

Excess Electron Attachment Induces Barrier-Free Proton Transfer in Anionic Complexes of Thymine and Uracil with Formic Acid

Maciej Harańczyk,^{†,‡} Iwona Dąbkowska,[‡] Janusz Rak,[‡] Maciej Gutowski,^{*,†,‡} J. Michael Nilles,[§] Sarah Stokes,[§] Dunja Radisic,[§] and Kit H. Bowen, Jr.[§]

Chemical Sciences Division, Pacific Northwest National Laboratory, Richland, Washington 99352,

Department of Chemistry, University of Gdańsk, Sobieskiego 18, 80–952 Gdańsk, Poland, and

Department of Chemistry, Johns Hopkins University, Baltimore, Maryland 21218

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The anionic complexes of formic acid with uracil and thymine reveal broad features in photoelectron spectroscopy (PES) experiments with maxima at 1.7 and 1.1 eV, respectively. The results of quantum chemical calculations suggest that electron vertical detachment energies (VDE) of 1.6–1.9 eV correspond to anionic structures in which a proton has been transferred from the carboxylic group of the formic acid to the O8 atom of uracil or thymine. Smaller values of VDE (0.8 to 1.3 eV) correspond to chemically untransformed complexes, in which anionic uracil or thymine interacts through two hydrogen bonds with the carboxylic group of the intact formic acid. The recorded spectra and the results of quantum chemical calculations suggest that both nucleic acid bases undergo barrier-free proton transfer in anionic complexes with formic acid. The difference in experimental spectra of UF^- and TF^- provides an indication that the methyl group of thymine could make a difference in the intermolecular proton transfer.

1. Introduction

Tautomerizations involving nucleic acid bases have long been seen as critical steps in mutations of genetic material. The intra- and intermolecular tautomerizations involving nucleic acid bases have long been suggested as critical steps in mutations of DNA.^{1–3}

Low-energy electrons are produced in copious quantities by ionizing-energy radiation interacting with condensed phases. These have been recognized as mutagenic agents that are responsible for single- and double-strand breaks in DNA.⁴ Recent theoretical studies addressed possible mechanisms of strand breaks.^{5,6} We have recently reported that excess electron attachment to complexes of uracil (U) with glycine (G) and alanine (A) leads to a barrier-free proton transfer (BFPT) from the carboxylic group of the amino acid to the O8 atom of U.^{7a,8}

Thymine (T) and uracil occur in DNA and RNA, respectively, with DNA storing genetic information and with RNA translating it to proteins. There is a basic question to which extent thymine is susceptible to the BFPT^{7a,8} or other tautomerization processes. If it were less susceptible than U, the result might shed new light on an intriguing question why U occurs in RNA and T in DNA. The current opinion is that T is preferable to U in DNA, because if U were a normal DNA base then the deamination of cytosine would be highly mutagenic.²

Here, we report the results of anion photoelectron spectroscopy (PES) measurements and quantum chemical calculations on anionic complexes of formic acid (F) with U and T. Formic acid serves as a model of weak organic acids abundant in living cells. F has a larger gas phase deprotonation enthalpy than glycine by ca. 3.5 kcal/mol⁹ and, therefore, the proton-transfer

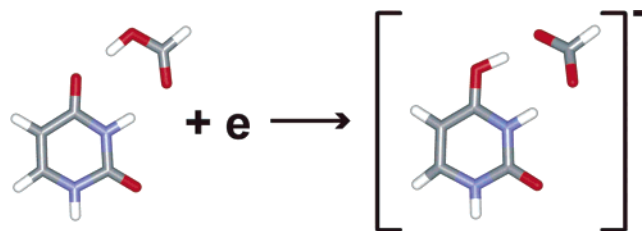


Figure 1. Barrier-free proton transfer upon an excess electron attachment to a uracil–formic acid complex.

process to the O8 atom of U or T should be *less facile* than in the case of complexes with glycine. We demonstrate, however, that BFPT occurs readily in anionic complexes of F with U, see Figure 1. It also occurs in anionic complexes of F with T, though less readily.

