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Theoretical studies on the tautomerization of guanine nucleobase

R.P.Deka, J.Basumatary, J.Deka, T.K.Barman, C.Medhi*
Department of Chemistry Gauhati University, P.O Gauhati University, Guwahati-781014, (INDIA)
E-mail : chitrani@sify.com

ABSTRACT

There are several tautomers of nucleobases. Schematic study on the tautomerization of guanine (G) has been analysed from certain reaction pathway. The differences of energies of several tautomers are found very small. Tautomerization of G has been analysed by using B3LYP/6-31+G(d,p) calculations. The formation of tautomers due to H shifting from compatible H-bonding sites is shown in this tautomerization pathway. Transformation of normal G to tautomer *cis*G1 is found most favourable and other tautomers are formed at narrow energy gaps. The activation energies of forming *trans*G4 and 1cisG4 tautomers is small compared to those of other tautomers having large differences of energies from normal G. The energy difference of *cis*G1 tautomer from normal G is small(0.720 kcal/mol). Hence the existence of this particular tautomer might be more feasible than others which also agree with the experimental reports.

KEYWORDS

Nucleobases;
Tautomers;
Ab initio;
Base pair;
DNA.

INTRODUCTION

Tautomerization of Nucleic acid bases (nucleobases) has been found in many literatures[1-10]. The changes in the molecular forms of these nucleobases may lead to damage of genetic code and unfavoured metabolism resulting development of several chronic diseases. The five nucleobases, Adenine (A), Cytosine (C), Guanine (G), Thymine (T) and Uracil (U) may exist in various tautomeric forms. Among these nucleobases, Guanine can easily tautomerize to form several molecular forms as shown in Figure 1. The generation of tautomers are shown from the intrinsic excitation state features using UV-spectroscopy and supersonic expansion techniques[1-8]. Hence, several experimental studies have reported the existence of quite a number of guanine tautomers, which may be relevant to biological reactions. There are numerous causes of tautomerization, however photo electron excitation and prototropic rearrangement are the major mechanisms of forming tautomers. It may be noted that intramolecular proton transfer in hydrated guanine that might invove in assisting tautomerization of G has been shown in some literature[8]. The formation of tautomers due to photo excitation and subsequent solvation by the surrounding water molecules are considered to be competitive reactions[10]. Here the transformations of groups like keto to enol, amine to imine or vice versa are indeed the common mechanisms of forming tautomers (Figure 2). It is to be noted that the guanine tautomers might affect on the structure and stability of GC base pair in DNA. On the other hand the individual tautomers could form tautomer base pairs through H-bonds in a

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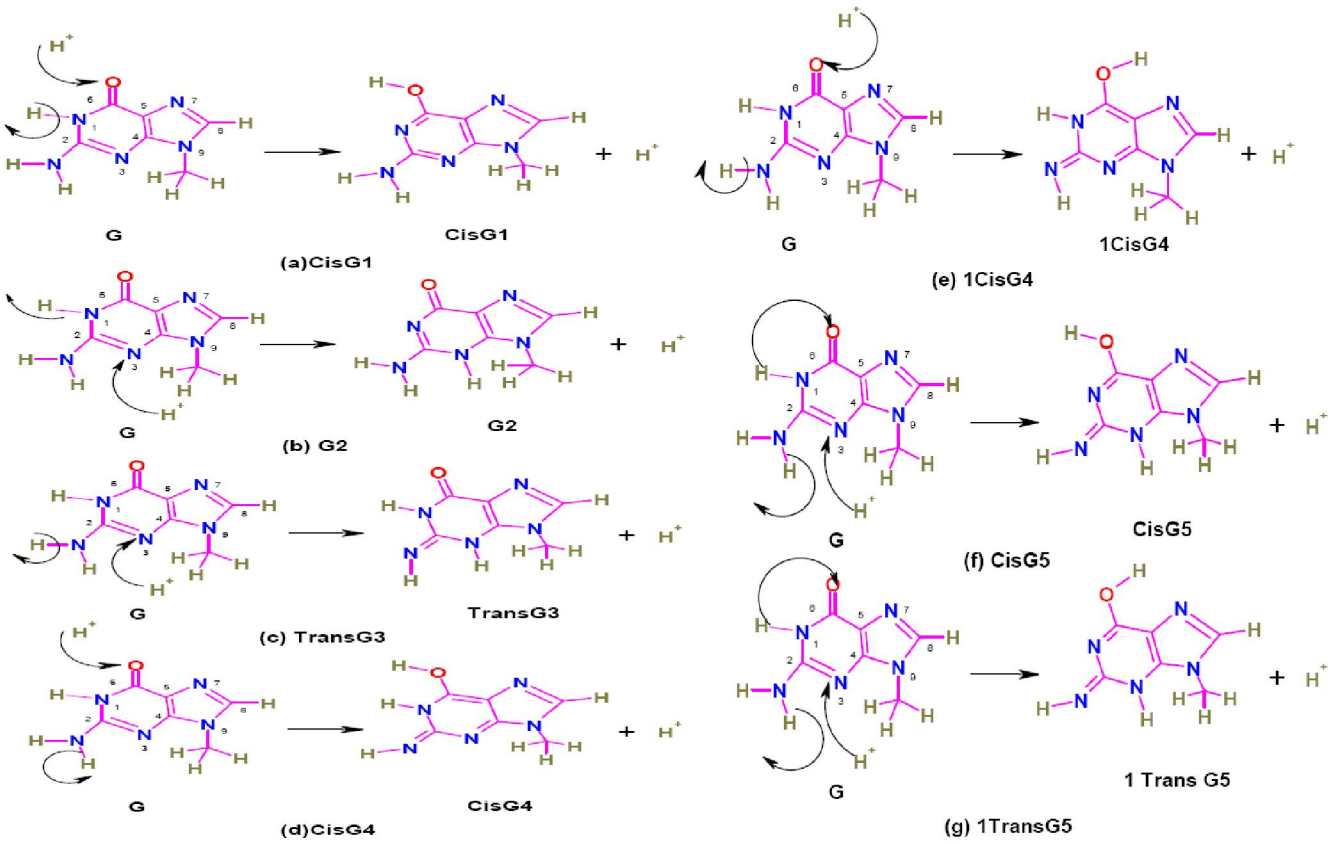

