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What Are the Differently Paired Forms of Adenine (A)-Thymine (T)?

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Abstract:
Some tautomers of adenine (A) and thymine (T) are selected to study the existence of differently paired TA pairs unlike Watson Crick (WC) AT. In this investigation DFT (B3LYP/6-31+G (d,p)) calculations are carried out to obtain the optimum structures of all tautomers and tautomer TA pairs. Schematic studies have been performed to analyse how the effect of proton on basic sites of A and T can destabilize the Watson Crick (WC) AT. Certain basic sites of A and T acquire strong affinity for proton that may lead to destabilization of hydrogen bonds in WC AT. Several tautomer TA pairs formed due to paring of A and T tautomers through H-bonding in a different manner are found quite stable. These tautomer TA pairs are formed through hydrogen bonding between compatible counter tautomers. So, destabilization of WC AT usually generates less stable tautomers of A and T, which in turn may combine to form quite stable tautomer TA pairs. Moreover, direct reaction pathways from WC AT to tautomer TA pairs are not feasible, since the reaction pathways pass through high energy barriers.

Keywords: *Nucleobases, Tautomers, DFT, Base pair, Hydrogen bonds.*

1. Introduction

The tautomeric forms of adenine and thymine are found under certain condition, which is perhaps responsible for the change of genetic code [1-10]. It is to be noted that the existence of tautomeric form of adenine might affect on thymine of AT base pair and thymine nucleobase may tautomerise simultaneously. Some of the adenine and thymine tautomers are shown in Figure 1. It is likely that the tautomers of A and T may pair up through H-bonds to form tautomer AT pair or it may convert to more stable forms. The rearrangement of H-atom at certain groups in adenine and thymine usually take place during tautomerization. Several tautomer TA base pair combinations may be formed from A and T tautomers under different conditions. Although the causes of tautomerization may be numerous, the prototropic rearrangement is considered as one of the major pathways. Intramolecular proton transfer in hydrated adenine has been discussed in some studies [9-10].

Some tautomers might not be stable enough to exist under normal condition and they may instantaneously interact with other nucleobases to form several mismatch base pairs other than WC AT base pairs. The pairing of tautomer nucleobases may depend on the H-bonding capacity of the counter tautomer nucleobase. Most of the available mismatched pairs may also be associated with prototropic mechanism of tautomerization because of the unambiguous small differences in the stability among tautomers [11-17].

Hydrogen bonding ability of nucleobases with surrounding water molecules, and the effect of ions might play important role in tautomerization. The changes of H-bonding from WC type H-bonding to NWC type are observed in several sequences of DNA. The metal ion interaction with certain sites of nucleobases might lead to generation of tautomers. The acid-base characteristics of various donor-acceptor sites of nucleobases are not largely different [9,11,18-27]. Hence there are possibilities of H-migration between counter sites having small variation of basicities, leading to tautomerization.

Subsequently, tautomer nucleobases may combine to form several base pairs other than WC GC and AT, which can lead to distortion of normal DNA structure. This aspect is very important in DNA mutation as well as in the evolution of chronic diseases. However, there may be situation where tautomers having equal stability may also resonate among several forms. All the previous studies had not

considered the basis of tautomer pairing leading to tautomer pairs. Hence the present study has been taken up to examine several strategies of forming tautomer pairs to generate tautomer TA base pairs.

Initially, it is essential to know how the destabilization of WC AT occurred in DNA, and further study on the pairing of complementary tautomers to form tautomer AT pairs may be investigated. Moreover, less stable tautomers may be short-lived and formed as minor product under certain environment. These rare A and T tautomers may interact to form stable tautomer TA pairs, which is in fact relevant to mutagenesis. So, it is worth analysing the pairing of some tautomers of A and T through hydrogen bonds.

2. Computational Methods

The standard geometry optimization of tautomers and DP base pairs have been carried out by using B3LYP/6-31+G(d,p) calculations. The corresponding interaction energies ΔE, changes of thermal (ΔH) and Gibbs free energies (ΔG) are estimated. The BSSE are also calculated to estimate the error due to insufficient basis set in the interaction energies of these H-bonded tautomer TA pairs. The changes of Gibb's free energies are calculated at 298K. The effect of proton on the basic sites of WC AT are also computed with B3LYP/6-31+G(d,p) to understand the stability of WC AT as a result of protonation at various basic sites. We have also examined the reaction pathway of WC AT to tautomer TA pairs. Before performing potential energy scan, the hypothetical transition state structures are carefully identified. All calculations were carried out with Gaussian 03 program code [28].

It is important to estimate the equilibrium constants (K_E) and the pK_E of tautomer TA pairs with respect to WC AT. The feasibility of conversion from WC base pairs to tautomer AT pairs may be analysed from these values. The pK_E and K_E values are calculated from the following equations.

$$pK_E = \frac{\triangle G}{2.303\ RT}$$

K_E = Equilibrium constant, ΔG = Gibb's free energy change, T = 298K, and R = Gas constant.

$$K_E = e^{-\Delta G/2.303RT}$$

The computed values may be taken to understand the mechanism for the reaction of WC AT base pairs to tautomer TA pairs.

