Excited-State Amino−Imino Double-Proton Tautomerism in Adenine Nucleotide Analogues Catalyzed by Carboxylic Acids

Pi-Tai Chou,* Yuan-Chan Chen, Ching-Yen Wei, and Wen-Sin Chen

Department of Chemistry, The National Chung-Cheng University, Chia-Yi, Taiwan, R.O.C.

Received May 10, 2000
Revised Manuscript Received August 8, 2000

The excited-state double-proton-transfer (ESDPT) reaction in 7-azaindole hydrogen-bonded dimer and complexes has received considerable attention[1a−m] and has served as a model to study the fundamental mechanism for the mutation due to a "misprint" induced by the proton transfer of a specific DNA base pair.[1b,2,3] Further focus on the ESDPT reaction relevant to the molecular nature of mutation requires the study of the proton-transfer reaction in 7AI analogues of biological importance. Among which, adenine possessing a similar structural moiety with respect to 7AI is of particular interest. Unfortunately, the reaction center incorporating the proton migration from the N(1) to the N(7) atom in 7AI does not take place in the adenosine due to the linkage of the N(9) atom (see Figure 1) in adenine with a sugar moiety. Instead, the proposed proton-transfer inducing mutation for the adenine (A)−thymine (T) pair is based on the amino−imino tautomerism incorporating a shift of the N^6 proton to the N(1) nitrogen in adenine coupled with a keto−enol tautomerism of thymine.[2,3] Numerous experimental and theoretical approaches have drawn the conclusion that the A(amino)−T(keto) → A(imino)−T(enol) double-proton-transfer tautomerism is thermally unfavorable in the ground state.[3−9] Likewise, a recent ab initio calculation also predicts a highly endothermic ESDPT of ∼10 and 15 kcal/mol for the locally excited A*(amino)−T(keto) and A(amino)−T*(keto) pairs, respectively (* indicates the first electronically excited state).[9] The results are in accordance with experimental progress up to this stage, showing no evidence of proton transfer for the A−T pair in the excited states.

The occurrence of double-proton transfer in the A−T pair forming their corresponding imino and enol forms can be specified as a noncatalytic process defined in the case of 7AI hydrogen-bonded complexes,[10] where the molecular structures for both A and T are altered simultaneously. Such a process should be energetically more unfavorable than that of a catalytic type of proton transfer in which only the host molecule (e.g., 7AI) is tautomerized, while the molecular structure of the guest species (such as carboxylic acids[10]) remains unchanged. On this basis, it is of importance to investigate the catalytic type of ESDPT for various adenine/guest hydrogen-bonded complexes to further explore the fundamental basis of the photoinduced mutagenesis. Consequently, 9-cyclohexylmethyl adenine (9CHA) was synthesized[11] (see Figure 1). On one hand, the cyclohexylmethyl functional group acts as a subsituent to simulate the linkage of the deoxyribose site at the N(9) position. On the other hand, the hydrophobic moiety of the cyclohexylmethyl group increases the solubility of 9CHA in nonpolar solvents (e.g., cyclohexane) so that the formation of host/guest hydrogen-bonded complexes can be free from the solvent perturbation. Excited-state carboxylic acid-catalyzed amino−imino tautomerism in 9CHA was first observed in which key results and discussion are presented as follows.

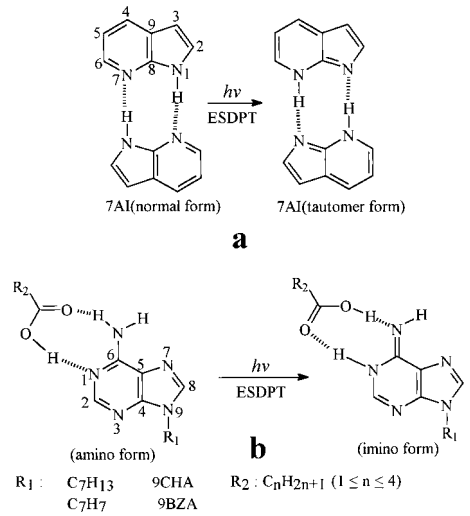

Figure 1. Proposed ESDPT mechanism of (a) the 7AI dimer and (b) adenines/carboxylic acid complexes.

