Proton Transfer in the Adenine–Thymine Base Pair

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Abstract: The transition state for the double proton transfer in the Watson–Crick type of adenine–thymine (AT) base pair was located by using the ab initio HF MINI-1 full gradient optimization technique. Allowing the geometry to relax in the calculation of the double proton transfer and interaction energies results in a substantial lowering of the energetic difference between AT and its imino–enol tautomer, A*T*. An energy barrier of 9.7 kcal/mol was calculated for the lowest energy reaction path from the canonical AT structure to the rare tautomeric A*T* structure, whereas a barrier of only 0.2 kcal/mol occurred for the reverse reaction. Although the relative energies may be affected by the theoretical level used, it is evident that there exist two energy minima which are separated by a low-lying transition state. Our results confirm the stability of the genetic code. More specifically, the possibility that proton transfer in the nucleic acid base pairs might be the cause of spontaneous point mutations in DNA and aging phenomena is not supported by the SCF/MINI-1 results for the isolated AT base pair. No minima which correspond to the zwitterionic structures A^+T^- and A^-T^+ were found. The effect of basis set extension was studied by including single-point SCF/MIDI-1 calculations. In addition, we suggest a possible role of the remarkably large interaction enthalpy of the A*T* base pair formed by imino–enol tautomers in RNA–ribozyme and codon–anticodon interactions.

Introduction

The tempting possibility that imino–enol tautomeric forms of DNA bases are involved in the process of mutagenesis, due to their ability to form mispairs with canonical DNA bases, has accompanied molecular genetics from its very beginning.[1,2] The fidelity of the replication process in vivo per one base pair was found[3,4] to be 10^8–10^{10}. The more recent study of Fersht and Knill-Jones[5] has shown the error rate in the procaryotic cell to be higher and close to the rate in vitro, i.e., one mistake in 10^6 to 10^7 base pairs. Extensive experimental and theoretical studies carried out in the past three decades[6–15] have disproved the presence of rare tautomers of DNA bases in both polar and nonpolar environments, in solution as well as in solid state. However, the experiments could not, in principle, detect any amount of rare tautomers below the limit of 10^{-3}%, due to the limited sensitivity of the methods. Consequently, the question of whether point mutations could be formed spontaneously via the tautomerism of free nucleotides in solution remains unanswered. Alternatively, the rare tautomers could be formed within the hydrogen-bonded base pair by the process of concerted transfer of two protons in two parallel bonds, proposed by Löwdin[16,17] (Figure 1). If the equilibrium ratio of rare to canonical tautomers in DNA formed in this way was larger than 10^{-4}–10^{-5}% and the rare tautomers remained stable during the time period needed for the replication process, double proton transfer would play an important role for the occurrence of spontaneous mutations. The feasibility of the first of the aforementioned necessary conditions is investigated in this study by ab initio methods.

A quantitative evaluation of the probability of the proton transfer in a hydrogen-bonded system must be based on knowledge of the dependence of the total energy of the system on the proton coordinates, i.e., on a part of the potential energy surface (PES). The potential energy curve for the proton transfer in the O—H···O, N—H···O, and N—H···N hydrogen bonds occurring in organic systems may exhibit both single and double well character. An authoritative review of this topic has been made by S. Scheiner.[18] The results of early semiempirical studies[19,20] which provided a double well potential for the transfer of a single proton in the adenine–thymine (AT) and guanine–cytosine (GC) base pairs at a time were questioned by subsequent ab initio[21] and PRDDO calculations[22] resulting in a single well potential. This was attributed to the strong electrostatic forces which tend to attract the proton back to the negatively charged base. The double proton transfer, which preserves the electroneutrality of both hydrogen-bonded species during the proton transfer, was calculated to be described by double well potential. Kong et al.[23] calculated by the ab initio STO-3G method the energy difference of canonical

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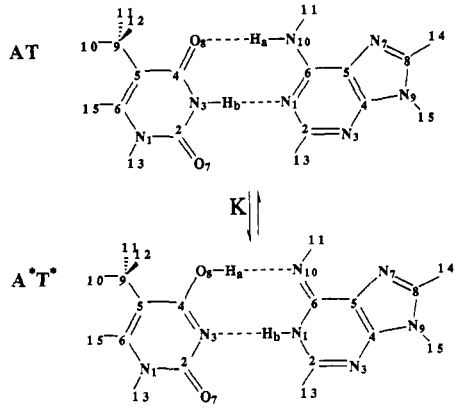

Figure 1. Structure and numbering of the canonical (AT) and imino–enol (A*T*) adenine–thymine base pair.

