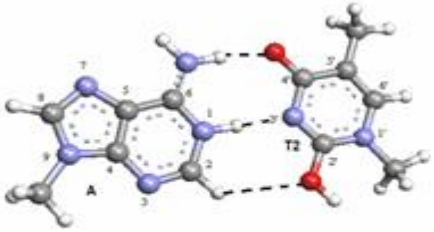
(a) Protonation at A3 of AT Base Pair.



(b) Protonation at A1 of AT Base Pair.



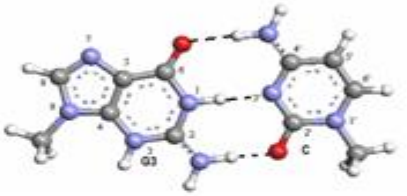
(c) Protonation at T1 of AT Base Pair.



(d) Protonation at T2 of AT Base Pair.

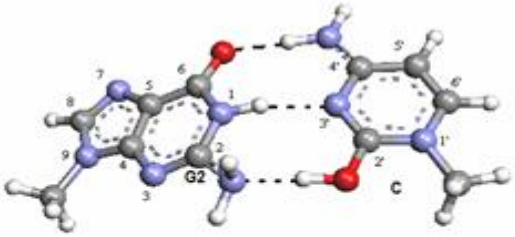
Figures 1(a-d): Protonation at various sites of AT.

(a) Protonation at G7of GC Base Pair.

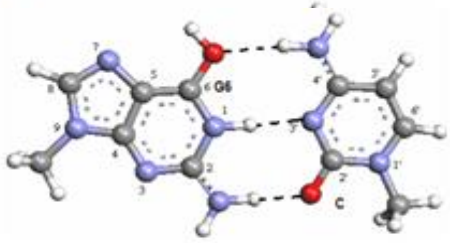


(b) Protonation at G3of GC Base Pair.

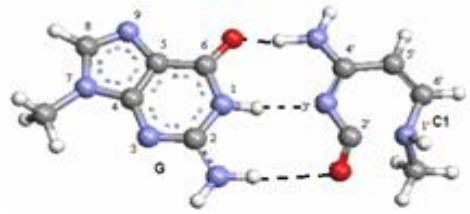
(c) Protonation at G1of GC Base Pair.



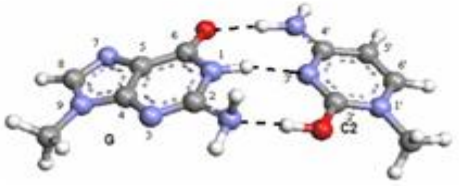
(d) Protonation at G2of GC Base Pair.



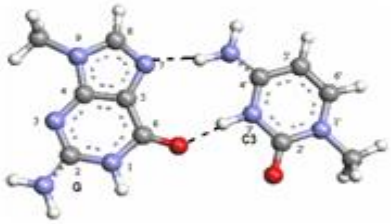
(e) Protonation at G6 of GC Base Pair.



(f) Protonation of GC Base Pair at position C1



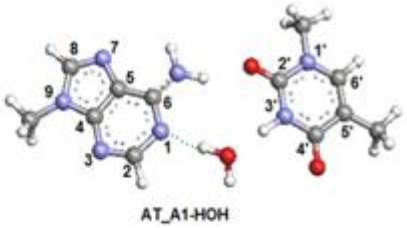
(g) Protonation of GC Base Pair at position C2



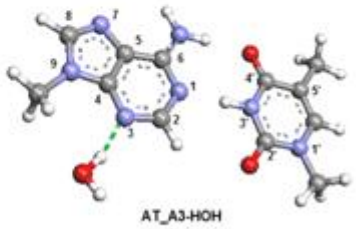
(h) Protonation of GC Base Pair at position C3

(i) Protonation of GC Base Pair at position C4.

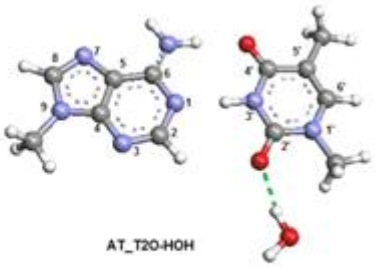
Figure 2(a-i): Protonation at several sites of GC.



(a) Interaction of water molecule at A1 site in AT.

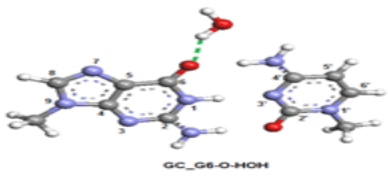


(b) Interaction of water molecule at A3 site in AT.