3. Results and Discussions

The tautomerization reactions of A and T are demonstrated in this study. The results obtained from quantum mechanical calculations can be used to estimate the comparative stability of several tautomers of A and T. Considering that, in certain cases, pairing of tautomers through H-bond may occur in DNA, one would like to explore the stability of several tautomer TA pairs. The search for A and T tautomers based on the mechanism of tautomerization shown in Figure 1 can be done from the potential energy plots [Figure 2]. The relative stability of these tautomers with respect to A and T along with the changes of Gibbs free energies, Enthalpy and Zero point energies are shown in Table 1. The energies of A tautomers are 12.344 kcal/mol to 19.305 kcal/mol higher than A, whereas for T tautomers the energy range of 12.112 kcal/mol to 30.240 kcal/mol is observed higher than T. Some tautomers of A and T are shown in Figure 1, and the stabilities of tautomers transA1, *cis*A1, *cis*T1 and *trans*T1 can be analysed from [Table 1]. As we know that A and T nucleobases are sensitive to solution pH, and subsequently they may undergo the relevant tautomerization pathway through protonation/deprotonation reactions at the basic sites. Considering the standard tautomerization reactions given in Figure 1, the computed ΔE, ΔH, ΔG and ΔZPE are shown in Table 1. The energy values of these tautomers shows appreciable differences from normal A and T, and the later is in turn found at lower energy level. From the computed values of equilibrium constants, tautomerization of A to *trans*A1 is found to be a dominant reaction, and *trans*A1 might be the major tautomer(K_E=3.2x10^{-10}). Similarly, the most feasible tautomerization is expected from T to *cis*T1 (K_E=5.2x10^{-10}) [Table 1]. It is possible that major tautomers may be formed subsequently from other less stable tautomers. Again, it can be argued that how the process of tautomerization under acidic condition (low pH) is different from the basic medium (high pH). There are possibilities of forming anionic tautomers unlike the keto/enol tautomers. Here, the study has been taken up mainly for the tautomerization due to H- migration mechanisms.

Figure 3 provides the structural features of the tautomer TA pairs formed through hydrogen bonding between two compatible sites of T and A tautomers. The intermolecular H-bonding patterns in these tautomer TA pairs are different from WC AT, and the nature of H-bonding is shown in Table 2. The H-bonds are either skipped or planar in these tautomer TA pairs depending on the H-bonding capacity of basic sites. Comparison of the H-bond lengths and angles of these tautomer TA pairs are relevant to the understanding of relative stability of stable tautomer TA pairs. The ΔE, ΔG and ΔH values of tautomer TA base pairs are shown in Table 3. Figure 4 illustrates the relative electronic energies of tautomer AT pairs with respect to WC AT.

The H-bond patterns and their characteristics may be used to extract information about the predominant H-bond in tautomer TA pairs. The H-bond strengths in these tautomer TA pairs may not be equal, and in fact the H-bond lengths are indicative of H bonding capacity between counter tautomers. Comparison of H-bonding patterns in tautomer TA pairs with respect to WC AT is given in Table 2. The arrangement of two hydrogen bonds, H_u (upper) and H_l (lower) in several tautomer TA pairs are different from that of WC AT. As we know that the two H-bonds in WC AT are unequal and bond lengths are less than 2 Å. We have noted elongated H-bonds in tautomer AT pairs, (a) TA-Q1 (c) TA-Q2, (d) TA-Q3 and (e) TA-Q4. The role of H-bonds in DNA structure is significant and the structure may drastically affect due to mispairing between nucleobases. Tautomerization is considered as an essential reason for generating several base pairs other than WC AT.

The interactions between the basic sites of these tautomers with the complementary acidic hydrogen atoms are usually considered in tautomer pairing. Hence, the extent of acidic/basic characters of these sites can determine the interaction energies of tautomer TA pairs. Again, we can explore the formation of unstable tautomers which may instantaneously pair up to form several stable tautomer pairs. No drastic changes in structures and stability of some tautomer TA pairs from WC AT are found. We have observed possibility of forming skipped H-bonding in certain tautomer AT pairs [Figure 3]. The results show that TA-Q1 is the more preferred pairing than WC AT, and the variation of interaction energies and other thermodynamic parameters are shown in Table 3.

For instance, the conversion of WC AT base pair to tautomer TA pairs may be hypothesised through some transition state. It is not necessarily the exact situation for tautomerization, since the nucleobases may tautomerise under different conditions and then subsequently expected to form stable tautomer TA pairs. In such cases, tautomerization of nucleobases and pairing may occur simultaneously. However, such processes can be examined with respect to a hypothesised transition state structure. The situation for protonation/deprotonation steps to form several tautomers can be chosen in the transition state. The activation energies for tautomerization through hypothesised intermediates are given in Table 4. We are fully aware of the basic mechanism of tautomerization of nucleobases from WC AT base pairs, but the activation energies for the reaction WC AT to tautomer TA pairs can be approximately estimated from the potential energy scan. Table 4 shows the variation of activation energies for the formation of tautomer TA pairs, and the potential energy plots shown in Figure 5 clearly indicate endothermic reaction. The computed activation energies of these tautomerization processes are found within the range 42.508 kcal/mol to 55.995 kcal/mol.