AT and tautomeric A*T* base pairs to be 51.5 kcal/mol. Scheiner and Kern[22] used the semiempirical method PRDDO to evaluate double proton transfer potentials and tunneling rates in both GC and AT base pairs. They obtained energy differences of 61.6 and 94.3 kcal/mol for GC and 82.8 kcal/mol for AT base pairs.

These results as well as other semiempirical studies[24] provided a theoretical explanation for the stability of the Watson–Crick genetic template, since such a model assumes implicitly that the protons forming the template are held in place by sufficiently high energy barriers. However, all previous calculations used the frozen intramolecular coordinates approximation for the evaluation of potential energy curves. The use of an adiabatic potential that allows us for geometry relaxation during the proton transfer process is necessary to calculate the equilibrium constant K that expresses the ratio between the canonical and tautomeric structures. This led in our previous SCF/MINI-1 study of energetics and harmonic vibrational spectra of AT and A*T* base pairs[25] to a significant decrease of the energy difference between the AT and A*T* structures to 9.5 kcal/mol ($K = 10^{-7}$). To estimate quantitatively the kinetics of the proton transfer, the energy barrier between both stationary structures has to be evaluated as well. Therefore, we optimized, in the present study, the geometry of the AT base pair corresponding to the transition and to the zwitterionic structures, and we also included enthalpies of AT and A*T* formation and basis set effects in the discussion. The closing paragraphs are devoted to the analysis of our computational results in the context of both the mutagenesis and of codon–anticodon recognition in proteosynthesis.

Computational Methods

The search for the transition state of the double proton transfer in the AT base pair was carried out at the HF SCF/MINI-1 level in three steps. First, intramolecular geometries were preoptimized holding the proton positions fixed in the middle of hydrogen bonds. This calculation was followed by the force constants matrix calculation, used in the subsequent full optimization to the transition state as implemented in the Gaussian 90 program.[26] The whole process had to be repeated twice until convergence in the last step was reached. The correspondence of the structure obtained to the saddle point of first order on the potential energy surface (PES) was verified by the calculation of harmonic vibrational frequencies from which, subsequently, the zero-point vibrational energy (ZPE) was determined. A single-point SCF calculation using the split-valence MIDI-1 basis set was done at the MINI-1 geometry of the transition state. The Huzinaga's MINI-1 and MIDI-1 basis sets[27] were used since they gave interaction energy values close to those obtained with DZP basis set; further, these basis sets yield relatively small basis set superposition error.[28] The same procedure and basis set were used in the unsuccessful search for the stationary points on the PES, corresponding to the zwitterionic A^+T^- and A^-T^+ structures.

Results and Discussion

In Table 1, the geometry of the transition state (TS) is compared with the AT and A*T* geometries. The double proton transfer into the rare tautomeric structure A*T* leads to a notable shortening of distances between heavy atoms forming hydrogen bonds. A further shortening was, as expected, found in the TS structure. The calculated results evidence the strong coupling of the symmetric intermolecular stretching vibration with the effective coordinate for the simultaneous double proton transfer. The same conclusion can be drawn by analyzing Cartesian components of the normal vibrational mode corresponding to the one imaginary harmonic vibrational frequency (496 cm^{-1}) in the transition state. The observation that the proton transfer is assisted by hydrogen bond compression was pointed out by several authors.[18,29] Evidence for this phenomenon has also been obtained experimentally.[30] The linearity of hydrogen bonds is conserved during the whole simultaneous double proton transfer mainly because protons do not occupy the middle points of the corresponding hydrogen bonds in the TS structure, thus avoiding their mutual electrostatic repulsion. Instead, the protons are shifted only 0.08 and 0.05 Å from their equilibrium A*T* positions with regard to the O8 and N1 atoms, respectively.

As far as the transfer of a single proton in one hydrogen bond is concerned, we may conclude that relaxation of geometry does not lead to the formation of the stationary state corresponding to the zwitterionic structure. The results of the previous calculations[21,22] that provided only single well potential for this type of proton transfer were consequently confirmed. Nevertheless, one may expect a certain stabilization of the zwitterionic structure when larger basis sets and contributions of correlation energy[31] and polar environment[32] are included in the calculation. Since the energy of the A^+T^--like structure (the position of the proton was hold fixed 1.04 Å from the N1 nitrogen of adenine, remaining geometry was optimized) was found to be only 17 kcal/mol above the main AT minimum, the possible significance of the single proton transfer cannot be ruled out.

