Feasibility of Adenine Photoinduced Mispairing of the Watson−Crick Pairing in DNA

J. Catalán

Departamento de Química Física Aplicada, Universidad Autónoma de Madrid, Cantoblanco, E−28049 Madrid, Spain

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The complexes potentially formed by 9-alkyl adenine with acetic acid were characterized, and the feasibility of them undergoing a two-proton transfer via the hydrogen bonds that bind the two components was determined, in light of the chemical simultaneity criterion. In this way, the ability of adenine to undergo a two-proton transfer in its first π,π^* excited state via Watson−Crick pairing was ruled out. On the other hand, the process is feasible when the adenine binds to acetic acid via Hoogsteen pairing.

Introduction

Ever since Watson and Crick[1] reported the pairing scheme for DNA bases in 1953, a great deal of the molecular specificity of nucleic acids has been accepted to reside in the manner in which purines and pyrimidines interact with each other through hydrogen bonding. As a result, the property of nucleic acids that is directly involved in the stable storage, transmission, and expression of genetic information is the specific association of the purine and the pyrimidine bases. Also, the base sequence of DNA is replicated and transcribed to messenger RNA by mechanisms that observe the strict complementarity of adenine with thymine or uracil and cytosine with guanidine.

The highly specific pairing between these bases relies on their occurring in their most stable forms, i.e., as keto-amino tautomers. As already pointed out by Watson and Crick,[1] the presence of forms of these bases such as the enol-imine tautomer might result in anomalous pairing. One way of obtaining these unwanted base forms in situ is by inducing simultaneous proton transfers via their hydrogen bonds, which would yield the above-mentioned tautomers. As a result, these prototropic tautomerisms might be involved in spontaneous mutagenesis because the H-bonding patterns of tautomers favour mispairing.[2−4]

For example, the in situ generation of the amino−imino tautomer of adenine in DNA would entail having the corresponding adenine/thymine pair undergo a two-proton transfer via the hydrogen bonds that link them. The process is so strongly endothermal in the ground electronic state that it is highly unlikely to occur; it may, however, be more feasible in excited electronic states,[5,6] which are usually much more acidic and basic.[7] A good photophysical instance of this situation is 7-azaindole dimer, which undergoes a two-proton transfer in its first excited singlet.[8,9]

Recently, Chou et al.[10] showed 9-cyclohexylmethyl adenine (9CHA) in its first π,π^* excited electronic state to undergo a two-proton transfer via one molecule of acetic acid linked to it through a Watson−Crick base pairing mechanism. They concluded that the results demonstrate the feasibility of an amino−imino tautomerism in the adenine analogues through a catalytic type of excited state double proton transfer (ESDPT) for the first time, and thus provide a more plausible biological model to explore the ESDPT dynamics related to mutation than does 7AI.

Reproducing the pairing situations proposed by Watson and Crick for adenine on the molecular complex scale using monomeric species is far from simple, even if the base bears a substituent on its N_9 atom, which is the site involved in glycosidic linkage. The structural difficulty lies in the fact that the adenine monomer can use its amino group as proton donor for pairing via a double hydrogen bond in two different ways: one (Watson−Crick base pairing, **WCbp**) involves its pyrimidine nitrogen, N_1; the other (Hoogsteen base pairing, Hbp), involves its azole nitrogen, N_7. In both situations, two hydrogen bonds capable of binding a thymine molecule are formed (see Figure 1).

In 1959, Hoogsteen[11] found an aqueous solution containing equimolar amounts of 9-methyl-adenine and 1-methyl-thymine to form a complex that did not crystallize via **WCbp** but rather via one involving its N_7 atom; such a mechanism is currently known as "Hoogsteen base pairing". In 1964, Mathews and Rich[12] also found an equimolar mixture of 9-ethyl-adenine and *N*-methyl uracil in DMSO to crystallize via **Hbp**, so they suggested that the complex formed might be more stable than the corresponding **WCbp** complex in these solutions. Obviously, if an effect of the molecular environment suffices to induce crystallization of an **Hbp** structure, the Hbp complex must be intrinsically more stable or nearly as stable as the **WCbp** complex.

The more common nucleic acid bases (viz. adenine, guanine, cytosine, thymine, and uracil) possess special photophysical features as chromophores (e.g., they are nonfluorescent at room temperature[13−17]) that prevent damaging photochemistries in the nucleic acids carrying the genetic information in DNA and RNA. Although it has attracted the interest of several research groups,[10,18] the possibility of an ESDPT process destabilizing the information stored through base pairing in these structures seems unlikely.

