Role of pH in The Stability of Cytosine-Cytosine Mismatch and Canonical AT & GC Base Pairs Mediated With Silver Ion: A DFT Study

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Abstract

Metallo-nucleic acids have been investigated for their applications in the field of nanodevices and genetic expansion. The cytosine- Ag^+ -cytosine mismatch base pair interactions and their stability in nucleic acids have attracted the attention of chemists. We report a systematic study of canonical, mismatch, Ag^+ mediated system with CC, AT, and GC base pairs computationally. The stability of such mismatch base pairs is dependent on the pH range ~ 5 to 9 and the duplexes beyond this range are unstable. The DFT calculations performed with the model DNA duplexes comprising of such mismatch pairs reveal the stability trend while varying the pH conditions. The stability of canonical Watson-Crick ATGC base pairs was compared with the CCAT and CCGC mismatch base pairs and the calculated results at B3LYP-D3/6-31G* level of theory in the aqueous phase suggest that the base stacking and hydrogen bonding are well maintained in the former case, however, the larger perturbations in the geometry are observed with the mispair and relatively unstable. The calculated binding energy at B3LYP/6-31G* level of theory of ATGC is energetically more stable (~ 15 kcal/mol) than the mismatch base pairs. The Ag^+ mediated mismatch base pairs i.e., C_CAT and C_CGC examined at the same level of theory suggest that the CC mismatch base pairs complexed with proper alignment to the Ag^+ ion and the AT and GC bases maintained the hydrogen bonding interactions. The mismatched base pair duplex systems i.e., C_CAT and C_CGC are structurally similar to the canonical Watson-Crick base pairs and energetically stable by ~ 40 and ~ 50 kcal/mol compared to the canonical ATGC base pairs. The experimental report on the thermal transition profile in 5'-(A)₁₀C(A)₁₀-3' and 5' (T)₁₀C(T)₁₀-3' duplexes showed remarkable stability and corroborate the calculated results.[1] The stability of Ag^+ mediated mismatch bases at the higher pH 9 was also examined and the nucleobases such as guanine and thymine would be deprotonated under this condition. The calculated results suggest that the CCA_T and CCG_C duplexes are largely distorted with the complexation of Ag^+ with the AT and GC base pairs and would in turn denature the duplex. The AIM analysis performed at B3LYP-D3/6-31G* level of theory for all the studied Ag^+ mediated complexes reveals that the Ag^+ interaction with the corresponding nucleobases was electrostatic in nature. The role of pH in governing the stability of C- Ag^+ -C complex formation in mismatch base nucleic acids is crucial for their application of genetic expansion and nucleic acid-based nanodevices.

Introduction

Metallo-nucleic acids have drawn attention toward biological purposes such as stability of secondary structures, nucleic acid folding (triplexes, quadruplex, and RNA folding), and their crucial role in catalytic activities.[1–6] The DNA duplexes having metal-mediated natural mismatch have been used in the development of bio-nano materials such as DNAzymes, DNA magnets, biosensors, logic gates, etc.[4, 5] Metal-mediated nucleic acids have been formed by replacing the Watson-Crick hydrogen bonding with metal-base coordination complexes. For example, thymine-thymine (TT) and cytosine-cytosine (CC) mismatch in duplexes selectively captures the Hg^{2+} and Ag^+ ions.[1–3] The other experimental techniques such as UV melting, electrospray ionization mass spectroscopy, circular dichroism, and NMR

have also confirmed the thermodynamic and structural property of the C-Ag⁺-C complex in DNA duplex.[7, 8] The Isothermal titration calorimetry (ITC) results also confirmed that the Ag⁺ ion specifically interacts with the CC mismatch at a 1:1 molar ratio and with a higher binding constant as compared to the other metal ions.[7, 8] The stability of Ag⁺ was also reported with cytosine-thymine(C-T) and cytosine derivative, 5-methylisocytosine base pairs.[9] The incorporation of cytosine derivative, 5-methylisocytosine-cytosine (m⁵iC-C) base pairs mediated with silver ion also showed comparable stability as compared to the naturally occurring CC mismatch.[9] Recently, the development of metal triggered replicating system and DNA nanodevice recognized by DNA polymerases was achieved by the formation of the C-Ag⁺-A complex. [10] Okamoto et al., have investigated the metal ion property of artificial nucleobases thiopyrimidine mediated with two equivalents of Ag⁺ ion, which leads to the significant stabilization of the DNA duplex. High-resolution crystal structure of consecutive dinuclear Ag⁺ mediated base pairs with artificial 4-thiothymine-2Ag⁺-4-thiothymine nucleobase by forming a rectangular network of silver ions has also been reported.[11] Experimentally, the silver arrays inside the DNA duplexes were also constructed by complexation with artificial cytosine analogs.[12, 13]