The energetics of the double proton transfer and formation of AT and A*T* base pairs from isolated molecules is presented in Figure 2 and Table 2. It is evident that the AT structure is more stable than the A*T* one. However, the energy difference (9.51 kcal/mol) calculated here is smaller than those published previously. Further, the TS is found to be only 0.2 kcal/mol above the A*T* isomer. The very small energy barrier should be viewed with caution because it may be a consequence of the theoretical level used.[33] Nevertheless, clearly the two minima (AT, A*T*) are separated by a low-lying TS. The ratio K of tautomeric to canonical base pairs is in thermal equilibrium

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Table 1. Calculated HF SCF/MINI-1 Geometries (in Å and deg) of the AT and A*T* Base Pairs and the Transition State for the Double Proton Transfer (TS)

bond[a]	AT	TS	A*T*	angle[a]	AT	TS	A*T*
				Adenine in the Complex			
N1–C2	1.422	1.428	1.429	N1–C2–N3	131.13	128.72	128.33
C2–N3	1.396	1.377	1.373	C2–N3–C4	109.38	108.99	108.91
N3–C4	1.429	1.453	1.458	N3–C4–C5	127.46	128.52	128.86
C4–C5	1.442	1.432	1.430	C4–C5–C6	116.80	118.33	118.69
C5–C6	1.480	1.505	1.514	C5–C6–N1	118.40	113.29	111.95
C6–N1	1.410	1.439	1.449	C6–N1–C2	117.67	122.14	123.26
C5–N7	1.463	1.456	1.455	C4–C5–N7	111.55	111.62	111.55
N7–C8	1.368	1.371	1.371	C5–N7–C8	103.30	103.57	103.66
C8–N9	1.453	1.451	1.450	N7–C8–N9	113.38	112.72	112.61
N9–C4	1.429	1.424	1.424	C8–N9–C4	106.42	106.59	106.58
C6–N10	1.409	1.363	1.354	N9–C4–C5	105.35	105.51	105.60
N10–H11	1.045	1.055	1.058	C5–C6–N10	122.19	127.06	128.71
C8–H14	1.141	1.140	1.139	C6–N10–H11	116.01	110.24	108.97
N9–H15	1.050	1.051	1.051	N3–C2–H13	115.94	117.83	118.15
C2–H13	1.151	1.154	1.153	N7–C8–H14	124.94	125.27	125.33
				C8–N9–H15	128.20	128.25	128.26
				Thymine in the Complex			
N1–C2	1.467	1.475	1.475	N1–C2–N3	115.44	117.70	117.80
C2–N3	1.457	1.464	1.470	C2–N3–C4	124.44	119.66	118.82
N3–C4	1.452	1.398	1.383	N3–C4–C5	116.76	122.42	123.79
C4–C5	1.551	1.539	1.535	N4–C5–C6	118.45	116.88	116.32
C5–C6	1.389	1.394	1.395	C5–C6–N1	122.57	121.33	121.23
C6–N1	1.447	1.440	1.439	C6–N1–C2	122.34	122.01	122.04
C2–O7	1.302	1.304	1.304	N1–C2–O7	121.48	119.93	120.02
C4–O8	1.307	1.364	1.385	N3–C4–O8	121.01	121.01	120.48
C5–C9	1.568	1.567	1.567	C4–C5–C9	116.75	118.03	118.73
C9–H10	1.134	1.134	1.134	C5–C9–H10	110.18	110.03	109.83
C9–H12	1.138	1.137	1.138	C5–C9–H12	109.34	109.38	109.44
N1–H13	1.046	1.047	1.048	C6–N1–H13	121.33	121.29	121.25
C6–H15	1.141	1.141	1.142	C5–C6–H15	121.60	122.03	122.04
				C4–C5–C9–H11	59.14	59.13	59.22
				Hydrogen Bonds[b]			
N10A–Ha	1.071	1.330	1.474	C6A–N10A–Ha	120.35	125.55	127.30
N3T–Hb	1.095	1.446	1.568	C2T–N3T–Hb	116.44	115.49	113.81
N1A–Hb	1.647	1.161	1.112	C6A–N1A–Hb	121.91	116.22	115.66
O8T–Ha	1.729	1.186	1.107	C4T–O8T–Ha	122.49	115.42	113.22
N10A–O8T	2.799	2.516	2.580	N10A–Ha–O8T	177.31–	177.92+	177.05+
N1A–N3T	2.742	2.605	2.676	N3T–Hb–N1A	178.41+	175.21+	173.70+

[a] For atom numbering, see Figure 1. [b] Atoms belonging to adenine (thymine) are denoted by the A(T) letter following the atom number. The symbol + (–) denotes the trans (cis) position of the C6A and O8T (C2T and N1A) atoms with respect to the N10A–Ha (N3T–Hb) bond.