This paper reports experimental and theoretical structural data for the complexes potentially formed between adenine and acetic acid, as well as the acidity and basicity changes undergone by the adenine sites involved in the complexes upon electronic excitation. The data were used to examine the likelihood of adenine being involved in ESDPT processes, destabilizing the information stored through the formation of highly specific hydrogen bonds in such important molecular structures.

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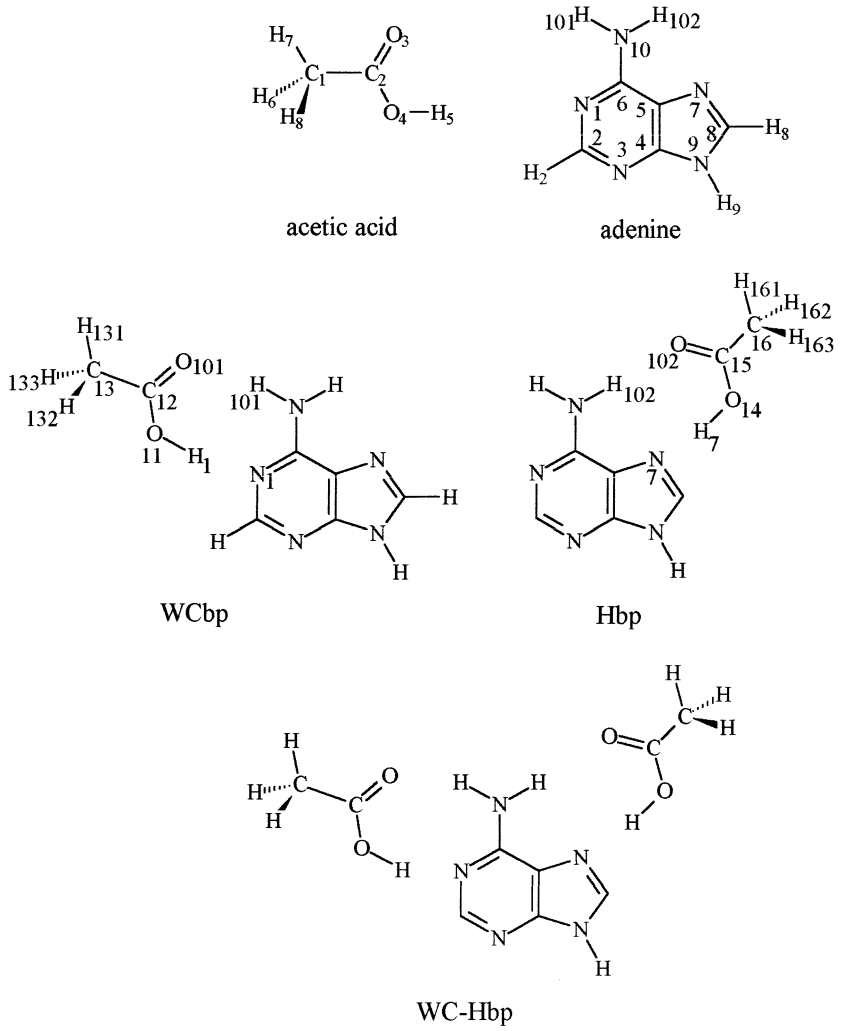

Figure 1. Molecular structures, and the corresponding numbering.

Theoretical and Experimental Section

All of the computations on the ground electronic state of the molecular structures studied were done within the framework of the methods of the density functional theory (DFT), using the Gaussian 98 software package.[19] Full geometry optimizations for the ground electronic state of the neutral molecular structures (viz. adenine and acetic acid, and their corresponding complexes), protonated adenine (with the proton on the pyrimidine nitrogen, N_1, or the azole nitrogen, N_7) and adenine with a deprotonated amino group, were carried out by using the hybrid functional B3LYP[20,21] in conjunction with the 6-31G** basis set. All of the calculated molecular structures were found to correspond to actual energy minima; in fact, all of them had positive vibrational frequencies. The optimized geometries for the ground state were used to compute the Frank−Condon excitation energies for the singlet excited state [$S_1(\pi,\pi^*)$] in the light of the recently developed time-dependent density functional theory (TDDFT), which has so far yielded excellent results.[22,23]