Some tautomers may be less stable and formed as minor product which can readily convert to more stable forms. Instead, two less stable tautomers may combine to form stable tautomer pairs. Comparison of tautomer TA pairs with WC AT base pairs can be made from Table 5, we find wide variation of interaction energies. The tautomer pair TA-Q1 acquire larger (-ve) interaction energies than other tautomer pairs, and particularly more stable than WC AT. A broad feature of H-bonding between tautomers T and A are possible. Note that Hu(upper) and H_l(lower) H-bonds can stabilize tautomer TA pairs. The variation of structures of tautomer TA pairs could be due to the H bonding capacity of several hydrogen bonds.

4. Conclusion

The tautomers of adenine and thymine are less stable than the normal forms of these nucleobases. Since the normal A and T are the most stable forms, the formation of less stable tautomers is possible under unusual condition. However, these less stable tautomer T and tautomer A can pair up through hydrogen bonds to form several stable tautomer pairs, and TA-Q1 is found to be the most stable form. The computed interaction energy of TA-Q1 is -24.705 kcal/mol, whereas the corresponding value for normal TA is -12.619 kcal/mol. The geometrical features of tautomer pairs usually depend on the pattern of H-bonding between tautomers in TA. Maximum tautomers are found at very close energy levels, and transformation of these tautomers among themselves might be easier within this region. The tautomerization pathway among canonical forms of A and T tautomers may be depicted with respect to the population of tautomers at certain energy levels. The keto/enol tautomerization pathways (H-atom migration) chosen in this study predict both endothermic and exothermic reactions from the activation energy values. The activation energies for the reaction pathways directly normal TA to tautomer TA pairs through hypothesized intermediates are appreciably large in most cases. So the feasible pathway of forming tautomer TA pairs can be formed only after tautomerization of normal A and T, but not directly from normal AT.

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Annexure

Nucleobase	Tautomers	Relative Energies (kcal/mol)	K_{eq}	ZPE (kcal/mol)
A→	*trans*A1	12.344[a],12.866[b], 12.960[c]	$3.2x10^{-10}$	0.643
	*cis*A1	19.305[a], 19.190[b], 19.176[c]	$9.1x10^{-15}$	0.233
T→	*cis*T1	12.112[a], 12.091[b], 12.683[c]	$5.2x10^{-10}$	-0.266
	*trans*T1	19.688[a], 19.419[b], 19.977[c]	$2.4x10^{-15}$	-0.581
	*trans*T2	30.240[a], 30.103[b], 29.670[c]	$1.9x10^{-22}$	-1.074

Table 1: Computed relative energies ΔE, ΔH, ΔG, K_{eq} and ZPE of different nucleobase tautomers with B3LYP/6-31+G(d,p) methods of calculations.

a→ ΔE, electronic energy change for the reaction, b→ ΔH, Enthalpy change for the reaction,
c→ ΔG, Free energy change for the reaction, Keq→ Equillibrium constant of the reaction and
ZPE→ zero point energy

Base pairs	Tautomer AT base pairs	H-bond distance (Å)	Planarity
AT	-	H_u →1.914 H_l→1.801	Planar
	(a) TA-Q1	H_u →1.471 H_l→1.713	Planar
	(b) TA-Q2	H_u →2.508 H_l→ 2.048	Planar
	(c) TA-Q3	H_u →1.931*	Planar
	(d) TA-Q4	H_u →1.940*	Planar

Table 2: Computed H-bond distances in AT and tautomerized pairs.

'*' indicates skipped H-bond

Nucleobase pairs	Tautomerized base pairs	Energies of the reactions (kcal/mol)	K_E	pK_E
AT	TA-Q1	13.143[a], 17.063[b], 18.443[c],1.380[d]	$3.1x10^{-14}$	13.504
	TA-Q2	40.054[a], 42.971[b], 43.222[c],0.627[d]	$2.2x10^{-32}$	31.648
	TA-Q3	50.017[a], 53.448[b], 52.883[c],0.878[d]	$1.9x10^{-39}$	38.722
	TA-Q4	46.181[a], 49.433[b], 48.743[c],0.376[d]	$2.0x10^{-37}$	35.690

Table 3: Computed ΔE, ΔH, K_E, ΔG and ZPE of different tautomerized base pairs with B3LYP/6-31+G(d,p) calculations.

a→ ΔE, Electronic energy change for the reaction, b→ ΔH, Enthalpy change for the reaction, c→ ΔG, Free energy change for the reaction, d→ ΔZPE, Zero point energy, K_E→ Equilibrium constants of the reaction,