Figure 1: Tautomers of guanine and H-migration sites.

different manner. It may be mentioned that most available mismatched pairing may be associated with the mechanism of prototropic tautomerization of the nucleobases because of the unambiguous small differences in the stability among tautomers[11-17].

The acidic nature of the hydrogen bonding site is also importantly a predominant factor for the changes of molecular forms in G. It has been shown in several studies how the atomic sites of N1, N2, N3, N7, N9 and O6 of guanine are protonated or hydrogen bonded with water molecules in solution environments (10-18, Figure 2). Tautomerization is considered to be the consequence of the migration of H atom from one site to various sites. It has been cited in several references that the interaction of metal ion with certain sites of G might lead to tautomers. It may be due to the change of acidity or basicity of these sites after coordination with metal ion[7,17]. The acid-base characteristics and hydrogen bonding capacity of various donor sites of G vary significantly. In turn the tautomerization process is likely to be through H-migration between counter sites having small variation of basicities.

Many dominant G tautomers have been identified from several experimental and theoretical studies[1,9,17,23]. However, some tautomers may not be easily detectable due to small differences in the stability. All the previous studies focused on the structure and stability of taunomers, but the tautomerization pathway had not been thoroughly considered. Hence, the present study has been taken up to analyse the strategies of forming G tautomers.

COMPUTATIONAL METHODS

The standard complete geometry optimization of different structures have been carried out by using B3LYP/6-31+G(d,p) calculations. The corresponding energies of tautomerization (ΔE), changes of thermal (ΔH) and Gibbs free energies (ΔG) of tautomers are estimated. The changes of Gibb's free energy are calculated at 298K. To construct the possible tautomerization reactions of forming various tautomers, the potential energy plots through a hypothetical transition state structures are also computed with B3LYP/6-31+G(d,p) calculations, and thereby estimated the re-