Protonation (eq 1) and deprotonation energies (eq 2) were both positive as they were expressed as the acidity of the protonated and neutral form, respectively. These values were computed as the differences between the total energy for the neutral and ionic forms directly involved in the equilibria in both the ground electronic state (ΔE_{prot} and ΔE_{deprot}) and the first π,π^* singlet excited state [$S_1(\pi,\pi^*)$] (ΔE^*_{prot} and ΔE^*_{deprot})-

$$(9EA)H^+ + \Delta E_{prot} \rightleftarrows 9EA + H^+ \quad (1)$$

$$9EA + \Delta E_{deprot} \rightleftarrows 9EA^- + H^+ (2) \quad (2)$$

UV spectra were recorded on a Cary-5 spectrophotometer, using rectangular quartz cells of 1 cm light path. Corrected fluorescence and excitation spectra were obtained by using a previously calibrated Aminco−Bowman AB2 spectrofluorimeter. Samples were excited at 260 ± 16 mm by using light from a continuous (CW) 150 W xenon lamp, with a slit width of 8 nm in the emission monochromator. Excitation spectra were recorded by monitoring the 460 or 360 nm wavelengths, using a slit width of 16 nm for emission and 4 nm for excitation.

9-Ethyl adenine (9EA) was purchased from Sigma and used as received. Cyclohexane (Cyh) was Merck Uvasol grade and contained less than 0.005% water. Glacial acetic acid (ACS reagent) was obtained from Sigma in 99.7% purity. All solutions were prepared from freshly opened bottles.

Results and Discussion

The calculated molecular structures for adenine and acetic acid reproduced their respective measured gas-phase rotational constants[24,25] quite acceptably (Table 1). Table 2 shows the theoretical and experimental bond distances and angles for the structures; as can be seen, there is close agreement between

TABLE 1: Theoretical and Experimental (in parentheses) Rotational Constants (A, B, and C in MHz) of Acetic Acid, Adenine, and Adenine-acetic Acid Complexes (Wcbp, Hbp, WC−Hbp)

compd	A	B	C
Acetic acid	11284.61 (11355.50)[a]	9420.37 (9478.64)[a]	5302.90 (5325.01)[a]
Adenine	2369.22 (2371.87)[b]	1566.19 (1573.35)[b]	943.03 (946.25)[b]
WCbp	1939.58	325.52	277.75
Hbp	1366.50	389.72	303.81
WC−Hbp	438.22	243.17	156.69

[a] Ref 24. [b] Ref 25.

both. Worth special note is the fact that the molecular structure of adenine, the vibrational frequencies of which are all positive, is nonplanar: it belongs to the C_1 symmetry group and its nonplanarity is due to its amino group, the protons in which are tilted by about 10° with respect to the molecular plane (see Table 2).

Specially relevant among the findings for adenine is that it is a polar molecule in the gas phase ($\mu = 2.41$ Dby), which is consistent with the reported evidence for its 9-methyl derivative in dioxane ($\mu = 3.25 \pm 0.20$ Dby)[28] and its 9-butyl derivative in CCl_4 ($\mu = 3.20$ Dby).[29] Also, as can be seen from Table 3, its amino protons are its acid sites (H_{101} being only 0.7 kcal/mol less acidic than H_{102}), and its pyridine (N_1 and N_3) and azole (N_7) nitrogen are its basic sites (N_1 being the most basic one). Worth special note is also the fact that the HOMO and LUMO of adenine are both π orbitals, whereas its HOMO−1 is an *n* orbital.

On the basis of the theoretical spectroscopic data, the first excited electronic state of adenine corresponds largely (70%) to the HOMO−1 → LUMO transition, i.e., it is an (n,π*) state. On the other hand, its second excited electronic state is of the (π,π*) type as it corresponds essentially to the HOMO → LUMO (56%) and HOMO → LUMO+1 (27%) transitions. These results are especially relevant as whether the first excited electronic state of adenine is of the (n,π*) or (π,π*) type has been a subject of debate since the 1960s.[30−32] Recently, Kim et al.[33] reported the jet-cooled spectrum for adenine, which they obtained using a resonant two-photon ionization (R2PI) technique, and concluded that the first excited electronic state was of the (n,π*) type and that the energy gap between the (n,π*) state and the first (π,π*) excited electronic state in adenine was in the region of 600 cm^{-1}.

Adenine−Acetic Acid Complexes. A 9-alkyl adenine molecule can use one of its amino protons (H_{101} or H_{102}) as a proton donor and one of its nitrogen atoms adjacent to the amino group (N_1 or N_7) as a proton acceptor to form complexes with acetic acid via a double hydrogen bond. These two compounds can thus potentially form two different complexes by hydrogen bonding: one (**WCbp**) involves the H_{101} and N_1 sites, and the other (Hbp) the H_{102} and N_7 sites in adenine.