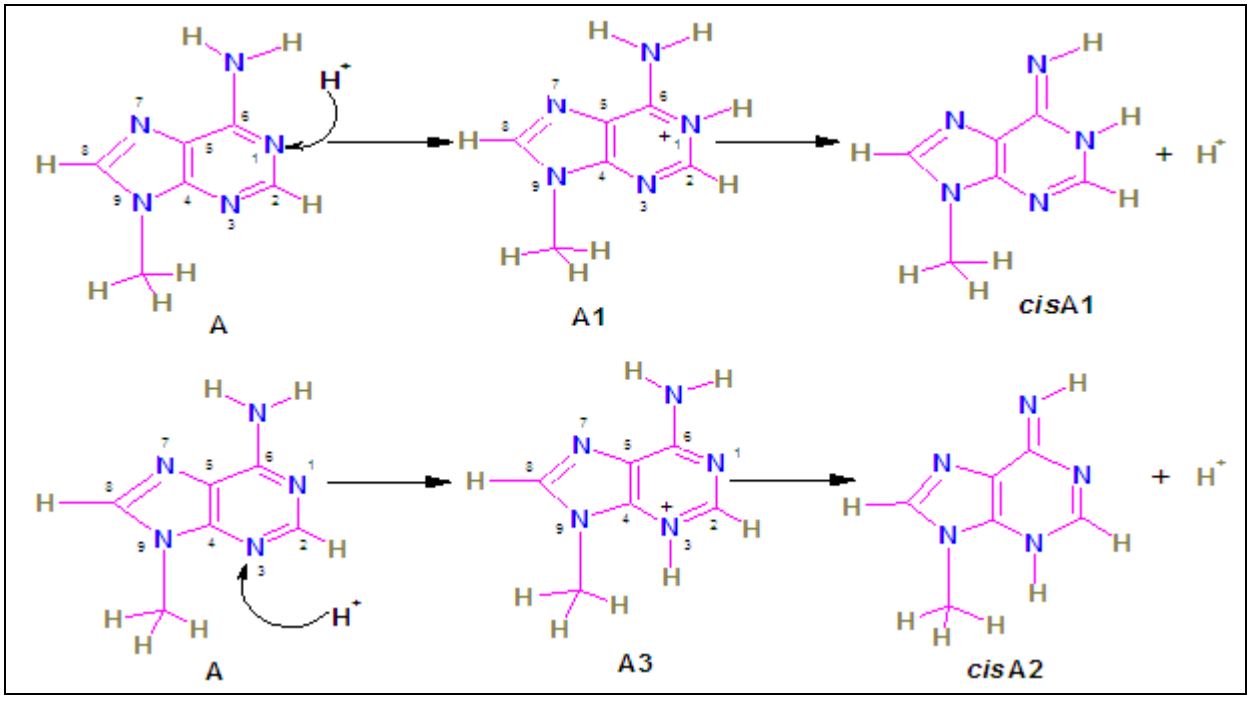
Base pairs	Tautomer TA base pairs	ΔA (kcal/mol)
		B3LYP/6-31+G(d,p)
AT	1. TA-Q1	55.995
	2. TA-Q2	45.384
	3. TA-Q3	42.508
	4. TA-Q4	45.177

Table 4: Variation of activation energies during conversion of normal base pair to tautomerized base pair.

Base pairs	Interaction energies, IE = E_{BP} - E_B - E_{BH}+ (kcal/mol) (B3LYP/6-31+G(d,p))		BSSE Energies (kcal/mol)
Normal →	AT	-12.619	
Tautomer pairs →	1. TA-Q1	-24.705	1.008
	2. TA-Q2	-11.474	0.664
	3. TA-Q3	-12.105	0.542
	4. TA-Q4	-8.991	0.466

Table 5: Computed interaction energies of normal AT and tautomer TA pairs and BSSE energies (B3LYP/6-31+G(d,p)).

E_{BP} → Energies of base pairs
E_B → Energies of tautomer Adenine(A)
E_{BH}+→ Energies of tautomer Thymine(T)
BSSE→Basis Set Superposition Error.