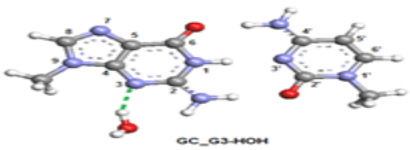


(c) Interaction of water molecule at T2O site in AT.

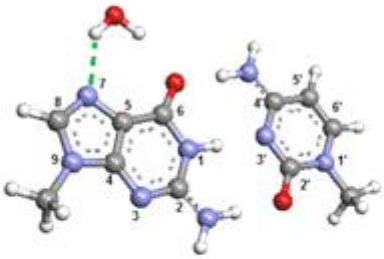
Figure 3 (a-c) Interaction of water molecule at several sites in AT.



(a)Interaction of water molecule at G6O site in GC .



(b) Interaction of water molecule at G3 site in GC.

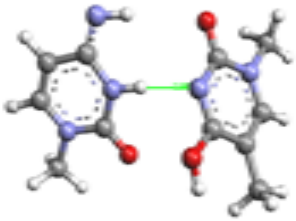


GC_G7-HOH

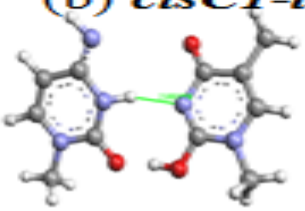
(c) Interaction of water molecule at G7 site in GC .

Figure 4 (a-c): Interaction of water molecule at several sites in GC .

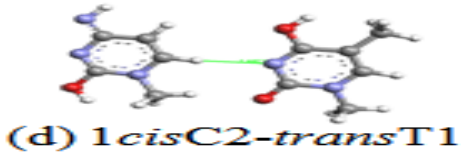
(a)*cis*C1-*cis*T2



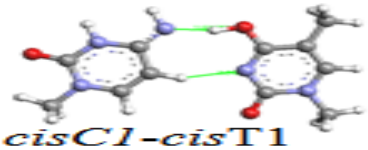
(b) ***cisC1-transT1***



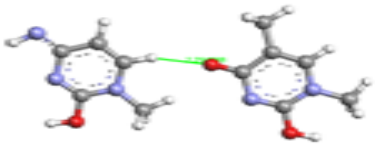
(c) *trans*C1-*cis*T2



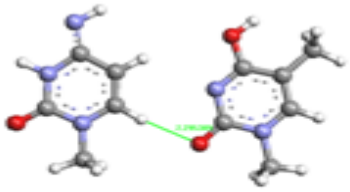
(d) 1*cis*C2-*trans*T1



(e) *cis*C*1*-*cis*T1



(f)*cis*C2-*trans*T2



(g) *trans*C1-*trans*T1

Figure 5 (a-g): Structures of CT mismatches.

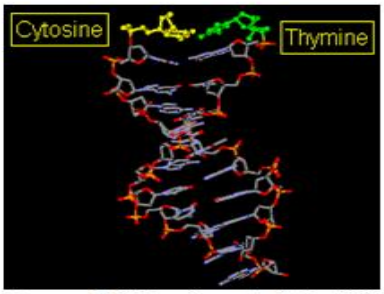


Figure 6: Structure of CT mismatch in DNA (1NJZ pdb)

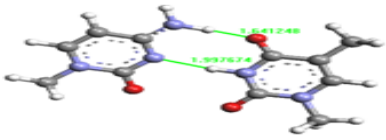


Figure 7: CT pairing in (1NJZ pdb).

Table 1: Protonation energies at different sites of GC.

Position of proton in GC base pairs	Protonation energies
	kcal/mol
G7	-247.584
G3	-249.377
G1	-257.495
G2	-213.700
G6	-241.854
C1	-252.839
C2	-241.852

C3	-257.520
C4	-251.934

Table 2: Protonation energies at different sites of AT.

Position of proton in AT base pairs	kcal/mol
A3	-244.349
A6	-239.319
A1	-201.220
T1	-238.002
T2	-243.126

Table 3: Type of hydrogen bonds in protonated GC, bond lengths, bond angles and dihedral angles.