An advantage of gas phase experimental studies of biological molecules is that the results can be directly compared with theoretical predictions, as will be presented below. Another advantage is that the gas phase results allow distinguishing between intrinsic and externally imposed properties of biological molecules. External effects, such as solvation, might be critical for biophysical processes. In the future study we will explore the effect of solvation on the occurrence of BFPT in anionic complexes of uracil and thymine with formic acid.

2. Methods

An anion PES is conducted by crossing a mass-selected beam of negative ions with a fixed-frequency laser beam and energy-analyzing the resultant photodetached electrons.¹⁰ To prepare the species of interest, mixtures of U and F and T and F were placed in the stagnation chamber of a nozzle source and heated to ~180 °C. Argon gas at a pressure of 1–2 atm was used as an expansion gas. Electrons were then injected into the emerging jet from a biased Th/Ir filament to produce the anions of interest.

* Corresponding author.

[†] Pacific Northwest National Laboratory.

[‡] University of Gdańsk.

[§] Johns Hopkins University.

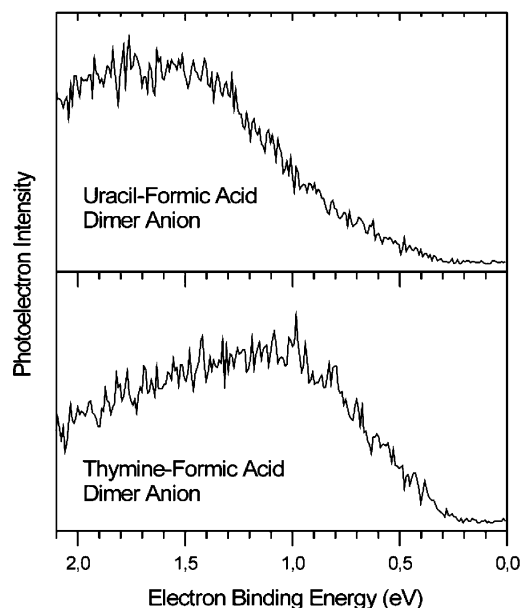


Figure 2. Anion photoelectron spectra recorded with 2.540 eV photons.

The computational studies were restricted to six structures, which are analogous to the structures labeled 1–4, 14, and 16 in our studies on the UG complexes.⁷ This set of geometries comprised the four most stable neutral conformers, labeled 1–4, while structures 2, 4, 14, and 16 led to the most stable anionic UG complexes. The neutral complexes between $X = U$ or T and F will be labeled XF_n , with n referring to the labeling of the aforementioned structures. The anionic structure resulting from the neutral structure XF_n will be labeled aXF_n .

We applied primarily the density functional theory method with a hybrid B3LYP functional¹¹ and 6-31++G** (5d) basis sets¹² to determine stabilization energies (E_{stab}) and Gibbs energies (G_{stab}) in the neutral and anionic complexes, and electron vertical detachment energies (VDE) for the anions. The values of G_{stab} were obtained for $T = 298$ K and $p = 1$ atm. The TF2 and TF16 structures have also been studied at the second-order Møller–Plesset (MP2) theory level. All calculations were carried out with the GAUSSIAN 98¹² and NWChem codes.¹³

The presence of many low energy structures for neutral and anionic complexes prompted us to determine the populations of these structures in the gas-phase equilibrium. First, we selected a reference structure R for a given species (neutral or anionic). Next, for every structure M other than the reference structure R we determined the equilibrium constant K_M

$$K_M = [M]/[R]$$

from the difference in Gibbs free energies for M and R . The fraction of M in the equilibrated sample is given by

$$x_M = K_M / (1 + K_1 + K_2 + \dots)$$

where the sum in the denominator goes through all structures for a given species. The fraction of R in the sample is

$$x_R = 1 / (1 + K_1 + K_2 + \dots)$$

3. Results

U in anionic complexes with F behaves much like U in anionic complexes with G .⁷ A broad PES feature with a maximum at 1.7 eV (see Figure 2) cannot be associated with intact U^- solvated by F .