The crystal structure of the RNA duplex with the C-Ag⁺-C linkage was also determined.[14] Similarly, structural determination of silver mediated with CC mismatch within the DNA duplex and their characterization of the C-Ag⁺-C complex was determined by NMR spectroscopy in solution.[15] The 3D structure of DNA-Ag⁺ has also revealed the structural conformation of the duplex with a notable propeller twist in C-Ag⁺-C formation.[15] Molecular dynamics simulations have been performed for the Ag⁺ mediated double helix.[16] The comparison of parallel and antiparallel strand orientation of the double helix of C₁₁-(Ag⁺)-C₁₁ was also examined by molecular dynamics simulations.[16] The stability of cytosine-Ag⁺-cytosine and cytosine-Ag⁺-adenine was also investigated by the quantum chemical study. [17] Gwinn et al., have also investigated the Ag⁺ ion interaction of mismatch systems such as AA, CC, GG, and TT using high-resolution electrospray ionization mass spectroscopy and quantum chemical calculations.[18] The experimental studies suggest that the formation of the C-Ag⁺-C complex is pH-dependent (5 to 9) and beyond this range, the disruption of the DNA duplexes was noticed.[1] Therefore, it would be interesting to examine the interaction of Ag⁺ ion mediated to the canonical base pair (A-Ag⁺-T and G-Ag⁺-C) and cytosine-cytosine mismatch-base pair (C-Ag⁺-C) and their stability in duplex formation with the involvement of mismatch mimicking the experimental pH condition. We have examined the stability of Ag⁺ mediated canonical base pairs (AT and GC) with the CC mismatch in the DNA duplex computationally. The calculated results would reveal the specific coordination of the Ag⁺ with the cytosine-cytosine mismatch-base pair in DNA duplexes under a certain pH condition range, which, however, is not fully understood in the literature.

Computational Methods

All geometries of canonical, mismatch and Ag⁺ mediated with CC base pairs were fully optimized using the B3LYP-D3/6-31G* level of theory in the aqueous phase using the polarized continuum solvation

(PCM) model.[19, 20] The analysis of positive vibration frequencies of all complexes confirmed the minimum optimized geometries. For the studied systems, the basis set superposition error (BSSE) was not considered because all the studied systems were investigated in the aqueous phase. Grimme's London dispersion corrected density functional theory DFT-D3 is also computed for all the studied systems. The DFT-D3 has a crucial role to provide accurate interaction energies.[21] The LANL2DZ i.e., effective core potential was employed for the Ag⁺ mediated systems. The binding energies were calculated using equation 1.

Binding energies, $\Delta E = E_{\text{complex}} - (E_{\text{DNA}} + E_{\text{Ag}^+})$ ..... (1)

E_{complex} refers to the energy of the complex of canonical, mismatch, and mediated Ag⁺ mediated ion, E_{DNA} refers to the energy of the isolated DNA counterparts, and E_{Ag^+} is the energy of studied silver ion. The corresponding wavefunctions were utilized for the molecular electrostatic potential (MESP) calculations using Molekel 4.3 software.[22] MESP calculations were performed using the following equation 2.

$$V(\mathbf{r}) = \sum_A \frac{Z_A}{|\mathbf{R}_A - \mathbf{r}|} - \int \frac{\rho(\mathbf{r}')}{|\mathbf{r}' - \mathbf{r}|} \text{..... (2)}$$

$V(r)$ is the potential at distance r' by the nuclei and electrons of the molecule, $\rho(r')$ is the electron density, and Z_A is the charge of nucleus A at point R_A .

The computational tool of Bader's atom in molecules (AIM) is crucial to unravel the behavior and nature of the atom-atom interactions in non-covalent & covalent in small molecules, macromolecules such as proteins, crystals, base pairing in DNA as well as stacking.[23] The Bader's atom in molecules (AIM) was examined for all the systems using Multiwfn software with the wave functions generated at the same level of theory.[24] All the calculations were performed with the Gaussian 09 suite of programme.[25]

Results And Discussion

The standard crystal structures of the studied DNA of canonical and mismatch systems were obtained from the protein data bank (<http://www.rcsb.org>) crystal structural database (CSD) PDB ID: 5XUV and 1BNA to model the study.[11, 26] The crystal structure of silver mediated cytosine-cytosine (CC) mismatch in DNA was obtained from PDB ID: 5XUV.¹¹ Experimentally, the specific interaction between the Ag⁺ ion and cytosine-cytosine mismatch in DNA duplexes has been investigated.[1] The experimental report suggests that the addition of Ag⁺ ion to the canonical Watson-Crick base pairs (AT & GC) does not influence the transition profile.[1] The studies with other metal ions which are known to bind nucleic acids (Hg²⁺, Cu²⁺, Ni²⁺, Pd²⁺, Co²⁺, Mn²⁺, Zn²⁺, Pb²⁺, Cd²⁺, Mg²⁺, Ca²⁺, Fe²⁺, etc) have shown no notable effect on the thermal transition profile.[1] The effect of pH in the C-Ag⁺-C complex formation is important to impart stability in DNA and helix adaptation. The C-Ag⁺-C complex in the DNA duplex is stable from the pH range between 5 and 9, however, the duplex was found out to be destabilized outside this pH range.[1]