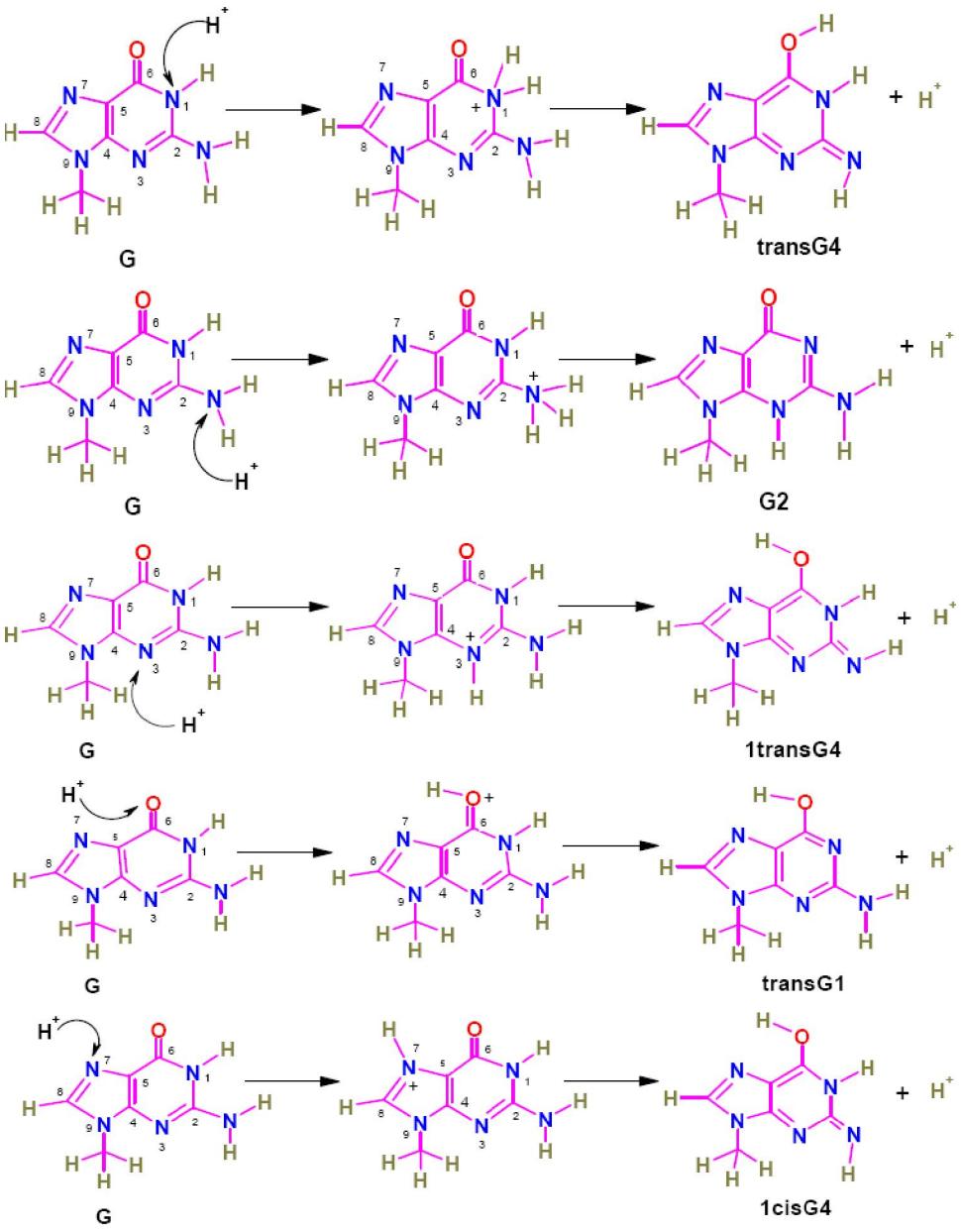

Figure 2 : Some mechanisms of tautomerization of guanine.

spective activation energies. Before performing potential energy scan, the hypothetical transition state structures are carefully identified. All calculations were carried out with Gaussian 03 program code[24].

It is important to estimate the equilibrium constants (K_E) and the pK_E of tautomers with respect to normal nucleobase. The values may be compared with the reported values of major tautomers, and the feasibility of equilibration among tautomers and with normal nucleobase may be analysed. The pK_E and K_E values are calculated from the following equation.

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

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

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

pK_E of several tautomers are not available, however the computed values may be taken to compare the possible tautomerization reactions.

RESULTS AND DISCUSSIONS

The formation of some tautomers of guanine is shown in Figure 1. These tautomers may be formed as

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TABLE 1: Computed ΔE, ΔH, ΔG, ZPE, K_E and pK_E of guanine tautomers with B3LYP/6-31+G(d,p) calculations.