Because the theoretical data (Table 3) suggest that N_1 is 8.4 kcal/mol more basic in the ground state than is N_7, one can expect hydrogen bonding interactions to be stronger in the **WCbp** complex than in the **Hbp** complex of adenine and the former to be more stable as a result. The theoretical results for the enthalpy and Gibbs free energy changes corresponding to the formation of the two complexes in the gas phase (Table 4) confirm the previous assumption; however, the stability difference between them is seemingly only 0.8 kcal/mol. Thermodynamically, the two complexes have negative ΔG^0 values (Table 4), so both will form spontaneously, the formation of the **WCbp** complex being slightly more likely.

One result of potential relevance to the problem addressed here is that the **Hbp** complex is much more polar ($\mu = 3.79$ Dby) and slightly less polarizable ($\alpha = 146.1$) than the **WCbp** complex ($\mu = 2.72$ Dby, $\alpha = 165.2$); as a consequence, the two will be more similarly stable in solution (even in hydrocarbon solvents). Accordingly, adding acetic acid to a dilute solution of a 9-alkyl adenine will result in the nearly simultaneous formation of both adenine−acetic acid complexes, so the evidence reported by Chou et al.[10] should be revised in the light of the concurrence of both complexes.

The **WCbp** complex is intrinsically more stable than the **Hbp** complex; however, the fact that the latter is substantially more polar results in it also being more stable than the former in such polar solvents as water and DMSO. This is consistent with the finding that equimolar mixtures of 9-methyl adenine and 1-methyl thymine in water,[11] and 9-ethyl adenine and *N*-methyl uracil in DMSO,[12] yield crystals consisting of **Hbp** complexes.

One other possibility worth considering is the formation of complexes involving both types of pairing such as that between one adenine molecule bound to two acetic acid molecules with its carbonyl oxygens bonded to N_1 and N_7, respectively, in adenine; we shall designate this complex WC−Hbp (see Figure 1), which is regarded as a feasible predecessor of the triple helix DNA[34−36]. The theoretical Gibbs free energy for this complex is −9.51 kcal/mol, see Table 4. Obviously, this complex will only form at higher acetic acid concentrations, where it will make an additional structure to be considered. Also worth noting is the fact that this complex is highly polar ($\mu = 4.44$ Dby) and polarizable ($\alpha = 189.8$), so it will become more stable (and possess a more negative ΔG^0 value) in the condensed phase. The principal structural data for the three complexes are compiled in Table 2.

The theoretical data suggest an interesting spectroscopic difference in these complexes from the above-described situation of adenine; in fact, their first excited electronic state is of the (π,π*) type. The calculated first $\pi \rightarrow \pi^*$ electronic transition in these complexes is bathochromically shifted (to 245.7, 252, and 253.3 nm for **WCbp**, **Hbp**, and **WC−Hbp**, respectively) from the corresponding $\pi \rightarrow \pi^*$ transition in adenine (which theoretically occur at 241.1 nm, this transition is detected at 249 nm in the gas phase[37]); on other hand, the ($n \rightarrow \pi^*$) transitions are hypsochromically shifted in **WCbp** and **WC−Hbp** (to 236.7 and 238.3 nm, respectively), but not in **Hbp** (which remains at 249.3 nm), from that in adenine (249.1 nm). This situation, together with the similar stability of the two adenine−acetic acid complexes, hinders distinguishing the **WCbp** and **Hbp** complexes on the basis of UV spectroscopic data.

Figure 2 shows the UV spectra for a 10^{-5} M solution of 9EA in pure cyclohexane (Figure 2, spectrum a) and cyclohexane with increasing amounts of acetic acid (Figure 2, spectra b−p). As can be seen, the spectrum for 9EA shifts bathochromically as the complexes form, consistent with the theoretical predictions. As previously noted by Chou et al.,[10] 9CHA in cyclohexane, like 9EA, exhibits when adding acetic acid, within the concentrations 6.7×10^{-5} and 7.3×10^{-4}, an isobestic point around 255 nm (see Figure 2). Careful analysis of the situation reveals that this is not the case because for solutions with low acetic acid content (i.e., those spectra gathered in the inset of Figure 2) the spectra intersect in the region between 253 and 257 nm. This suggests that, as the amount of acetic acid in the solution increases, the acid forms more than one type of complex with 9EA, so accurately identifying an isobestic point is impossible.