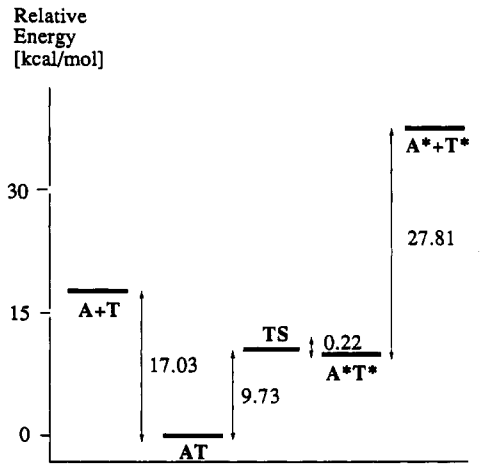

Figure 2. Relative energies (HF/MINI-1) of the significant stationary points on the potential energy surface of the adenine–thymine complex. A+T and A*+T* denote isolated monomers, TS denotes the transition state for the double proton transfer, and AT and A*T* denote the energies of canonical and rare tautomeric base pairs, respectively. For the interaction enthalpies at 0 K, i.e., the relative HF/MINI-1 energies corrected for BSSE and ZPE, see the text.

governed by Boltzman statistics:

$$K = \exp[-(E_{A^*T^*} - E_{AT})/RT]$$

where the product of the gas constant R and temperature T[K]

Table 2. SCF/MINI-1//SCF/MINI-1 and SCF/MIDI-1//SCF/MINI-1 Energies and Zero-Point MINI-1 Energies of Canonical and Rare Tautomers of Adenine and Thymine, Their Base Pairs, and the Transition State for the Double Proton Transfer (TS)

	MINI-1		MIDI-1
molecule	RHF [au]	ZPE [kcal/mol]	RHF [au]
A	–461.087 980	71.20	–461.629 351
A*	–461.064 604	72.26	–461.607 153
T	–448.199 419	73.81	–448.730 463
T*	–448.190 466	73.73	–448.696 481
AT	–909.314 540	146.84[a]	–910.392 404
A*T*	–909.299 389	146.47[a]	–910.360 340
TS	–909.299 035	144.30	–910.360 863

[a] In ref 25, page 1555, the incorrect (9.88 kcal/mol) SCF/MINI-1 + ZPE energy difference between AT and A*T* was given. The correct value is 9.14 kcal/mol.

has the magnitude of 0.62 kcal/mol at T = 310 K. Substitution yields the equilibrium constant K (310 K) = 10^{-7} for the system under study. Such a ratio might account for the observed frequency of spontaneous point mutations that fall into the 10^{-6}–10^{-10} range.[3-5]

The mutation hypothesis proposed by Löwdin assumes the occurrence of a certain small number of base pairs in the stable, rare tautomeric structure during the replication event as a consequence of the ability of protons to tunnel through the potential energy barrier. The notion "energy barrier" denotes here the energy difference between energies of the transition state (TS) and the upper stationary state (A*T*). The height and

width of the energy barrier for the double proton transfer determines the mean "lifetime" of the molecules in the tautomeric state. For temperatures near 310 K and small barriers, i.e., barriers less than about 2 kcal/mol, the proton transfer above the barrier, utilizing excited vibrational levels, dominates the rate of quantum mechanical tunneling. Thus, the lifetime of tautomeric molecules corresponds to the frequencies of molecular vibrations, i.e., 10^{-13} s. This time is much shorter than the time required for the opening of two DNA strands during the replication process, which can be described as a large, slow, and damped anharmonic motion. Moreover, any lengthening of the distance between adenine and thymine energetically disfavors the minimum corresponding to the A*T* structure.

The interaction enthalpies of the AT and A*T* base pairs[25] are 11.3 and 18.2 kcal/mol, respectively. These values were calculated with respect to the isolated A,T and A*,T* subsystems, including the counterpoise correction for the basis set superposition errors. They may be considered as energies required in the replication process for unpairing the canonical and tautomeric structures of adenine and thymine. The 60% greater interaction enthalpy (in absolute value) of the A*T* pair compared with the AT one implies the strengthening of hydrogen bonds by double proton transfer. This difference is manifested also by higher frequencies of intermolecular stretching vibrations.[25] The difference of 7 kcal/mol in pairing enthalpies originates mainly from the geometry relaxation effect.[25] It is notable that if the additional instant relaxation toward the AT structure is taken into account, as much as 27 (i.e., 9.1 + 18.2) kcal/mol is released by the pairing of imino–enol tautomers. Of course, this value must be reduced in the case of base pairing in the aqueous solution, but the ratio 3:1 of interaction enthalpy of tautomeric and canonical species should not be substantially changed. Similar behavior can be expected for the GC base pair.