Nucleobase(B)	Tautomers(T)	Energies (kcal/mol)	K_E	pK_E
G→	G2	19.477[a], 19.522[b], 19.929[c],-0.423[d]	$2.5x10^{-15}$	14.590
	*1cis*G4	21.628[a], 21.610[b], 22.083[c],-0.078[d]	$7.9x10^{-17}$	16.169
	*cis*G1	0.720[a], 0.336[b], 1.043[c],-0.392[d]	0.172	0.764
	*cis*G5	22.232[a], 22.255[b], 22.828[c],-0.232[d]	$1.9x10^{-17}$	16.715
	*1trans*G5	23.844[a], 23.956[b], 24.411[c],-0.299[d]	$1.3x10^{-18}$	17.874
	*cis*G4	36.127[a], 36.234[b], 36.176[c],-0.227[d]	$3.2x10^{-27}$	26.488
	trans G3	15.985[a], 15.492[b], 15.494[c],-0.496[d]	$4.5x10^{-12}$	11.345

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→Equillibrium constant of the reaction.

a result of sequential H-migration from the H bonding sites of guanine. The mechanisms of tautomerization of G through hypothetical transition states are shown in Figure 2. Typical guanine tautomers, such as G2, *cis*G1 and transG3 are found to be significantly stable and the various energies of forming these tautomers from G are shown in TABLE 1. The energy levels of these tautomers are shown in Figure 3. The tautomers formed at small ΔE values may exist in equilibrium with G in several reaction steps. However, the ΔA values of some tautomers formed at much higher energy levels than normal G have been computed from the potential energy plots (Figure 4). The activation energies of tautomerization pathways for selected tautomers are given in TABLE 2. The ΔE values of tautomers shown in TABLE 2 indicate variation of energies from 0.720 to 36.127 kcal/mol. The tautomers having small ΔE values may be sensitive to H-migration pathway to form different tautomers as shown in the relevant mechanism of tautomerization. The population of certain tautomers within close energy levels can be visualized in Figure 3.

Based on the tautomer population within certain energy levels shown in Figure 2, it is possible that some tautomers may form subsequently from major stable tautomers. Resonance among those tautomers that exist within small ΔE values is expected. The changes of energies (ΔE, ΔH, ΔG and ΔZPE) for tautomerization from the normal nucleobases are shown in TABLE 2. Nevertheless, the present study also concerns about the tautomerization through hypothetical transition state leading to less stable tautomers.

It is generally known that guanine is most basic nucleobase and acquires hydrogen bonding characteristic with water or proton easily in solution environment. The topic usually is a dynamical motion problem where the tautomers are converted from one form to another

TABLE 2: Variation of activation energies (ΔA) for the conversion of normal guanine to few less stable tautomers.

Conversions	ΔA kcal/mol
	B3LYP/6-31+G(d,p)
G→transG4	6.445
G→G2	63.897
G→1transG4	18.819
G→transG1	16.386
G→1cisG4	5.123

through H- migration, where less stable tautomers cannot be detected during experimental studies. So the results obtained from quantum mechanical calculations can be used to understand the stability of several G tautomers. The situation for H-migration steps leading to less stable tautomers has been analysed by choosing appropriate transition state structure which is feasible for the conversion of normal G to the desired tautomers. The activation energies of tautomers having similar stability with normal G are not calculated, since the reaction proceeds through direct H-migration pathway.

For instance, the conversion of normal G to other less stable tautomers may be hypothesised through some transition state (Figure 2). It may not necessarily that all tautomerization proceeds through this pathway, since G nucleobase can tautomerise under various conditions. In such cases, tautomerization from normal G to a tautomer can simultaneously convert to other more stable tautomers. Such processes can be examined focusing on particular mechanism through a hypothesised transition state structure which is appropriate for that pathway. Based on this mechanism of tautomerization, the activation energies have been estimated from the potential energy plots. TABLE 2 shows the variation of activation energies for the formation of some tautomer G, and the potential energy plots are shown in Figures

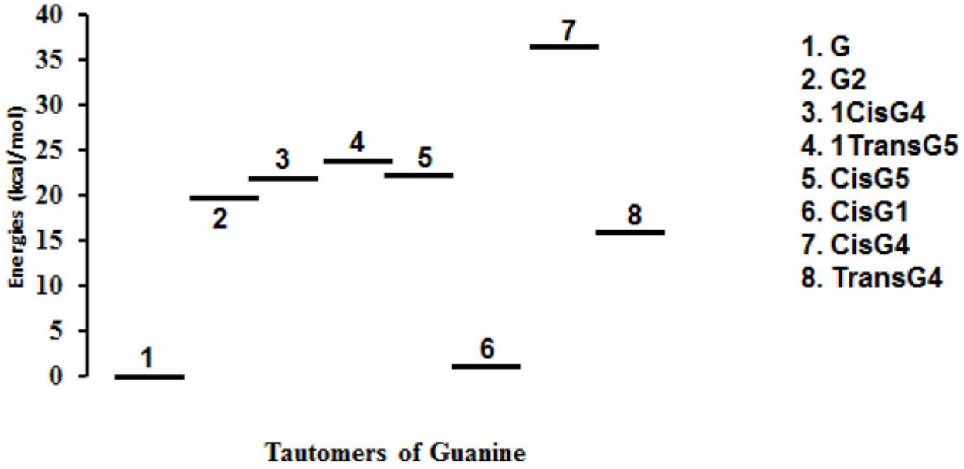

Figure 3 : Variation of energies of tautomers with respect to normal G.