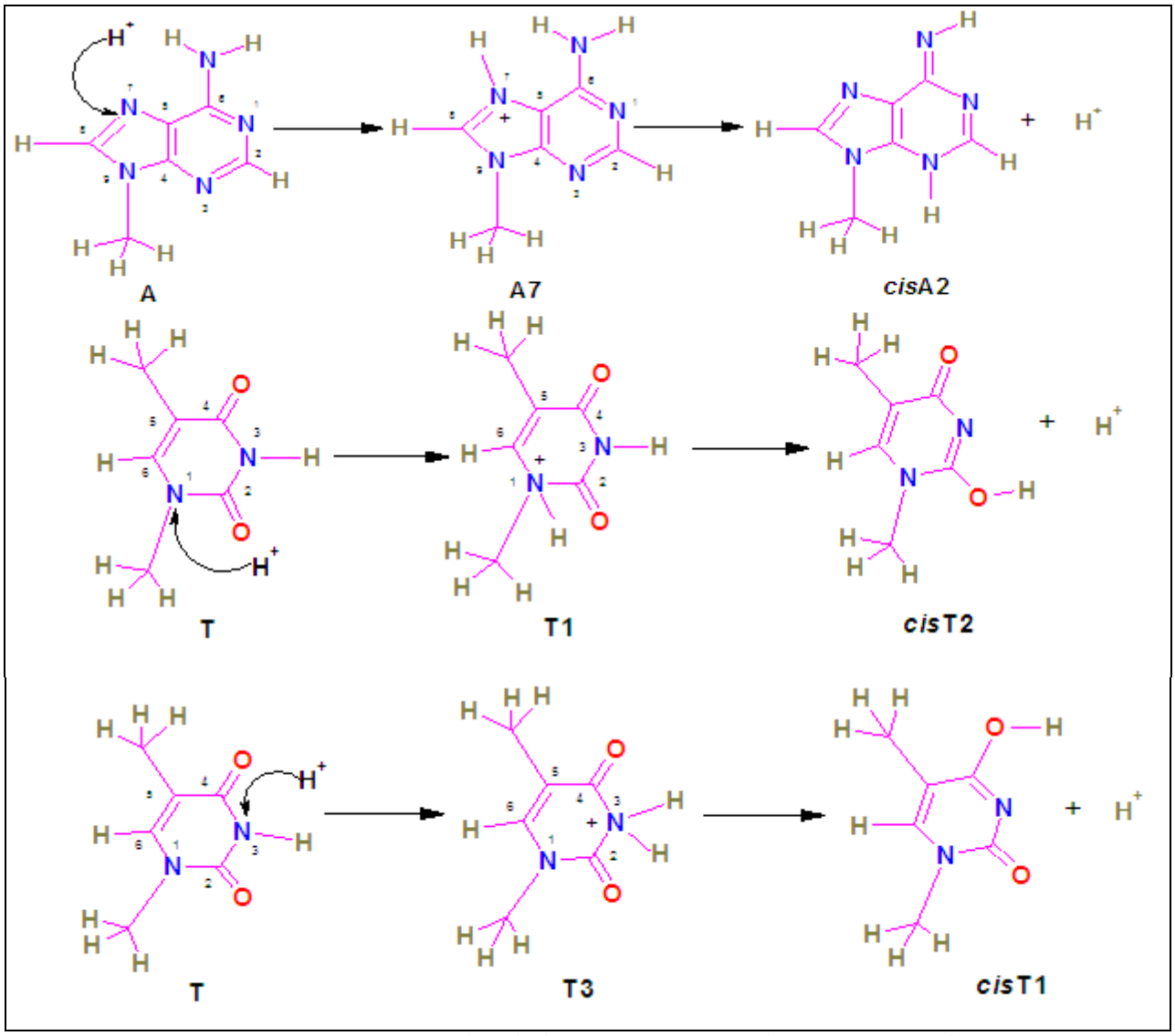
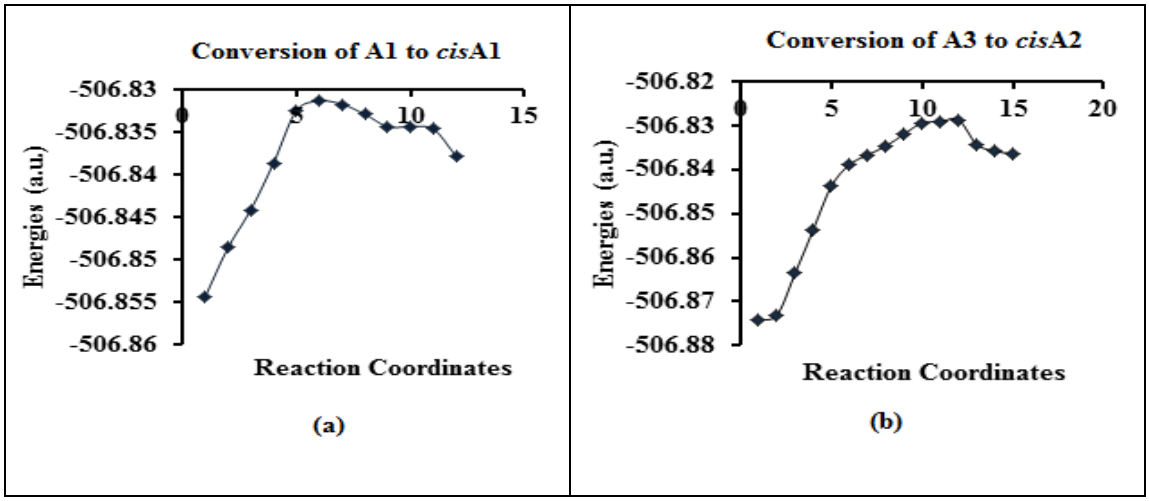


Figure 1: Conversion of normal nucleobase to tautomers via different protonated forms.