TABLE 2: Experimental and Theoretical Bond Distances (Å), Bond and Dihedral Angles (in degrees) of Acetic Acid, Adenine, and the Complexes WCbp, Hbp, and WC−Hbp[a]

Acetic acid

	electron diffraction[27]	theory		electron diffraction[27]	theory
C_1-C_2	1.520 ± 0.005	1.507	$C_1C_2O_3$	126.6 ± 0.6	126.1
C_2-O_3	1.214 ± 0.003	1.210	$C_1C_2O_4$	110.6 ± 0.6	111.4
C_2-O_4	1.364 ± 0.003	1.358	$O_3C_2O_4$	122.8	122.5
C_1-H_6	1.102 ± 0.012	1.094	$H_5O_4C_2$	107.0	105.9
O_4-H_5	0.97	0.972			

Adenine

	theory	X-ray of 9MeA[26a]	X-ray of 9MeA[26b]		theory	X-ray of 9MeA[26a]	X-ray of 9MeA[26b]
N_1C_2	1.344	1.335	1.348	$C_2N_3C_4$	111.1	110.0	112.4
C_2N_3	1.337	1.326	1.322	$N_3C_4C_5$	127.0	127.4	126.6
N_3C_4	1.339	1.351	1.338	$C_4C_5C_6$	115.8	116.8	117.2
C_4C_5	1.399	1.380	1.365	$C_5C_6N_1$	118.8	117.3	117.4
C_5C_6	1.411	1.411	1.395	$C_6N_1C_2$	118.3	118.7	119.4
C_6N_1	1.345	1.357	1.348	$C_5N_7C_8$	103.9	103.5	104.2
C_5N_7	1.381	1.389	1.379	$N_7C_8N_9$	113.5	114.4	112.0
N_7C_8	1.311	1.311	1.311	$C_8N_9C_4$	106.7	105.4	107.9
C_8N_9	1.381	1.365	1.354	$N_9C_4C_5$	104.4	106.4	104.7
C_4N_9	1.378	1.370	1.359	$C_4C_5N_7$	111.5	110.3	111.2
C_6N_{10}	1.355	1.329	1.348	$N_{10}C_6C_5$	122.3	124.3	125.7
$N_{10}H_{101}$	1.007	0.98		$C_5C_6N_{10}H_{101}$	−11.2		
$N_{10}H_{102}$	1.007	0.87		$N_1C_6N_{10}H_{102}$	10.2		
$N_1C_2N_3$	128.9	129.8	126.5				

Complexes Adenine−Acetic acid

	WCbp	Hbp	WC−Hbp		WCbp	Hbp	WC−Hbp
N_1C_2	1.348	1.339	1.344	H_1O_{11}	1.018		1.015
C_2N_3	1.329	1.339	1.331	$O_{11}C_{12}$	1.323		1.326
N_3C_4	1.343	1.337	1.341	$O_{101}C_{12}$	1.227		1.226
C_4C_5	1.396	1.398	1.395	$H_{102}O_{102}$		1.866	1.882
C_5C_6	1.415	1.419	1.424	N_7H_7		1.723	1.742
C_6N_1	1.357	1.352	1.363	H_7O_{14}		1.009	1.006
C_5N_7	1.383	1.389	1.387	$O_{14}C_{15}$		1.326	1.328
N_7C_8	1.311	1.314	1.314	$O_{102}C_{15}$		1.225	1.224
C_8N_9	1.381	1.370	1.370	$C_6N_{10}H_{101}$	120.1	117.0	118.6
C_4N_9	1.376	1.381	1.379	$C_6N_{10}H_{102}$	118.6	123.0	121.4
C_2H_2	1.087	1.088	1.088	$H_{101}\ O_{101}\ C_{12}$	124.5		124.5
C_8H_8	1.081	1.081	1.081	$N_{10}H_{101}O_{101}$	170.9		172.1
C_6N_{10}	1.338	1.341	1.329	$N_1H_1O_{11}$	173.6		173.0
$N_{10}H_{101}$	1.025	1.008	1.024	$H_1O_{11}C_{12}$	111.4		110.6
$N_{10}H_{102}$	1.007	1.021	1.021	$N_7H_7O_{14}$		169.4	169.8
$H_{101}O_{101}$	1.846		1.881	$H_7O_{14}C_{15}$		111.0	110.3
N_1H_1	1.694		1.706	$C_5C_6N_{10}H_{101}$	−180.0	180.0	−180.0

[a] The numbering corresponds to that of Figure 1.

TABLE 3: Protonation (ΔE_{prot}) and Deprotonation Energies (ΔE_{deprot}) for the Ground and $1(\pi,\pi^*)^1$ States of Adenine, together with the Energy Differences upon Electronic Excitation ($\Delta\Delta E_{prot}$ and $\Delta\Delta E_{deprot}$), in kcal/mol[a]

grp	ΔE_{prot}	ΔE_{deprot}	ΔE^*_{prot}	ΔE^*_{deprot}	$\Delta\Delta E_{prot}$	$\Delta\Delta E_{deprot}$
N_1	239.2		242.9		3.7	
H_{101}		374.8		351.3		−23.5
N_7	230.6		251.3		20.7	
H_{102}		374.1		352.3		−21.8

[a] The centers N_1 and H_{101} are to be involved in the **WCbp** type interaction, whereas the centers N_7 and H_{102} are to be involved in the **Hbp** type interaction.