4. Hence, in most cases tautomerization may not follow direct pathway from normal G. The activation energies are large for tautomers G2, *1trans*G4 and *trans*G1, whereas the corresponding values for *1trans*G4 and *1cis*G4 are small despite of acquiring less stability (TABLES 1 and 2).

The change of zero point (vibrational) energies of most tautomers are negative and correlates well with the changes of ΔH and ΔG values. So, the dominant tautomeric form of G is found to be *cis*G1 which may form directly from normal G to *cis*G1 and the mechanism is shown in Figure 2. Here, enolization of –C=O group to form corresponding enol tautomer might be feasible mechanism, and the energies for these reactions are listed in TABLE 2.

From the computed values of equilibrium constants, tautomerization of G to *cis*G1 is found to be the dominant reaction with pK_E value of 0.764, and *cis*G1 is also reported to be the major tautomer of G. Similarly, the other tautomerization reactions from normal G are not found to be feasible pathway. The pK_E value supports the existence of cisG1 as major tautomer as reported in literature(1,9). For the standard tautomerization reaction given in Figure 1, the ΔE, ΔH, ΔG and ΔZPE are given in TABLE 2. Comparison of the energy values of these tautomers with normal G shows appreciable energy difference and the latter is in turn found at lower energy level. The population of tautomers at certain energy levels with small energy gap shows the advantages of transformation among these. However, some tautomers may exist as minor product and can readily convert to more stable forms.

Comparison of several guanine tautomers can be made from the relative stabilities with respect to normal form of guanine. The variations of the relative stabilities, enthalpies and Gibbs free energy among few guanine tautomers are significant and most stable tautomer is *cis*G1. The existence of these tautomers is already reported from the experimental studies[1,9]. The predominance of few isomers within the certain energy range with small difference in the relative stabilities is observed. As we can see in TABLE 1, the calculated activation energies of forming arbitrarily selected less stable tautomer is ~ 5-6 kcal/mole, whereas the values for other tautomers are substantially higher than that of others. For the tautomerization of comparatively stable tautomers, the activation energies are not calculated since these tautomeric form can convert to normal G easily. Moreover, the tautomerization pathway through H-migration as shown in Figure 1.

The calculated values for the equilibrium constant and between normal G and *cis*G1 shows more feasible pathway compared to the other pathways. Tautomerization of G to *trans*G4 is also possible since the estimated activation energy is ~5 kcal/mole. Furthermore, comparison of equilibrium constants and pKE values for the tautomerization pathways shown in Figure 1 shows that the formation of some tautomer species from normal G may not be the direct pathway of generating other less stable tautomers. Hence, the relative stability of the tautomers may apparently indicate the specific tautomers that exist within small chemical stability.

The hydrogen bonds between water and guanine are some of the important features in local hydration of nucleobases in oligonucleotides. The hydrogen bond-