Further, in the absence of Ag^+ ion, the CC mismatch was reported to be stable around pH 5, due to the protonation of the cytosine nucleobases.[27] Generally, the guanine and thymine nucleobases have a pK_a value at $\sim 9\text{--}10$ and act like weak acids (Scheme 1).[28]

There are reports available of CC mismatch mediated with Ag^+ with canonically paired with AU/AT base pair in RNA & DNA duplex.[1] However, the CC mismatch mediated with Ag^+ with GC base pairs in the duplex was not reported. The destabilization of canonical base pairs with Ag^+ mediated CC mismatch at a higher pH range has been examined computationally. The deprotonated canonical base pairs at higher pH (~ 9.0) can offer an opportunity to complex with the Ag^+ and stabilize the duplexes. Nevertheless, the stabilization in such duplexes was not observed. To examine the stronger basicity of such deprotonated nucleotides, we have analyzed the molecular electrostatic potential (MESP) of deprotonated DNA nucleotides (Adenosine, Thymidine, Guanosine & Cytidine). The MESP generated for the DNA deprotonated nucleotides was computed at the B3LYP-D3/6-31G* level of theory in the aqueous phase (**Figure 1**). The negative potential (V_{min}) was calculated for the imine nitrogens to coordinate with the metal ions of deoxyadenosine monophosphate (dAMP), deoxyguanosine monophosphate (dGMP), deoxythymidine monophosphate (dTMP) & deoxycytidine monophosphate (dCMP) suggests that dCMP would have lesser ability to bind in DNA duplexes.

All geometries with canonical base pairs (ATGC), mismatches (CCAT, CCGC), and CC mismatch mediated with canonical base pairs (C_CAT, C_CGC, CCA_T, CCG_C) have been optimized at B3LYP-D3/6-31G* level of theory in the aqueous phase. The optimized geometries of canonical base pairs and CC mismatch-base pairs are shown in figure 2. The Watson-Crick hydrogen-bonding pattern was observed in the case of canonical ATGC base pairs. The distance of canonical base pairs has been found out to be ($1.98 \text{ \AA}$ and $1.80 \text{ \AA}$) in AT and ($1.88 \text{ \AA}$, $1.87 \text{ \AA}$, and $1.88 \text{ \AA}$) in GC base pairs respectively (Figure 2a). The ATGC canonical base pairs corroborate well with the crystal structure of dodecamer DNA (PDB ID: 1BNA).[26] The stacking interactions are also between the range of $\sim 3.5 \text{ \AA}$ which is well in agreement with the available DNA dodecamer structure.²¹ The torsional angles calculated at B3LYP-D3/6-31G* level for the model system in the aqueous phase have been found out to be in good agreement with the 1BNA ATGC base pairs PDB structure (Table 1).

Table 1: The comparison of the torsional angle of standard PDB canonical ATGC pairs and our calculated ATGC pairs calculated at B3LYP-D3/6-31G* level of theory in the aqueous phase.

System	PDB (1BNA)	Our model
	Torsional angle	Torsional angle
ATGC	$\alpha = -73.1^\circ$	$\alpha = -50.0^\circ$
	$\beta = 171.0^\circ$	$\beta = 158.5^\circ$
	$\gamma = 73.2^\circ$	$\gamma = 59.1^\circ$
	$\delta = 135.9^\circ$	$\delta = 148.3^\circ$
	$\epsilon = 174.1^\circ$	$\epsilon = 151.4^\circ$

In the case of the duplex containing CC mismatch (CCAT & CCGC), the hydrogen bonding distance between AT base pairs in CCAT are 1.90 Å and 1.81 Å, respectively, whereas, the hydrogen bonding distance between GC base pairs in CCGC are 1.93 Å, 1.84 Å, and 1.89 Å, respectively (Figure 2b & 2c). The presence of CC mismatch in these duplexes caused perturbations in the alignment of base pairs. The perturbations influenced the hydrogen bonding between the AT and GC base pairs in (CCAT & CCGC) systems (Figure 2). The CCAT mismatch showed larger distortions in the alignment of CC mismatch and appears that would significantly destabilize such DNA duplexes. The calculated binding energies using B3LYP-D3/6-31G* level of theory of ATGC, CCAT, and CCGC are -45.6, -30.5, and -39.3 kcal/mol respectively. The canonical ATGC base pairs are energetically more stable than the studied mismatch systems (Table 2).