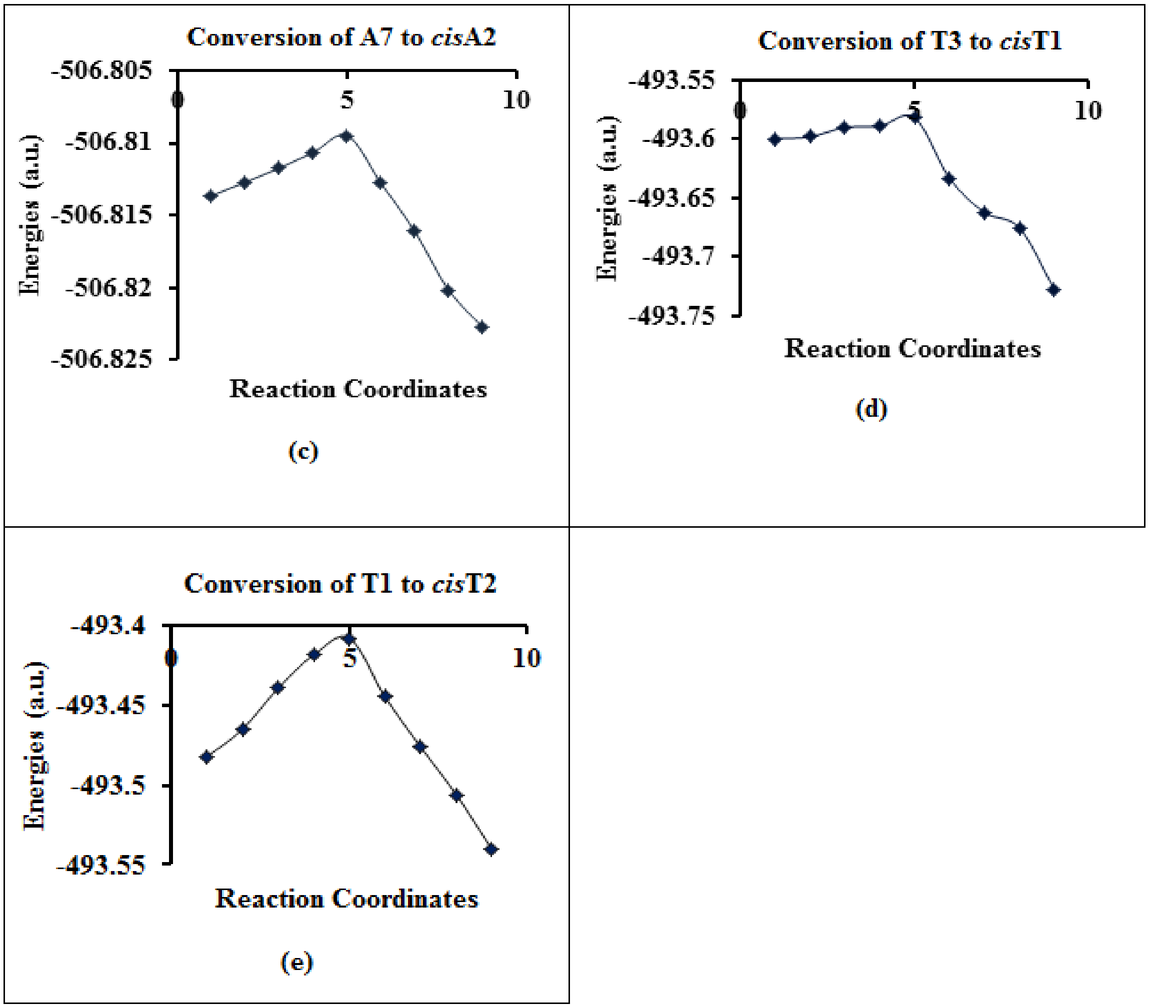
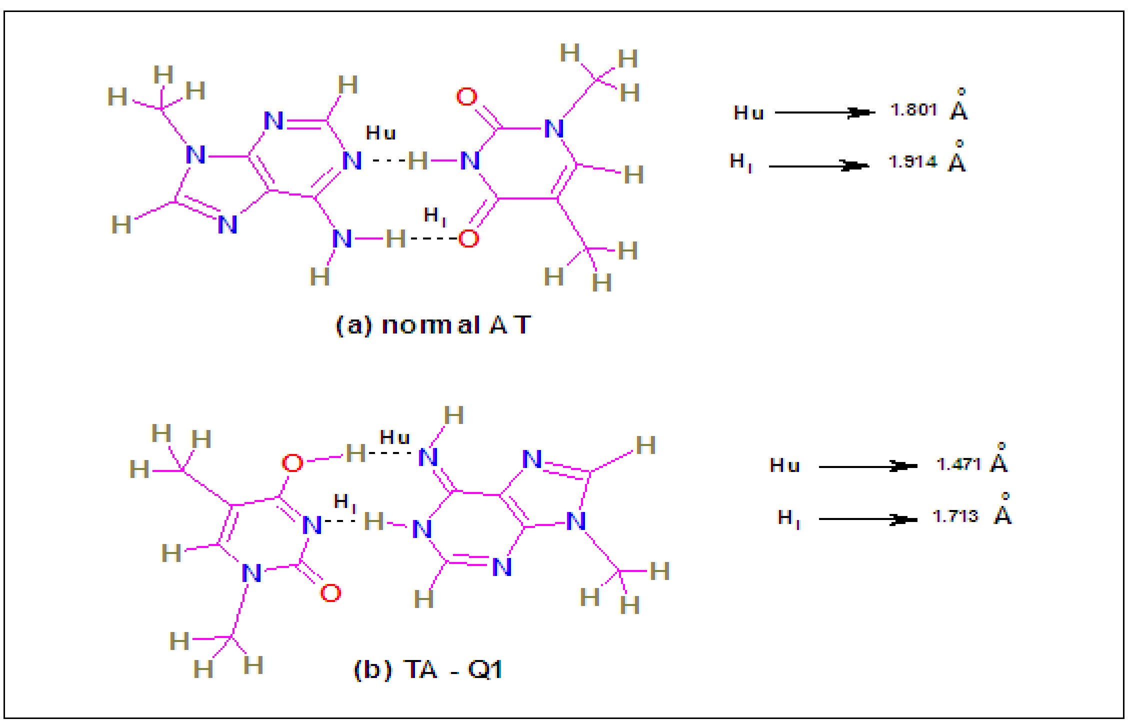