Acid concentration-dependent electronic absorption spectra were observed for 9CHA in which the 258-nm peak characterized for the $S_0-S_1(\pi\pi^*)$ transition gradually disappeared upon increasing the acetic acid concentration, accompanied by a red shift of the absorption profile and an appearance of isosbestic point at ∼255 nm throughout the titration in cyclohexane (see Figure 2). In comparison, N^6,N^6-(dimethylamino)-9-cyclohexylmethyl purine (6DCP),[13] which is treated as a non-proton-transfer model due to the lack of N^6 protons, exhibits identical absorption profiles in the same range of added acetic acid concentration. The comparative experiments unambiguously conclude the formation of a 9CHA/acetic acid complex incorporating the N^6 proton. On the basis of a dominant complex formation possessing a 1:1 9CHA/acetic acid dual hydrogen-bonding configuration (see Figure 1), the association constant, K_a, was calculated to be 1.1×10^4 M^{-1}

(1) (a) Taylor, C. A.; El-Bayoumi, M. A.; Kasha, M. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *63*, 253. (b) Ingham, K. C.; El-Bayoumi, M. A. *J. Am. Chem. Soc.* **1974**, *96*, 1674. (c) Chapman, C. F.; Maroncelli, M. *J. Phys. Chem.* **1992**, *96*, 8430. (d) Chou, P. T.; Martinez, M. L.; Cooper, W. C.; McMorrow, D.; Collin, S. T.; Kasha; M. *J. Phys. Chem.* **1992**, *96*, 5203. (e) Chen, Y.; Gai, F.; Petrich, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 10158. (f) Douhal, A.; Kim, S. K.; Zewail, A. H. *Nature* **1995**, *378*, 261. (g) Goodman, M. F. *Nature* **1995**, *378*, 237. (h) Smirnov, A. V.; English, D. S.; Rich, R. L.; Lane, J. Teyton, L.; Schwabacher, A. W.; Luo, S.; Thornburg, R. W.; Petrich, J. W. *J. Phys. Chem. B* **1997**, *101*, 2758. (i) Mente, S.; Maroncelli, M. *J. Phys. Chem. A* **1998**, *102*, 3860. (j) Takeuchi, S.; Tahara, T. *J. Phys. Chem. A* **1998**, *102*, 7740. (k) Chaban, G. M.; Gordan, M. S. *J. Phys. Chem. A* **1999**, *103*, 185. (l) Chou, P. T.; Wu, G. R.; Wei, C. Y.; Cheng, C. C.; Chang, C. P.; Hung, F. T. *J. Phys. Chem. B* **1999**, 103, 10042. (m) Fiebig, T.; Chachisvillis, M.; Manger, M.; Zewail, A. H.; Douhal, A.; Garcia-Ochoa, I.; de La Hoz Ayuso, A. *J. Phys. Chem. A* **1999**, 103, 7419.

(2) Watson, J. D.; Crick, F. H. C. *Nature (London)* **1953**, *171*, 964.

(3) Lowdin, P.-O. *Adv. Quantum Chem.* **1965**, *2*, 213.

(4) Scheiner, S.; Kern, C. W. *J. Am. Chem. Soc.* **1979**, *101*, 4081.

(5) Kong, Y. S.; John, M. S.; Lowdin, P. O. *Int. J. Quantum Chem., Quantum Biol. Symp.* **1987**, *14*, 189.

(6) Katrizky, A. R.; Karelson, M. M. *J. Am. Chem. Soc.* **1991**, *113*, 1561.

(7) (a) Hrouda, H.; Florian, J.; Hobza, P. *J. Phys. Chem.* **1993**, *97*, 1542. (b) Florian, J.; Hrouda, V.; Hobza, P. *J. Am. Chem. Soc.* **1994**, *116*, 1457.

(8) Florian, J.; Leszczynski, J. *J. Am. Chem. Soc.* **1996**, *118*, 3010.

(9) Gullar, V.; Douhal, A.; Moreno, M.; Lluch, J. M.*J. Phys. Chem. A* **1999**, *103*, 6251.

(10) Chou, P. T.; Wei, C. Y.; Chang, C. P.; Chiu, C. H. *J. Am. Chem. Soc.* **1995**, *117*, 7259.

(11) 9CHA was synthesized according to ref 12. NMR analyses: ^{1}H NMR-(DMSO-d_6, 400 MHz) δ 1.0−1.85 (m, 11H), 3.95 (m, 2H), 8.09 (s, 1H), 8.12 (s, 1H).

(12) Hedayatullah, M. *J. Heterocycl. Chem.* **1982**, *19*, 249.