The perturbations observed in the CCAT and CCGC duplex model systems, we have examined such metal-mediated duplexes to stabilize with proper alignment of the base pairs. Earlier experimental reports concluded that CC mismatch selectively captures the Ag^+ ion and stabilizes DNA/RNA duplex.[1] Wang et al. reported the interaction of Ag^+ with CC mismatches and their derivatives in an α -hemolysin nanopore and concluded that the silver ion stabilizes the double-stranded DNA duplex with CC mismatches.[29] Molecular dynamics simulations corroborate the cation binding site and the CC mismatch conformation in DNA duplex.[29]

Recently, the 3D structure of DNA containing C- Ag^+ -C complex in the duplex was investigated by the nuclear Overhauser effect (NOE).[15] The study was carried out in anti-parallel of C- Ag^+ -C (cis-conformation) was not distorted in the duplex and maintained the most stable B-form conformation in such duplexes.[15] We have also modeled the cis conformation of C- Ag^+ -C in the DNA duplexes. The binding of Ag^+ ion with the CC mismatch calculated at B3LYP-D3/6-31G* level of theory in the aqueous phase (Figure 2b & 2c). The geometries of C- Ag^+ -C with canonical base pairs (C_CAT, C_CGC), A- Ag^+ -T & G- Ag^+ -C with CC mismatch (CCA_T, CCG_C) have been optimized using B3LYP-D3/6-31G* level of theory in the aqueous phase (**Figure 3**). The base pairs designated with the underscore “_” represent that Ag^+ is coordinated with the imine nitrogen of such bases.

The computational studies have been performed with Ag^+ mediated cytosine base pairs and mismatch assuming that the pH of the medium is neutral, however, the effect of pH at the range of ~ 9 and above were not explored with the DNA duplexes.[1] The bases like thymine and guanine would be deprotonated at this pH range and hence the coordination site of Ag^+ would change from cytosine base pairs to canonical base pairs (CCA_T, CCG_C). In the case of the C_CAT model duplex system, the distance between the imine nitrogens of cytosines (N-Ag^+) was found out to be 2.21 Å, which is in good agreement with the literature reports.[16] The Watson-Crick hydrogen-bonding interactions between the adenine and thymine were found to be 1.83 Å and 1.92 Å, respectively. The propeller twist can be observed to corresponding cytosine nucleobases when mediated with the Ag^+ ion. A similar propeller twist in the crystal structure was also observed in the C- Ag^+ -C formation.[14] The angle of N-Ag^+ -N lies between 165° - 168° of C- Ag^+ -C complex formation in C_CAT. The lone pair repulsions of the imine nitrogen's of cytosines in CCAT is compensated with the inclusion of the Ag^+ , which provides an electrostatic attraction in C_CAT, and hence the base pair distortions are less significant in the former case (Figure 2b & 3a). The stacking in the C_CAT between the corresponding base pair in the model system was also found to be ~ 3.5 Å (Figure 3). Similarly, in the case of C_CGC duplex system, the distance between the imine nitrogen of cytosines (N-Ag^+) was found out to be 2.20 Å and the angle lies between them was 167° . The three Watson-Crick hydrogen bonding was maintained in the GC base pairs with the distances of 1.88 Å, 1.86 Å, and 1.86 Å respectively. These hydrogen-bonded GC pairs were well corroborated with the canonical hydrogen bonding in the ATGC base pairs system. The stacking interaction between the corresponding nucleobases was also observed in the range of ~ 3.5 Å which is in good agreement with the ATGC base pair system. The calculated results revealed that the CC mediated with the Ag^+ ion (C_CAT and C_CGC) have higher stability of the duplexes than the canonical ATGC and the studied CC mismatch-base pair (C_CAT and C_CGC). The C_CAT is ~ 40 kcal/mol more stable than canonical ATGC base pairs whereas C_CGC is ~ 50 kcal/mol more stable than that of canonical ATGC base pairs. The Gibbs free energies, ΔG and change in enthalpy, ΔH were also calculated using B3LYP-D3/6-31G* level of theory in the aqueous phase and showed a similar trend of stability (Table 2).