Figure 2: Potential energy plots for conversions nucleobases through protonated intermediates (a) A1→ cisA1 (b) A3→ cisA2 (c) A7→ cisA2 (d) T3→ cisT1 (e) T1→ cisT2



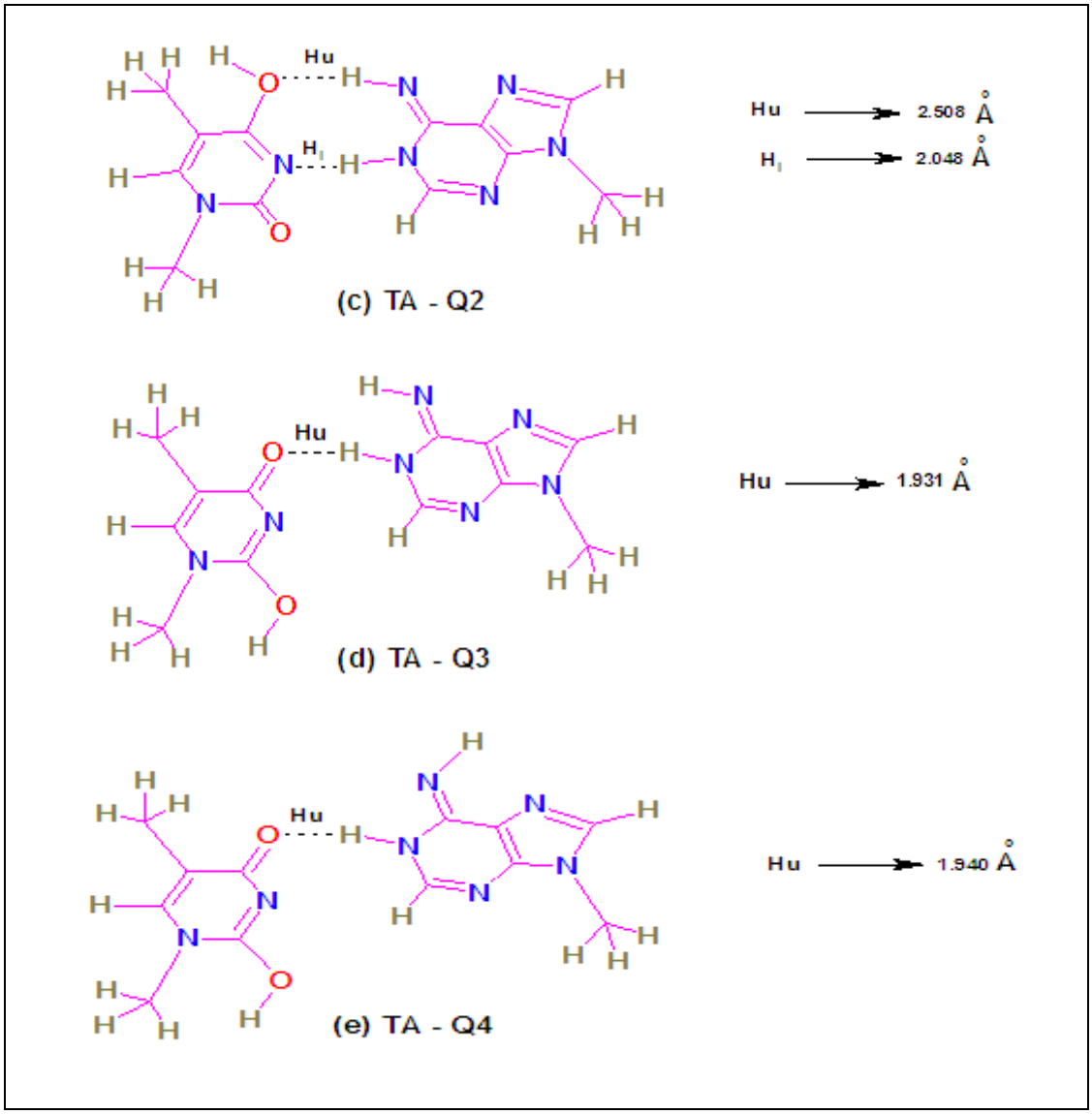


Figure 3: Structures of tautomerised AT base pairs (a) normal AT (b) TA-Q1 (c) TA-Q2 (d) TA-Q3 (e) TA-Q4