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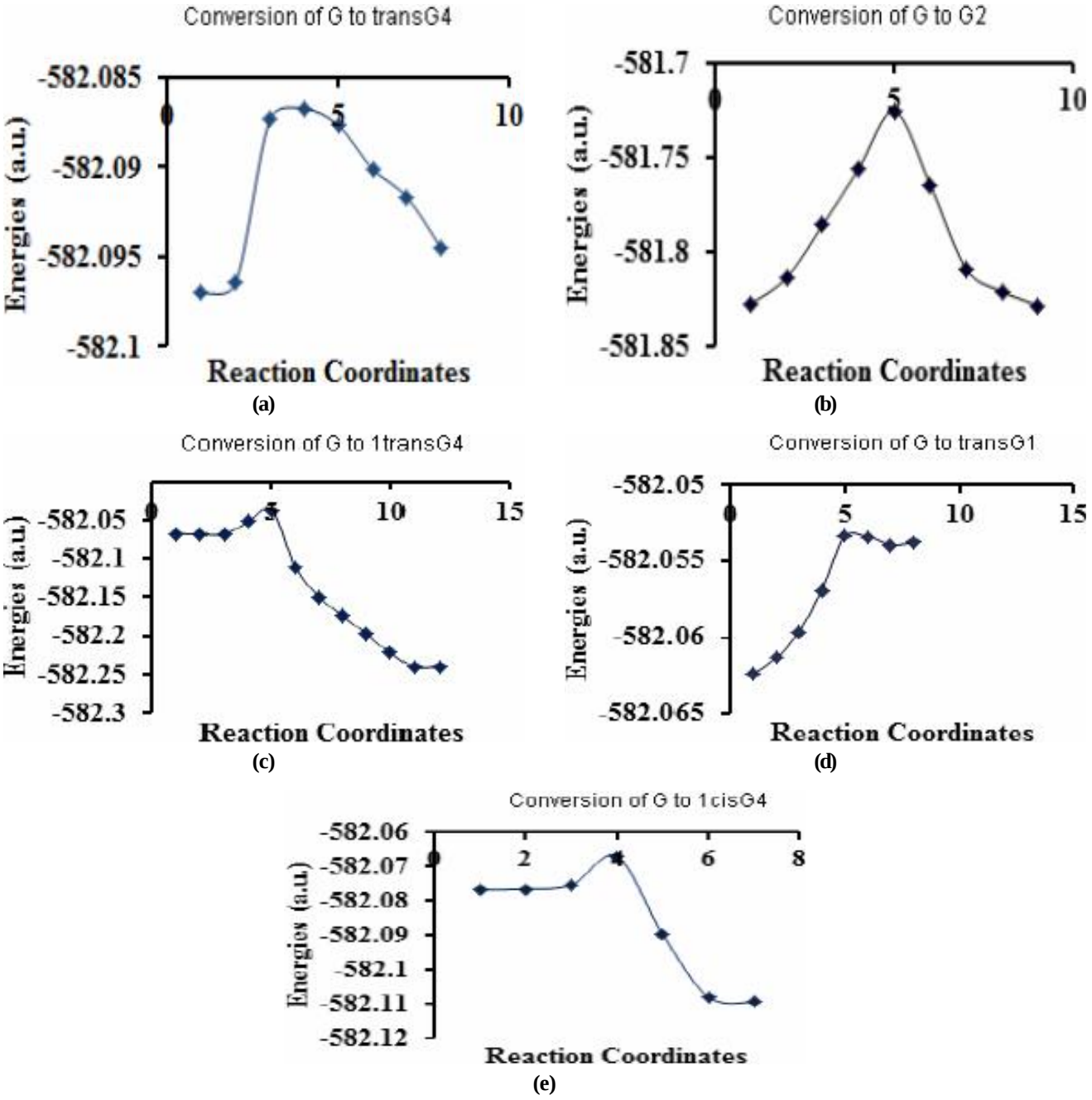

Figure 4: Potential energy plots for conversion of normal nucleobase to tautomers through protonated intermediates:- (a) G→*trans*G4, (b) G→G2, (c) G→*1trans*G4, (d) G→*trans*G1, (e) G→*1cis*G4.

ing ability usually depends on the electron donor and acceptor sites of guanine. The influence of these water molecules on guanine through hydrogen bonds may produce considerable change in the structure and stability of guanine in DNA. Moreover several mispairing and imperfect pairing of Watson crick GC base pairs manifests itself the existence of tautomeric form of guanine or cytosine. The participation of intermolecular hydrogen bonds between guanine and water molecule is indeed the major feature which may be strong, medium or weak. Regional variation of these hydrogen bond strengths may lead to simultaneous proton acceptor or donor capability of guanine. The existence of stable guanine-water complex has been reported in some literatures[21,23]. The information is quite relevant to understand the existence of tautomeric forms of guanine which involved H-bonds or proton. With these assumptions several mechanisms of tautomerization analysed

in this study may be useful.

The model study revealed that the position of proton taken to represent hydrogen bonding site between guanine and water leading to variation of activation energies through internal protrotopic rearrangement to form tautomers of guanine. Among these mechanisms, some of the pathways proceed through low activation energies and moreover, the observed small values of equilibrium constant and pKE values compared to other mechanisms might indicate the predominance of certain tautomers. In this investigation the key question is which mechanism best represents the tautomerization of guanine through H-migration pathway. Here, the intermediate species chosen for calculating activation energies are based on the concept of H bond formation between guanine and water molecule which proceeds via interchange of the position of H-bond leading to several tautomers. Therefore, the results may be used to analyse such processes to gain insight into the origin of guanine tautomers.