Further, we have examined the interaction of Ag^+ with the canonical bases i.e., the $\text{pH} > 9$, and the calculations have been performed with deprotonated canonical nucleobases mediated with silver ion in the duplex system (CCA_T, CCG_C). In CCA_T mismatch, one of the hydrogen bonding's in the AT base pair was replaced with Ag^+ mediated interactions of imine nitrogen's and the calculated distances were 2.26 Å and 2.19 Å, respectively. The Watson-Crick hydrogen bonding was also observed with the Adenine- NH_2 and thymine- O_2 with a distance of 1.96 Å. The CC mismatch was distorted significantly and the hydrogen bonding in the CC mismatch with a distance of 1.97 Å was observed. The angle of N-Ag^+ -N was found to be $\sim 146^\circ$ in the A- Ag^+ -T complex. The calculations performed with Ag^+ mediated CCG_C duplex model showed larger distortions in the system. The Ag^+ is displaced in the middle of the duplex and the GC base pairs experience the repulsions of imine nitrogen's, which in turn perturbs the system. The larger ionic radii of Ag^+ ion (1.65 Å) than the hydrogen (1.20 Å) is not appropriate to fit in the Watson-crick AT and GC base pairs and therefore the silver ion is not aligned to the base pairs as observed with the

hydrogen-bonded DNA duplexes. The calculated binding energies using the same level of theory in CCA_T and CCG_C are -83.2 and -103.5 kcal/mol, respectively. The higher stability of CCG_C compared to C_CGC due to the additional interactions of Ag^+ with imine nitrogens and hydrogen bonding upon severe perturbation in the duplex system (Figure 3b and 3d). The phosphate backbone also folds and contributes to the stabilization of CCG_C (Figure 3d). The free energies (ΔG) and change in enthalpy (ΔH) were also calculated using the same level of theory in the aqueous phase and predicted a similar trend of stability (Table 2). These results suggest that the formation of Ag^+ mediated CCA_T would be less favored compared to C_CAT even at a higher pH range and the geometrical perturbations in the former case destabilize the duplexes.[1] The experimental reports are not available with Ag^+ mediated CCG_C, however, the computational results suggest larger perturbations in the duplexes and would disfavor the formation of such complexes.

The binding behavior of the silver ion to the corresponding cytosine bases in the C- Ag^+ -C complex has been analyzed by atom in molecules (AIM) analysis at the same level of theory in the aqueous phase (Table 3). The critical points (CPs) have been generated to explore the binding behavior of Ag^+ to the studied complexes. The interaction of silver ion to the imine region of canonical base pairs and studied mismatch complexes can be characterized by using other topological approaches such as gradient (Laplacian) of the electron density ^2r , total energies $H(r)$, the potential energies $V(r)$, and Langrangian kinetics energies $G(r)$. The non-covalent interaction i.e., (3, -1) critical point determines the bonding behavior of Ag^+ metal ion and its complex with the studied systems. When $|V(r)| > G(r)$ and $H(r)$ have a negative value, the interaction is said to be shared whereas when $|V(r)| < G(r)$ and $H(r)$ have a negative value, the interaction would be a closed shell. The interaction would be covalent in nature, when the ratio is $|V(r)| / G(r) > 2$ whereas the interaction would be a closed shell when the ratio is $|V(r)| / G(r) < 1$. If the ratio falls between the value of 1 and 2, the intermediate situation would occur. In the case of canonical hydrogen-bonded ATGC, the critical points for the hydrogen-bonded AT base pairs are CP-348 and CP-285 whereas for GC base pairs are CP-209, CP-287, and CP-355 respectively. The ratio, $|V(r)| / G(r)$ of hydrogen bonding between the base pairs is less than 1 which means the interaction is found out to be a closed shell. The stacking interaction for the ATGC base pairs (CP-242, CP-284, CP-251, CP-327, CP-203, and CP-360) between the corresponding nucleobases is also observed and these interactions are found out to be the intermediate situation.

Table 2: Binding energies (ΔE), change in enthalpy (ΔH), and Gibbs free energies of canonical mismatch, and Ag^+ mediated systems optimized at B3LYP-D3/6-31G* level of theory in the aqueous phase. Values are given in kcal/mol.

DNA model systems	B3LYP-D3/6-31G* ΔE (kcal/mol)	B3LYP-D3/6-31G* ΔH (kcal/mol)	B3LYP-D3/6-31G* ΔG (kcal/mol)
ATGC	-45.6	-41.5	-26.9
CCAT	-30.5	-26.3	-13.4
CCGC	-39.3	-35.0	-21.8
C_CAT	-85.7	-78.7	-70.4
C_CGC	-96.1	-87.3	-82.4
CCA_T	-83.2	-77.5	-66.5
CCG_C	-103.5	-103.7	-95.4