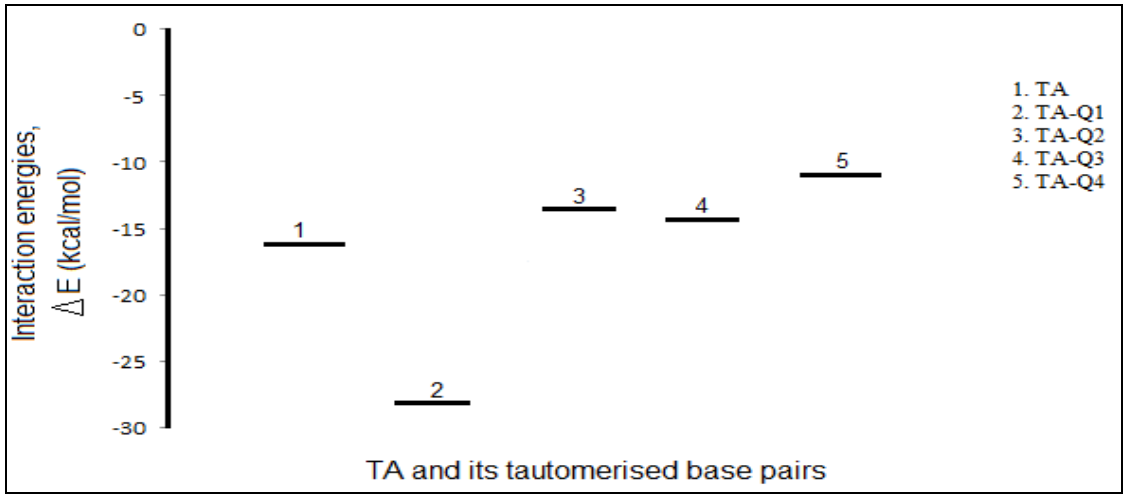


Figure 4: Comparison of interaction energies of normal AT and tautomer TA pairs.

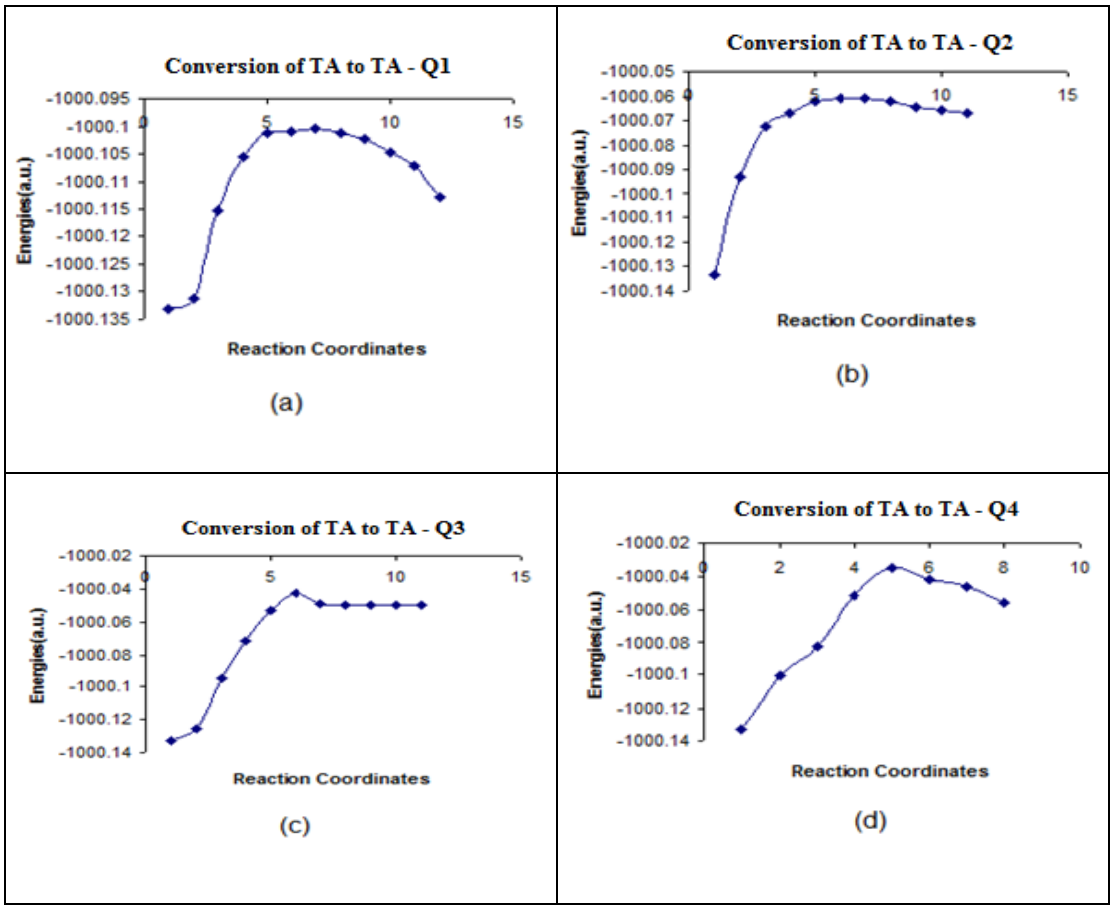


Figure 5: Potential energy plot for conversions of (a) AT → (TA-Q1) (b) AT → (TA-Q2) (c) AT → (TA-Q3) (d) AT → (TA-Q4)