The computed values of equilibrium constants and pKE values of tautomers clearly indicate the more feasible tautomer. The feasibility of tautomerization to *cis*G1 tautomer is indicated from the pKE value of 1.7, whereas other tautomers with large pKE values may not be the predominant species through the chosen mechanism. Again it is not possible to trace the tautomerization pathway among these tautomers without specific idea of reaction pathway. However comparison of pKE values of tautomerization processes can predict the feasibility of these mechanisms.

CONCLUSION

The selected tautomers of guanine are less stable than the normal guanine. Population of tautomers at certain energy levels is a clear indication of the existence of some tautomers in equilibrium. The difference of energies between normal G and *cis*G1 is smaller than those of other tautomers. The formation of this tautomer may be the primary reaction, which may undergo further reactions to form other tautomers. Otherwise the other tautomers may be generated under different processes Large differences in the stability of other tautomers compared to normal G are found. The equilibrium constants (K_E) and pK_E values indirectly demonstrate the existence of major tautomer *cis*G1. The tautomerization pathways chosen for estimating the activation energies show that some of the less stable tautomers may form from normal G since the reactions passe through low energy barriers.

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REFERENCES

[1] J.R.Blas, F.J.Luque, M.Orozco; Unique tautomeric properties of isoguanine, J.Am.Chem.Soc., **126**, 154–164 **(2004)**.

[2] M.Rueda, J.F.Luque, J.M.Lopez, M.Orozco; Amino-imino tautomerism in derivatives of cytosine: effect on hydrogen-bonding and stacking properties, J.Phys.Chem.A., **105**, 6575–6580 **(2001)**.

[3] A.Perez, M.E.Tuckerman, H.P.Hjalmarson, O.A.V.Lilienfeld; Enol tautomers of Watson Crick base pair models are metastable because of nuclear quantum effects, J.Am.Chem.Soc., **132**, 11510–11515 **(2010)**.

[4] K.Tomic, J.Tatchen, C.M.Marian; Quantum chemical investigation of the electronic spectra of the keto, enol and keto-imine tautomers of cytosine, J.Phys.Chem.A., **109**, 8410–8418 **(2005)**.

[5] W.Wang, H.W.Hellinga, L.S.Beese; Structural evidence for the rare tautomer hypothesis of spontaneous mutagenesis, J.Am.Chem.Soc., PNAS, **(2011)**.

[6] I.Faustino, A.Avino, I.Marchan, J.F.Luque, R.Eritja, M.Orozco; Unique tautomeric and recognition properties of thioketothymines, J.Am.Chem.Soc., **131**, 12845–12853 **(2009)**.

[7] Y.Podolyan, L.Gorb, J.Leszczynski; Ab initio study of the prototropic tautomerism of cytosine and guanine and their contribution to spontaneous point mutations, Int.J.Mol.Sci., **4**, 410–421 **(2003)**.

[8] M.Vonderach, T.O.Ehrler, K.Matheis, P.Weis, M.M.Kappes; Isomer-selected photoelectron spectroscopy of isolated DNA oligonucleotides: phosphate and nucleobase deprotonation at high negative charge states, J.Am.Chem.Soc., **134**, 7830-7841 **(2012)**.

Full Paper

[9] C.F.Guerra, F.M.Bickelhaupt, S.Saha, F.Wang; Tautomers: relative stabilities, ionization energies, and mismatch with cytosine, J.Phys.Chem.A., **110**, 4012–4020 **(2006)**.

[10] E.Pluharova, P.Jungwirth, S.E.Bradforth, P.Slavícek; Ionization of purine tautomers in nucleobases, nucleosides, and nucleotides: from the gas phase to the aqueous environment, J.Phys.Chem.B., **115**, 1294–1305 **(2011)**.

[11] J.W.Ho, H.C.Yen, W.K.Chou, C.N.Weng, L.H.Cheng, H.Q.Shi, S.H.Lai, P.Y.Cheng; Disentangling intrinsic ultrafast excited-state dynamics of cytosine tautomers, J.Phys.Chem.A., **115**, 8406–8418 **(2011)**.

[12] R.Wu, T.B.McMahon; Investigation of proton transport tautomerism in clusters of protonated nucleic acid bases (cytosine, uracil, thymine, and adenine) and ammonia by high-pressure mass spectrometry and Ab initio calculations, J.Am.Chem.Soc., **129**, 569–580 **(2007)**.

[13] B.Lippert, D.Gupta; Promotion of rare nucleobase tautomers by metal binding, Dalton Trans., , 4619–4634 **(2009)**.