In the case of CCAT, the critical points generated with hydrogen-bonded CP-304 and CP-233 are closed-shell interactions. The intermediate situation arose for the stacking interactions in which generated CPs are CP-213, CP-261, CP-201, CP-292, CP-218, and CP-245 respectively. Similarly, the critical points generated for the CCGC systems having closed-shell interactions (CP-302, CP-242, CP-161, and CP-241) and the ratio $|V(r)| / G(r)$ falls between the value of 1 and 2 (CP-333, CP-174, CP-299, CP-236, and CP-215) whereas the intermediate situations occur. In the case of silver ion mediated with the canonical AT and GC with CC mismatch (CCA_T & CCG_C), the hydrogen-bonded critical points are CP-308 for AT, CP-163 for GC are electrostatic in nature. The critical points (CP-219 and CP-222) generated for Ag^+ mediated with AT (Adenine N- Ag^+ -N Thymine) are electrostatic in nature whereas the CPs (CP-230 and CP-240) generated for the Ag^+ mediated with GC (Guanine N- Ag^+ -N Cytosine) are also electrostatic in nature. The CPs (CP-227, CP-297, CP-166, CP-315, CP-200, CP-212, CP-342, CP-310, and CP-347) generated for the stacking interactions were found to be the intermediate situation.

Furthermore, in the case of Ag^+ mediated systems with cytosine-cytosine base pairs (C_CAT & C_CGC), the critical points of Ag^+ interacting with the nitrogen of the cytosine base pairs are CP-295 & CP-285. These interactions between the cytosine base pairs and the Ag^+ metal ions are electrostatic in nature. The critical points of hydrogen-bonded AT base pairs are CP-286, and CP-224 found out to be electrostatic in nature. The critical points of stacking interactions in C_CAT generated such as CP-202, CP-331, CP-321, CP-253, and CP-297 fall under the intermediate situation. In the case of C_CGC, the critical points generated for the Ag^+ mediated with the CC mismatch (CP-285 and CP-296) are electrostatic in nature and the CPs (CP-215, CP-170, and CP-277) of hydrogen-bonded with GC pairs are found out to be electrostatic in nature. The stacking interactions in the C_CGC mainly fall between the value of 1 and 2 which is the intermediate situation.

Table 3: Atoms in molecules (AIM) analysis of canonical and CC mismatch-base pairs mediated with Ag^+ calculated at B3LYP-D3/6-31G* level of theory in the aqueous phase.

DNA Systems	Critical Points (CPs)	Density of all electrons	Laplacian of electron density (r)	Total Energy density H(r)	Potential energy density V(r)	Lagrangian kinetic energy G(r)	$\frac{V(r)}{G(r)}$
ATGC	CP-348	0.02509	0.07369	-0.00101	-0.02046	0.01944	0.95
		0.04461	0.11649	-0.00275	-0.03463	0.03188	0.92
	CP-285	0.03076	0.09313	-0.00114	-0.02558	0.02443	0.96
	CP-209	0.03717	0.09776	-0.00183	-0.02810	0.02627	0.93
		0.03623	0.11109	-0.00120	-0.03017	0.02897	0.96
	CP-287	0.00541	0.01732	0.00083	-0.00266	0.00349	1.31
	CP-355	0.00586	0.01922	0.00069	-0.00340	0.00410	1.21
		0.00459	0.01567	0.00051	-0.00289	0.00340	1.18
	CP-242	0.00556	0.01895	0.00081	-0.00311	0.00392	1.26
	CP-284	0.00670	0.01987	0.00037	-0.00421	0.00459	1.09
		0.00236	0.01303	0.00077	-0.00170	0.00248	1.46
	CP-251						
	CP-327						
	CP-203						
	CP-360						
CCAT	CP-304	0.03030	0.09059	-0.00120	-0.02505	0.02385	0.95
		0.04201	0.11045	-0.00236	-0.03234	0.02997	0.93
	CP-233	0.00791	0.02814	0.00085	-0.00532	0.00617	1.16
	CP-213	0.00650	0.02115	0.00067	-0.00392	0.00460	1.17
		0.00666	0.02379	0.00066	-0.00461	0.00527	1.14
	CP-261	0.00489	0.01562	0.00058	-0.00273	0.00332	1.22
	CP-201	0.00596	0.01827	0.00072	-0.00310	0.00383	1.24
		0.00678	0.01970	0.00064	-0.00362	0.00427	1.18
	CP-292						
	CP-218						