TABLE 4: Enthalpy ($\Delta H°$) and Free Energy ($\Delta G°$) Variations for the Adenine−Acetic Acid Complex Formation: WCbp, Hbp, and WC−Hbp

complex	$\Delta H°$ (in kcal/mol)	$\Delta G°$ (in kcal/mol)
WCbp	−16.6	−6.2
Hbp	−15.7	−5.4
WC−Hbp	−31.3	−9.5

After a certain amount of acetic acid has been added to the 9EA solution, the spectrum profile hardly changes (i.e., those spectra between spectra *k* and *l* in Figure 2); however, if the acid content in the solution is then substantially increased, then the spectrum exhibits a significant bathochromic shift, (see spectra included between spectra *ll* and *o* in Figure 2). It is noteworthy that these spectra exhibit an isosbestic point at 259 nm, which is indicated in Figure 2. On the basis of the above-described theoretical results, this may indicate that the **WC−Hbp** complex forms within this concentration range.

Acidity and Basicity Changes upon Electronic Excitation: *Feasibility of the Process.* Once we have established (*a*) that the **WCbp** and **Hbp** complexes form at low acetic concentrations, (*b*) that, at high enough concentrations, such complexes incorporate an additional acid molecule to form the **WC−Hbp** complex, and (*c*) that all these complexes are potentially fluorescent -the nature of their first two excited electronic states is inverted with respect to adenine and the first state acquires

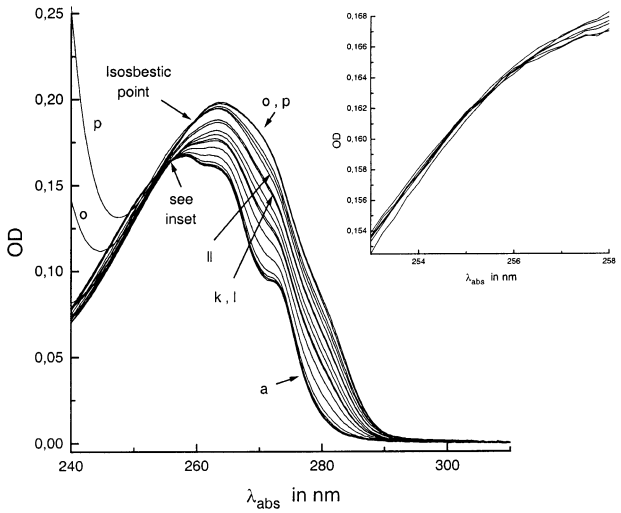

Figure 2. UV absorption spectra of 9-ethyladenine in pure cyclohexane, 10^{-5} M, (*a*), and cyclohexane with various quantities of acetic acid: Spectra between those *b* and *k* are ascribed to simultaneous formation of **WCbp** and **Hbp** complexes, and spectra between *l* and *p* are ascribed to **WC**−**Hbp** complex formation. Total concentrations (M) of acetic acid employed: (a) 0; (b) 7×10^{-5}; (c) 1.4×10^{-4}; (d) 2.8×10^{-4}; (e) 5.1×10^{-4}; (f) 7.2×10^{-4}; (g) 1×10^{-3}; (h) 1.7×10^{-3}; (i) 4.1×10^{-3}; (j) 9×10^{-3}; (k) 1.9×10^{-2}; (l) 2.4×10^{-2}; (ll) 0.23; (m) 0.46; (n) 0.69; (o) 3.9; (p) 11.5• It should be noted that the spectra *o* and *p* manifest clearly below 250 nm the corresponding acetic acid absorption in cyclohexane. The six spectra gathered in the inset possess the concentrations (M): 0, 7×10^{-5}, 1.4×10^{-4}, 2.1×10^{-4}, 2.8×10^{-4}, and 3.5×10^{-4}. These concentrations cover the concentration range employed by Chow et al.[10]

(π,π^*) nature-, let us examine the feasibility of these complexes taking part in ESDPT processes.

In 1956, Weller[38] established the excited-state intramolecular proton transfer (ESIPT) mechanism for salicyl compounds and assigned it to the dramatic acidity increase in a phenol group and the basicity increase in a carbonyl group linked via an intramolecular hydrogen bond upon electronic excitation.[39] Our group recently found the feasibility of a two-proton transfer in dimers involving two hydrogen bonds of the C_{2h} type in 7-azaindole to rely on fulfillment of the chemical simultaneity criterion,[40] viz. on the donor and acceptor groups in the monomer fragment involved in the process undergoing a dramatic, similar acidity and basicity increase upon electronic excitation.