(13) 6DCP was synthesized according a procedure similar to that for 9CHA except that 6-dimethylpurine was used as a starting reactant: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.3 (s, 1H), 7.6 (s, 1H), 3.8 (m, 2H), 3.4 (s, 6H), 1.1−1.9 (m, 11H). Note that like N^6,N^6-dialkylamino derivatives of purines,[14] 6DCP exhibits charge-transfer emission and thus cannot be applied as a non-proton-transfer model in the emission study.

(14) Andreasson, J.; Holmen, A.; Albinsson, B. *J. Phys. Chem. B* **1999**, *103*, 9782.

10.1021/ja001601t CCC: $19.00
Published on Web 09/09/2000

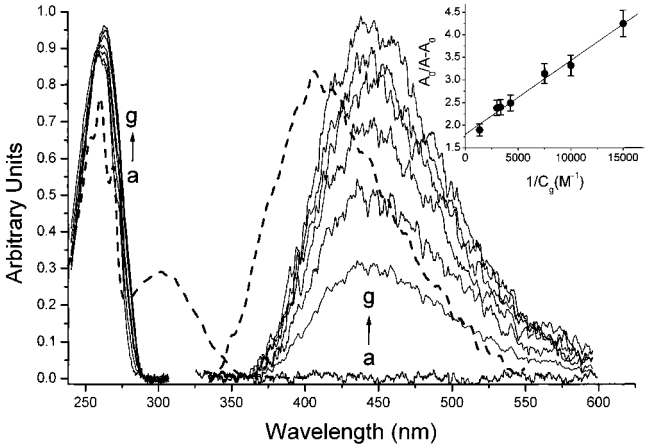

Figure 2. (—) absorption and emission spectra of 9CHA (1.2×10^{-5} M) in cyclohexane by adding acetic acid concentrations of (a) 0, (b) 6.7×10^{-5}, (c) 1.0×10^{-4}, (d) 1.4×10^{-4}, (e) 2.3×10^{-4}, (f) 3.0×10^{-4}, and (g) 7.3×10^{-4} M. (- - -) Absorption and emission spectra of 1MCA in cyclohexane (5.0×10^{-5} M). Inset: plot of $(A_0/A - A_0)$ at 272 nm as a function of $1/[C_g]$ (b−g**)** and a best least-squares fitting curve using the equation depicted in ref 15.

at 298 K.[15] Acetic acid concentration-dependent fluorescence spectra were also observed in 9CHA (see Figure 2). At sufficiently low concentration so that only monomer exists, negligible emission was detected under our detection limit. The lack of fluorescence in 9CHA may be rationalized by its close lower-lying $^1\pi\pi^*$ and $^1n\pi^*$ states, inducing a fast internal conversion followed by a dominant intersystem crossing.[16] Upon increasing the acetic acid concentration, a unique fluorescence maximum at ∼440 nm gradually appeared. The decay of the 440-nm emission band follows a single-exponential kinetics of $k_f \sim 8.3 \times 10^9$ s^{-1} ($\tau_f \sim 120$ ps), while the rise time is beyond the response of our current photon-counting system of ∼30 ps.[17] The excitation spectrum maximum of ∼270 nm monitored at 440 nm was red shifted by ∼12 nm with respect to that of the uncomplexed species, indicating that the emitting band originates from the 9CHA/acetic acid complex. The emission with a large Stokes shift relative to the excitation maximum ($>10^4$ cm^{-1}) accompanied by a system response limit rise time leads us to conclude the occurrence of a fast ESDPT in the 9CHA/acetic acid complex, resulting in an imine-like tautomer. To further verify this viewpoint, a model compound of the imino tautomer, 1-methyl-9-cyclohexylmethyl adenine (1MCA), was synthesized.[18] 1MCA exhibits a normal Stokes shifted fluorescence maximum at ∼410 nm ($\phi_f \sim 0.002$, $\tau_f \sim 115$ ps in cyclohexane) of which the spectral features and relaxation dynamics resemble the 440-nm emission band, supporting the occurrence of ESDPT in the 9CHA/acetic acid complexes. The red shift of the 9CHA/acetic acid tautomer emission with respect to that of 1MCA can be rationalized by its strong dual hydrogen bond formation.