	CP-245						
CCGC	CP-302	0.02784	0.08470	-0.00094	-0.02306	0.02212	0.95
		0.03973	0.10505	-0.00208	-0.03042	0.02834	0.93
	CP-242	0.03028	0.08995	-0.00131	-0.02512	0.02380	0.94
	CP-161	0.03142	0.08523	-0.00125	-0.02382	0.02256	0.94
		0.02080	0.07949	0.00155	-0.01676	0.01832	1.09
	CP-241	0.00717	0.02448	0.00062	-0.00486	0.00549	1.12
	CP-333	0.00619	0.02090	0.00086	-0.00350	0.00436	1.24
		0.00067	0.02254	0.00110	-0.00341	0.00452	1.32
	CP-299						
	CP-236						
	CP-215						
CCA_T	CP-308	0.02554	0.07909	-0.00062	-0.02103	0.02040	0.97
		0.06251	0.28672	-0.00657	-0.08483	0.07825	0.92
	CP-219	0.07329	0.33411	-0.01078	-0.10509	0.09431	0.90
	CP-222	0.00613	0.01841	0.00041	-0.00378	0.00419	1.11
		0.00691	0.02172	0.00071	-0.00400	0.00471	1.18
	CP-227	0.00128	0.00761	0.00053	-0.00082	0.00136	1.66
	CP-297	0.01001	0.03254	0.00078	-0.00656	0.00734	1.12
		0.02475	0.08764	0.00030	-0.02129	0.02160	1.01
	CP-166						
	CP-315						
	CP-200						
CCG_C	CP-230	0.05314	0.23771	-0.00368	-0.06679	0.06311	1.05
		0.04805	0.21545	-0.00225	-0.05836	0.05611	1.04
	CP-240	0.03509	0.11769	-0.00029	-0.03000	0.02971	1.00
	CP-163	0.00989	0.02912	0.00760	-0.00575	0.00652	0.88
		0.03685	0.12185	-0.00050	-0.03147	0.03096	1.01

	CP-301	0.00365	0.01280	0.00047	-0.00225	0.00272	0.82
		0.00355	0.01433	0.00086	-0.00184	0.00271	0.67
	CP-363	0.00739	0.02638	0.00114	-0.00430	0.00545	0.78
							0.80
	CP-212	0.00402	0.01524	0.00062	-0.00256	0.00318	
	CP-342						
	CP-310						
	CP-347						
C_CAT	CP-295	0.06974	0.31839	-0.00933	-0.09827	0.08893	0.90
		0.06989	0.31679	-0.00940	-0.09799	0.08859	0.90
	CP-285	0.02852	0.08507	-0.00102	-0.02331	0.02229	0.96
	CP-286	0.04057	0.10931	-0.00190	-0.03114	0.02923	0.94
		0.01312	0.03917	0.00049	-0.00879	0.00929	1.06
	CP-224	0.00572	0.02097	0.00075	-0.00372	0.00448	1.20
	CP-264	0.01766	0.05166	0.00010	-0.01269	0.01280	1.01
		0.00189	0.00679	0.00035	-0.00098	0.00133	1.36
	CP-202	0.00738	0.02140	0.00035	-0.00465	0.00500	1.08
	CP-331	0.00571	0.02178	0.00098	-0.00347	0.00446	1.29
	CP-321						
	CP-253						
	CP-297						
C_CGC	CP-215	0.03784	0.10117	-0.00167	-0.02865	0.02697	0.94
		0.03183	0.09850	-0.00090	-0.02643	0.02553	0.97
	CP-170	0.03090	0.09368	-0.00112	-0.02567	0.02454	0.96
	CP-277	0.07194	0.03258	-0.01021	-0.10189	0.09167	0.90
		0.07109	0.32459	-0.00986	-0.10087	0.09101	0.90
	CP-285	0.00648	0.01992	0.00047	-0.00403	0.00450	1.12

CP-296	0.00654	0.01865	0.00060	-0.00345	0.00405	1.17
	0.00618	0.01638	0.00023	-0.00363	0.00386	1.06
CP-276	0.02685	0.08890	-0.00024	-0.02271	0.02247	0.99
CP-241	0.00792	0.02130	0.00017	-0.00497	0.00515	1.04
	0.31576	-0.01587	-0.43573	-0.47470	0.03896	0.08
CP-244						
CP-318						
CP-265						
CP-335						

We have further examined the non-covalent interaction (NCI) between the stacked nucleobases at the same level of theory using Multiwfn software. The NCI analysis is supported by electron density and reduced density gradient (RDG). The color-filled RDG isosurface of all the studied canonical, mismatch and Ag⁺ mediated systems with their side view and the top view are represented in figure 4. The green circle is showing non-covalent interactions of the stacked nucleobases whereas the light brown circle depicts the low electron density (Figure 4). From the color-filled RDF isosurface, the identification of van der Waals interactions (vdW) can be seen clearly in ATGC base pairs by the presence of green circle between the stacked bases. The blue circle was also observed in ATGC which represents the strong attraction i.e., H-bonding between the adenine-thymine (AT) and guanine-cytosine (GC) base pairs. In the case of mismatch systems CCAT & CCGC, the NCI plots reveal weaker interaction between the base pairs due to the perturbation in CC mismatch in the duplex. (Table 2 and Figure 4). The electron density (λ_2) characterizes the behavior of stacked nucleobases and hydrogen bonding between them. The electron density (λ_2) divided into three categories, $\lambda_2 = 0$ tells about the weak interactions, when $\lambda_2 < 0$, strong interactions such as hydrogen bonding would occur, whereas, when $\lambda_2 > 0$, steric repulsive interaction will occur. The scatter plots of gradient isosurface (0.5 a.u) of canonical base pairs and mismatch systems are given in Figures 5 and 6.