Table 3 shows the acidity and basicity values for the first excited electronic state as computed from eqs 1 and 2, as well as the corresponding acidity and basicity changes with respect to the ground state for the sites of interest in the adenine system (viz. H_{101}, H_{102}, N_1 and N_7). Note that, whereas the acidity of the amino protons (H_{101} and H_{102}) increases dramatically upon excitation (by 23.3 and 21.8 kcal/nol, respectively), only the basicity of the azole nitrogen (N_7) increases to a similar extent (21.1 kcal/mol)—by contrast, that of the pyridine nitrogen (N_1) increases by only 3.5 kcal/mol. Because the chemical simultaneity criterion is only fulfilled by the H_{101}−N_7 proton donor−acceptor pair, see Figure 3, one can exclude the possibility of the **WCbp** complex undergoing a two-proton transfer in its excited electronic state.

We failed to detect any fluorescence over the range 290−600 nm range in a 10^{-5} M solution of 9EA in cyclohexane at RT. The addition of small amounts of acetic acid resulted in the formation of adenine−acetic acid complexes, as reflected in the bathochromic shift observed, see Figure 2; however, the fluorescence was only detected in the 450 nm region (via the

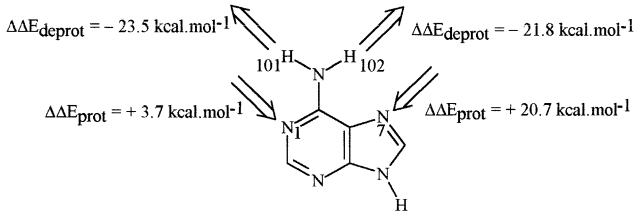

Figure 3. Basicity and acidity changes for the adenine groups involved in the Watsom&Crik pairing (N_1 and H_{101}) and in the Hoogsteen pairing (N_7 and H_{102}).

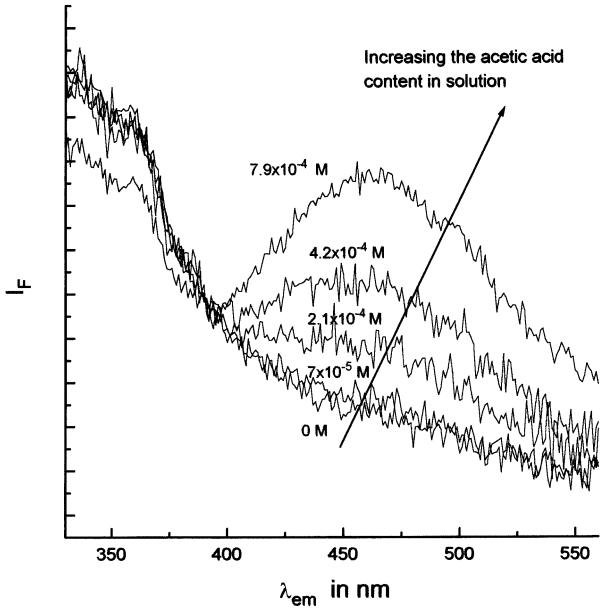

Figure 4. Fluorescence of the Hoogsteen (Hbp) complex of 9-ethyladenine with acetic acid acid at room temperature. The acetic acid concentration values are depicted inside the Figure.

excitation spectra) as the emission band was still visible. As the acetic acid concentration was raised, the emission band at ca. 460 nm became apparent and grew stronger as more acetic acid was added (see Figure 4). The increase was seemingly not proportional to the acid concentration throughout the studied range, which suggests the initial formation of a complex not emitting in that region giving rise to one more complex fluorescing in it as more acid was added to the solution. The corresponding excitation spectrum, obtained by monitoring the 460 nm wavelength, exhibited an unstructured band with its onset at 290 nm and peaking at 272 nm (Figure 5). The excitation spectrum obtained by monitoring the 360 nm wavelength showed the 9EA−acetic acid solution does not exhibit emission, consistent with the results for the 9EA solution in cyclohexane.

Higher concentrations of acetic acid (spectra included between those spectra *ll* and *p* in Figure 2), altered the situation: the emission at 460 nm decreased as the acid concentration increased (Figure 6); however, the variation passed by an isoemissive point at 412 nm. Also, the solution ceased to emit in the 460 nm region when the acid concentration reached a high enough level. The excitation spectrum obtained by monitoring the 460 nm wavelength was very similar to that of Figure 5; however, that obtained by monitoring the 360 nm wavelength consisted of an unstructured band with its onset at 310 nm and its peak at 280 nm.