The higher interaction energy of rare tautomers may become important when molecular recognition on the level of base pairing exhibits much higher specificity than might be expected from the number of interacting base pairs. Two illustrative examples from molecular biology can be pointed out. First, the association between an RNA enzyme (ribozyme) and substrate that is mediated by base pairing of the sequence of five base pairs was found to be accompanied by a binding energy greater than that of the standard RNA–RNA base pairing by about 4 kcal/mol.[34] To explain this phenomenon, T. Cech et al. introduced the model of participation of 2'-OH group of ribose in this tertiary interaction.[35] However, even this mechanism was not able to account for the whole observed excess of the interaction energy. The codon–anticodon recognition during the protein biosynthesis in ribosomes represents another, even more striking example of this type. Binding between effectively two pairs of RNA bases is decisive for the incorporation of the correct amino acid into the growing peptidic chain here.[36] We consider that the role of a single base tautomerization as an individual reaction step providing an explanation for the observed energy excess cannot be excluded here. We are aware that the assumption of such a hypothetic step makes the mechanism less probable. Nevertheless, the enzymatic machinery involved in the processes mentioned might be capable of such a function.

Since our results are based on purely theoretical methodology, the necessity appears for an evaluation of possible errors originating from accepted approximations. The MINI-1 basis set belongs to the group of minimal basis sets. It yields reasonable interaction energies of hydrogen bonding. Indeed, after the counterpoise correction, the calculated interaction enthalpy of the canonical AT base pair of 11.3 kcal/mol agrees within 10% with the gas-phase experimental data.[37] The main shortcoming of the MINI-1 basis set stems from its undersestimation of the lengths of hydrogen bonds. The error is in the range of 0.1–0.2 Å for hydrogen bonds between nucleic acid bases. On the other hand, intramolecular bond lengths are overestimated by this method. To evaluate the effect of basis set extension, we calculated the energies of AT and A*T* ($\Delta E_{A^*T^*} = E_{A^*T^*} - E_{AT} = 20.1$ kcal/mol) and transition state (TS) structures ($\Delta E_{TS} = E_{TS} - E_{AT} = 19.8$ kcal/mol) by the split valence MIDI-1 basis set at their MINI-1 geometries. From this calculation and from our calibration results from formamide dimer,[38] we estimate the true $\Delta E_{A^*T^*}$ to be in the 15–19 kcal/mol range and the transition state to lie slightly (0–2 kcal/mol) above the A*T* structure. Nevertheless, we are aware that only the optimization with an extended basis set can give the definitive answer about the relative energies of AT and A*T* structures. The effect of the polar environment should not influence the energy differences because both AT and A*T* structures have very small dipole moments.[25] Therefore, our conclusion, concerning the stability of the Watson–Crick template with respect to the double proton transfer in the AT base pair, seems to be reasonable also from this point of view. This, however, does not disprove a possible applicability of the Löwdin mutation hypothesis in the case of the guanine–cytosine base pair, where a smaller difference in the energy of ordinary and rare tautomers (as compared with the AT pair) was previously calculated[21,22] and the contribution of geometry relaxation still remains to be determined. It should be noted at this place that the proposition of Löwdin is not based on pure energetic grounds. It also assumes that after double proton transfer and the opening of the double stranded DNA, the bases remain non-hydrogen bonded until complex formation with another base. This assumption is not completely realistic because of the presence of water molecules, which are capable of forming hydrogen-bonded complexes with the rare tautomers in the template and which can possibly promote the reverse tautomeric transition toward canonical forms of the bases.

Conclusions

(1) The geometry and energy of the transition state for the double proton transfer in the AT base pair are surprisingly close to those found for the rare imino–enol tautomer of the AT base pair.

(2) The relaxation of geometry significantly lowers the energy of the A*T* base pair and increases the interaction enthalpy of A* and T* so that it is 60% larger (in absolute value) than those for the canonical bases. On the other hand, the contribution of the geometry relaxation is not large enough for the zwitterionic A^+T^- and A^-T^+ structures to appear as the stationary points on the SCF/MINI-1 potential energy surface of the adenine–thymine base pair.

(3) The very small energy barrier for the double proton transfer, together with the large energy needed for separation of the A*T* base pair and the nonexistent zwitterionic A^+T^- and A^-T^+ structures, represents the origin of the stability of the AT component of the genetic code.

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