Further, we have examined the NCI of Ag⁺ mediated with the canonical bases (CCA_T, CCG_C) and results reveal that the base pair stacking interactions are weaker in this case. In the case of C_CAT and C_CGC, the vdW interactions can be seen marked by green circle, which is well stacked to the corresponding nucleobases similar to the ATGC duplex.[30] The blue circle was also observed due to Watson-Crick between AT and GC base pairs. The NCI results corroborate well with the stability trend of these mismatches. The scatter plots of gradient isosurface (0.5 a.u) of C_CAT and C_CGC are given in figure 6.

Conclusion

In this work, we have examined the role of pH in canonical, mismatch, Ag^+ mediated with canonical base pairs (CCA_T and CCG_C) and Ag^+ mediated with CC mismatch-base pair (C_CAT and C_CGC) using B3LYP-D3/6-31G* level of theory in the aqueous phase. The calculated results reveal that the canonical ATGC base pairs are energetically stable by ~ 15 kcal/mol from CCAT and ~ 6 kcal/mol from CCGC mismatch base pairs. The structural analysis shows that the torsional angles of the canonical ATGC model were well corroborated with the standard PDB ID: 1BNA. The mismatch-base pair systems, CCAT & CCGC showed larger distortion in the alignment of the CC base pairs, which lead to the destabilization of the duplexes. The Ag^+ mediated CC mismatch base pair (C_CAT and C_CGC) showed remarkable stability ~ 40 -50 kcal/mol than the canonical Watson crick ATGC duplex model system. The Gibbs free energies and enthalpy results also corroborated the stability trend. The effect of higher pH ($\text{pH} > 9$) in the duplex model systems and their stability was also examined. The nucleobases such as guanine and thymine nucleobases would be deprotonated under such pH conditions. The MESP analysis of DNA deprotonated nucleotides calculated at the same level of theory in the aqueous phase revealed the maximum negative potential of dCMP and dTMP nucleotides reside around the imine regions. The dTMP has maximum negative potential to the imine region in the deprotonated situation than the dCMP. The larger atomic radii of Ag^+ in the canonical base pairs (A- Ag^+ -T and G- Ag^+ -C) showed larger perturbations in the model duplex systems and would denature the duplexes. The binding nature of Ag^+ interaction with the corresponding nucleobases was confirmed by the AIM analysis. All Ag^+ mediated complexes (C- Ag^+ -C, A- Ag^+ -T, and G- Ag^+ -C) have shown the electrostatic interactions in the model duplex systems. The NCI analysis revealed the stacking and hydrogen bonding between the canonical, mismatch-base pairs and Ag^+ mediated systems by electron density and color-filled RDG. The computational results revealed the dependence of Ag^+ mediated with CC mismatch-base pair (C- Ag^+ -C) in DNA mismatch base pair duplexes and rationalized the observed experimental results.

Declarations

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Associated Content:

Supporting Information (SI)

SI contains Optimized Cartesian coordinates of canonical, mispair and Ag⁺ mediated duplex model systems.

Conflict of Interest

The authors declare no conflict of interest.

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Figures

Figure 1

The MESP of deprotonated nucleotides namely dAMP, dCMP, dGMP, and dTMP calculated at B3LYP-D3/6-31G* level of theory in the aqueous phase. Arrow represents the maximum negative potential ($V_{\min}$) near the imine nitrogen of individual nucleotides.

Figure 2

The optimized geometries of canonical base pairs a) ATGC, canonical base pairs with CC mismatch, b) CCAT and c) CCGC using B3LYP-D3/6-31G* level of theory in the aqueous phase. The distances are given

Figure 4

The noncovalent interactions (NCI) plots for the stacked nucleobases of the canonical, mismatch, and CC-mismatch-basepair with Ag^+ mediated systems. Green color: non-covalent interactions, Brown color: density of electrons, Blue color: hydrogen bonding. Green ball: Ag^+ , Cyan ball: Carbon, Blue ball: Nitrogen, Red ball: Oxygen, Purple ball: Sodium-ion, and Light orange: Phosphorus.

Figure 5

The scatter plots of canonical ATGC with the gradient isosurface ($s=0.5$ a.u.) Green color: vdW interactions, Blue color: Hydrogen bonding, and Red color: steric repulsion interactions.

Figure 6

The scatter plots of mismatch (CCAT & CCGC), Ag+ mediated systems with AT (A-Ag+-T) GC (G-Ag+-C) and CC (C-Ag+-C) with the gradient isosurface ($s=0.5$ a.u.). Green color: vdW interactions, Blue color: Hydrogen bonding, and Red color: steric repulsion interactions.

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