Table 1. Thermodynamic and Photophysical Properties of Adenine Analogue/Acid Complexes in Cyclohexane (298 K)

	absorption λ_{max} (nm)	emission λ_{max} (nm)	F_{obs}	τ_f (ps)	$K_a(M^{-1})$
9CHA	258	na[a]	na	na	
9CHA/acetic acid	270[b]	440	3.5×10^{-3}	120	1.1×10^4
1MCA	270, 300, 340[c]	410	2.0×10^{-3}	115	
9CHA/BIHP	274[b]	370	8.2×10^{-3}	630	2.3×10^4
9BZA	258	na	na	na	
9BZA/acetic acid	270[b]	442	1.6×10^{-3}	125	1.0×10^4

[a] na, not available [b] Values were obtained from the fluorescence excitation spectrum. [c] The first vibronic peak of the S_0-S_1 ($\pi\pi^*$) transition.

The formation of a 1:1 dual hydrogen-bonded complex was also observed in other linear carbon-chain carboxylic acids as well as the biologically interesting carboxylic acids such as thioglycolic acid ($HSCH_2COOH$). Such a carboxylic acid-catalyzed ESDPT reaction is selective. When a phosphoric acid-related guest molecule such as bis(2-isopropyl)$_2$ hydrogen phosphate ($(CH3)_2CHO)_2P(O)OH$, BIHP) was added, the formation of a hydrogen-bonded complex was also observed (see Table 1). However, only the cationic type of emission maximized at 370 nm was observed, possibly due to the much higher acidity of BIHP of $pK_a \sim 2.1$. Thus, an excited-state protonation takes place instead of the ESDPT reaction.[19] ESDPT was also observed in other adenine nucleotide analogues such as 9-benzyladenine (9BZA). Table 1 summarizes thermodynamic and photophysical properties for 9CHA(or 9BZA)/carboxylic acids complexes. The results on one hand demonstrate for the first time the feasibility of an amino−imino tautomerism in the adenine analogues through a catalytic type of ESDPT and thus provide a more plausible biological model than 7AI to explore the ESDPT dynamics related to the mutation. On the other hand, it is important to note that many amino acid side chains have abnormal pK_a values in proteins.[20] Therefore, un-ionized carboxylic acids might be involved in the hydrogen bond interaction under biological conditions. Formation of cyclic dual hydrogen bonds allows an amino acid side chain incorporating carboxylic acid to discriminate between different nucleic acid bases, which serves as an important role in one of the interactions involved in selective recognition of nucleic acid bases by proteins. Therefore, it is also of great importance to probe the dual hydrogen-bonding protein−nucleic acid interaction[21] based on the excited-state proton-transfer tautomerism. These fundamental issues should initiate a broad spectrum of interest in the field of proton-transfer studies.

Acknowledgment. This work was supported by National Science Council NSC (89-2113-M-194-009).

JA001601T

(15) An equation, $A_0/(A - A_0) = (\epsilon_M/(\epsilon_C - \epsilon_M))[1/K_a[C_g] + 1]$, was applied to calculate the formation constant of the 1:1 9CHA/guest complex,[11] where K_a is the association constant, $[C_g]$ denotes the acid concentration, A_0 and A are the absorbance at a selective wavelength with and without adding acetic acid, and ϵ_M and ϵ_C are molar extinction coefficients of the 9CHA monomer and complex at that wavelength, respectively.

(16) An extremely weak fluorescence with a quantum yield of 5×10^{-5} at ∼320 nm has been reported for adenosine: Callis, P. R. *Annu. Rev. Phys. Chem.* **1983**, *34*, 329.

(17) Lifetime measurements were performed by using a third (260−275 nm) harmonic of the Ti−Sapphire oscillator (Spectra Physics) as an excitation source. An Edinburgh OB 900-L time-correlated single-photon counter was used as a detecting system.

(18) 1MCA was synthesized by stirring 9CHA (0.1 g) and CH_3I (0.4 g) in *N*,*N*-dimethylacetamide (3 mL) under N_2 at 50 °C for 2 h. NaOH (2.5 N, 3 mL) was then added and the mixture was stirred for ∼ 20 min: ^1H NMR (DMSO-d_6, 400 MHz) δ 3.72 (s, 3H), 4.06 (s, 2H), 1.0−1.85 (m, 11H), 8.41 (s, 1H), 8.56 (s, 1H).

(19) The result is similar to 9CHA in the acidified aqueous (or methanol) solution (pH ∼1.0) where 9CHA exhibits an N(1)-protonated cationic emission maximum at 370 nm.

(20) (a) Blow, D. M.; Steiz, T. A. *Annu. Rev. Biochem.* **1970**, *39*, 63. (b) Lancelot, G. *J. Am. Chem. Soc.* **1977**, *99*, 7037.

(21) Stormo, G. D.; Fields, D. S. *Trends Biochem. Sci.* **1998**, *23*, 109.