Position of H in GC	Type of H-bonds	Bond length (Å)	CH_3-CH_3 Distance (Å)	G(α) bond angle (º)	C(α1) bond angle (º)
GC (Unprotonated)	G6O- HNC4	2.26	11.07	52.51	54.82
	G1H-C3	2.35			
	G2NH- OC2	2.34			
GC_C1H	G6O-C4NH	1.60	10.91	44.91	64.13
	G1H-C3	2.11			
GC_C2OH	G6O- HNC4	1.81	10.31	55.2	57.8
	G1H-C3	2.04			
	G2NH-OC2	1.90			
GC_C4NH2H	G1H- OC2	1.73	10.48	Distorted	Distorted
GC_G1H	G6O- HC3	1.55	11.11	Distorted	Distorted
GC_G2NH2H	G6O- HNC4	1.93	10.35	54.5	59
	G1H-C3	1.82			
	G2NH- OC2	1.46			
GC_G3H	G6O- HNC4	1.95	10.59	52	57.3
	G1H-C3	1.85			
	G2NH- OC2	1.65			
GC_G6OH	G6O- NHC4	2.11	10.58	52.1	58.9
	G1H-C3	1.93			
	G2NH- OC2	1.73			
GC_G7H	G6O-H4N	1.97	10.57	54.7	57.4
	G1H-C3	1.89			
	G2NH-C2O	1.773			

Table 4: Type of hydrogen bonds in protonated AT, bond lengths, bond angles and dihedral angles.

Position of H in AT	**Type of H-bonds**	**Bond length (Å)**	CH_3-CH_3 Distance (Å)	**A(□) bond angle (º)**	**T(□ 1) bond angle (º)**
AT (unprotonated)	A6NH-OT4	2.08	11.39	54.2	52.4
	A1- HT3	1.99			
AT_T1H	A6NH-OT4	2.08	11.18	53.1	67.5
	A1-HT3	1.58			
AT_T2OH	A6NH-OT4	1.97	11.16	56	55.5
	A1- HT3	1.53			
AT_A1H	A6NH-OT2	2.08	13.22	Distorted	Distorted
	A1- OT2	1.82			
AT_A3H	A6NH-OT4	1.93	11.34	53.9	53.9
	A1- H T3	1.88			

Table 5: Interaction site in AT with water molecule and interaction energies

Position of water in AT base pairs	Interaction energies (kcal/mol)	Distances**(Å)**
AT_A1	-7.638	1.81
AT_A3	-8.166	1.92
AT_A7	-10.360	1.87
AT_T2-O	-7.203	1.88
AT_T4-O	-6.386	1.88

Table 6: Interaction sites in GC with water molecule and interaction energies.

Position of water in GC base pair	Interaction energies(kcal/mol)	**Distance (Å)**
GC_G3	-8.646	1.89
GC_G6-O	-9.166	1.89
GC_G7	-9.825	2.21

Table 7: Computed Interaction energies and BSSE energies of metastable CT base pairs with B3LYP/6-31+G(d,p)

CT mismatch	Interaction energies in kcal/mol B3LYP/6-31+G(d,p)	BSSE energies in kcal/mol B3LYP/6-31+G(d,p)
*cis*C1-*cis*T2 (a)	-21.263	0.895
*cis*C1-*trans*T1 (b)	-8.204	1.207
*trans*C1-*cis*T2 (c)	-13.113	0.982
1*cis*C2-*trans*T1 (d)	-8.557	0.547
*cis*C1-*cis*T1 (e)	-14.697	0.842
*cis*C2-*trans*T2 (f)	-6.995	0.307
*trans*C1-*trans*T1 (g)	-6.625	0.537

Table 8: Computed H-bond distances and Planarity of metastable CT mismatch.

CT mismatches	H-bond distances (Å)	Planarity
*cis*C1-*cis*T2	H_u →2.251 Hm →1.870 H_l →1.570	Planar
*cis*C1-*trans*T1	H_u →2.013	Twisted(83.99˚)
*trans*C1-*cis*T2	H_u →2.002 H_l →1.599	Twisted(47.60˚)
1*cis*C2-*trans*T1	H_u →2.442* H_l →2.309*	Skipped
*cis*C1-*cis*T1	H_u →1.674 H_l →2.249	Planar
*cis*C2-*trans*T2	H_u →2.295	Skipped
*trans*C1-*trans*T1	H_u →2.295	Planar

The values inside the parenthesis ( ) are torsional angle and '*' is skipped H-bond
H_u, Hm and H_l indicate upper, middle and lower hydrogen bonds in metastable CT mismatches.

T.K.Barman." The Effect Of Proton And Water Molecule On Watson Crick Hydrogen Bonds And The Formation Of Cytosine(C)-Thymine(T) Base Pairs" International Journal of Research in Engineering and Science (IJRES), vol. 06, no. 04, 2018, pp. 54–65.