It is important to point out that the excitation spectra measured by monitoring the emission at 460 nm may be regarded as posing equal spectral envelop for the whole range of concentra-

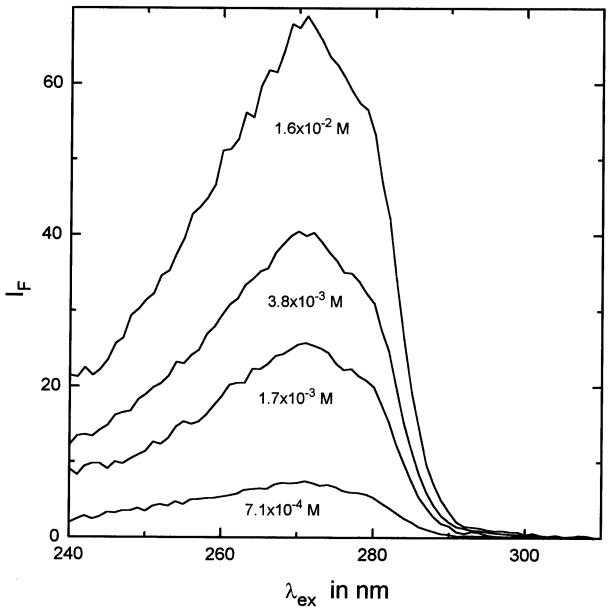

Figure 5. Excitation spectrum of the emission monitorized at 460 nm for the Hoogsteen complex (**Hbp**) in cyclohexane at room-temperature The corresponding acetic acid concentrations, which cover a significant wide range, are indicated inside the Figure.

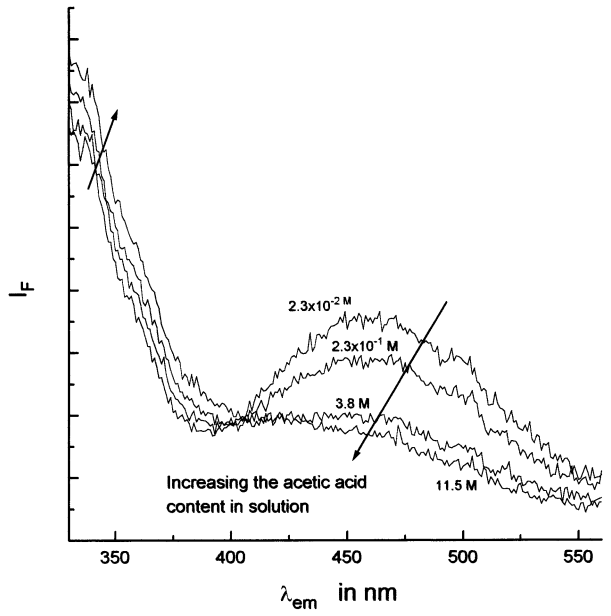

Figure 6. Fluorescence of the 9-ethyladenine and acetic acid complexes (**Hbp**, and **WC**−**Hbp**) in concentered solutions of acetic acid in cyclohexane at room temperature. The corresponding acetic acid concentrations are shown inside this Figure.

tions from 10^{-4} to 10^{-1} M, in which the emission ascribed to double proton transfer is observed.

In summary, the analysis of the emission and excitation spectra revealed that (*a*) the **WCbp** complex between adenine and acetic acid emits no fluorescence; (*b*) the **Hbp** complex emits light centered at 460 nm in accordance with a feasible two-proton transfer; and (*c*) the **WC**−**Hbp** complex emits in the 350 nm region but undergoes no two-proton transfer.

Conclusions

Adenine bearing an alkyl substituent at its glycoside linkage can yield three different types of complexes with acetic acid. Two consist of one adenine molecule and one acetic acid molecule coupled via a Watson−Crick or Hoogsteen pairing mechanism, the former complex being only 0.8 kcal/mol more stable than the latter. The third complex contains one adenine molecule that undergoes simultaneous pairing with two molecules of acetic acid via both mechanisms.

Although **WCbp** complexes are intrinsically more stable than **Hbp** complexes—which are considerably more polar than the former—highly polar solvents such as water and DMSO can easily reverse the stability sequence; this is consistent with the fact that equimolar mixtures of adenine and thymine in water or adenine and uracil in DMSO crystallize as complexes of the Hoogsteen type only.

One photochemically relevant finding is that the adenine−acetic acid complex of the **WCbp** type can give no tautomers via a two-proton transfer; in fact, this complex cannot follow such a mechanism. On the other hand, the Hoogsteen complex can undergo a two proton-transfer and hence introduce photochemical artifacts in a hydrogen bonding code.

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