[14] Y.F.Wang, S.X.Tian; Shape resonance states of the low-energy electron attachments to DNA base tautomers, Phys.Chem.Chem.Phys., **13**, 6169–6175 **(2011)**.

[15] A.Domingo, A.R.Fortea, C.de.Graaf; The absorption spectrum of cytosine tautomers: beyond the static approach, J.Chem.Theo.Comput., **8**, 235–244 **(2012)**.

[16] V.Sauri, J.P.Gobbo, J.J.Serrano-Peìrez, M.Lundberg, P.B.Coto, L.Serrano-Andreìs, A.C.Borin, R.Lindh, M.Merchaìn, D.Roca-Sanjuaìn; Proton/hydrogen transfer mechanisms in the guanine-cytosine base pair: photostability and tautomerism, J.Chem.Theo.Comput., **9**, 481"496 **(2013)**.

[17] D.Kosenkov, Y.Kholod, L.Gorb, O.Shishkin, D.M.Hovorun, M.Mons, J.Leszczynski; Ab initio kinetic simulation of gas-phase experiments: tautomerization of cytosine and guanine, J.Phys.Chem.B., **113**, 6140–6150 **(2009)**.

[18] J.Gu, A.Tian, W.K.Li, N.B.Wong; Intramolecular proton transfer in the tautomers of C8 oxidative adenine: a DFT study, J.Phys.Chem.B., **104**, 10692–10698 **(2000)**.

[19] V.I.Danilov, V.M.Anisimov, N.Kurita, D.Hovorun; MP2 and DFT studies of the DNA rare base pairs: The molecular mechanism of the spontaneous substitution, mutations conditioned by tautomerism of bases, Chem.Phys.Lett., **412**, 285–293 **(2005)**.

[20] V.Feyer, O.Plekan, R.Richter, M.Coreno, M.de.Simone, K.C.Prince, A.B.Trofimov, I.L.Zaytseva, J.Schirmer; Tautomerism in cytosine and uracil: a theoretical and experimental X-ray absorption and resonant auger study, J.Phys.Chem.A., **114**, 10270–10276 **(2010)**.

[21] A.K.Chandra, D.Michalska, R.Wysokinsky, T.Z.Huyskens; Theoretical study of the acidity and basicity of the cytosine tautomers and their 1:1 complexes with water, J.Phys.Chem.A., **108**, 9593–9600 **(2004)**.

[22] Y.Huang, H.Kenttamaa; Theoretical estimations of the 298 K gas-phase acidities of the pyrimidine-based nucleobases uracil, thymine, and cytosine, J.Phys.Chem.A., **107**, 4893–4897 **(2003)**.

[23] R.Krishnamurthy; Role of pKa of nucleobases in the origins of chemical evolution accounts of chemical research, **45**, 2035–2044 **(2012)**.

[24] M.J.Trucks, G.W.Schlegel, H.B.Scuseria, G.E.Robb, M.A.Cheeseman, G.R.Montgomery, J.A.Vreven, T.Kudin, K.N.Burant, J.C.Millam, J.M.Iyengar, S.S.Tomasi, J.Barone, V.Mennucci, B.Cossi, M.Scalmani, G.Rega, N.Petersson, G.A.Nakatsuji, H.Hada, M.Ehara, M.Toyota, K.Fukuda, R.Hasegawa, J.Ishida, M.Nakajima, T.Honda, Y.Kitao, O.Nakai, H.Klene, M.Li, X.Knox, J.E.Hratchian, H.P.Cross, J.B.Bakken, V.Adamo, C.Jaramillo, J.Gomperts, R.Stratmann, R.E.Yazyev, O.Austin, A.J.Cammi, R.Pomelli, C.Ochterski, J.W.Ayala, P.Y.Morokuma, K.Voth, G.A.Salvador, P.Dannenberg, J.J.Zakrzewski, V.G.Dapprich, S.Daniels, A.D.Strain, M.C.Farkas, O.Malick, D.K.Rabuck, A.D.Raghavachari, K.Foresman, J.B.Ortiz, J.V.Cui, Q.Baboul, A.G.Clifford, S.Cioslowski, J.Stefanov, B.B.Liu, G.Liashenko, A.Piskorz, P.Komaromi, I.Martin, R.L.Fox, D.J.Keith, T.Al-Laham, M.A.Peng, C.Y.Nanayakkara, A.Challacombe, M.Gill, P.M.W.Johnson, B.Chen, W.Wong, M.W.C.Gonzalez, J.A.Pople; Gaussian 03, Gaussian, Inc.Pittsburgh, PA, **(2003)**.