TABLE 1: Properties of the Neutral (N) and Anionic (A) Uracil–Formic Acid (UF) and Thymine–Formic Acid (TF) Complexes Determined at the B3LYP/6-31++G** Level of Theory^a

XF _n	$E_{\text{stab}} + E_{\text{vib}}^{\circ}$		G_{stab}		VDE -0.2^b	BFPT	x (%)	
	N	A	N	A			N	A
UF1	0.66	0.96	0.19	0.49	0.88	no	97.42	0.00
UF2	0.56	1.20	0.09	0.73	1.67	yes	2.10	56.65
UF3	0.51	0.78	0.05	0.34	0.90	no	0.42	0.00
UF4	0.44	1.11	−0.00	0.69	1.75	yes	0.06	11.49
UF14	0.31	1.11	−0.10	0.68	1.91	yes	0.00	6.21
UF16	0.30	1.11	−0.08	0.71	1.82	yes	0.00	25.65
TF1	0.72	0.95	0.26	0.48	0.79	no	97.71	0.02
TF2	0.60	1.17	0.15	0.70	1.57 ^c	yes	1.61	95.40
TF3	0.58	0.77	0.13	0.33	0.82	no	0.68	0.00
TF4	0.40	1.00	0.00	0.55	1.76	yes	0.00	0.15
TF14	0.36	1.09	−0.02	0.67	1.82	yes	0.00	4.33
TF16	0.33	0.99	−0.01	0.63	1.33 ^d	no	0.00	0.09

^a The stabilization energies E_{stab} are corrected for zero-point vibrational energies E_{vib}° . The energies, Gibbs stabilization energies, G_{stab} , and electron vertical detachment energies VDE in eV. ^b See ref 15 for a 0.2 eV shift. ^c 1.4 eV (MP2). ^d 1.0 eV (MP2).

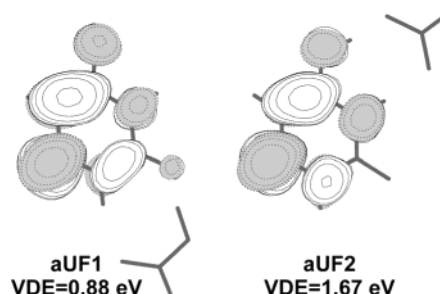


Figure 3. The excess electron charge distribution in a π^* anionic state of aUF1 and aUF2.

The valence π^* state of U^- is characterized by a calculated value of VDE of 0.507 eV.⁷ The solvation energy by F would have to be approximately larger by 1.2 eV for the anionic than for the neutral base to be consistent with a maximum at 1.7 eV. This is rather improbable, given that the VDE of the $U^-(H_2O)_1$ solvated anion is only 0.9 eV.¹⁴ The computational results provide an interpretation of the PES feature for UF^- : the four most stable aUF_n structures, i.e., those with the largest values of the anionic G_{stab} , undergo BFPT from the carboxylic group of F to the O8 atom of U^- (see Figure 1 and Table 1). The driving force for proton transfer is the need to stabilize the excess electron on a π^* orbital localized in the neighborhood of O8 (see Figure 3). The B3LYP values of VDE for these aUF_n structures are as large as 1.9–2.1 eV. After correcting downward by 0.2 eV,¹⁵ the resulting range coincides well with the maximum of the broad PES peak. There is no BFPT for aUF1 and aUF3, in which F is coordinated to O7 of U^- , and their values of VDE are smaller by 0.7 eV than for the structures with BFPT. Due to the small values of G_{stab} , the aUF1 and aUF3 structures are not populated in the anionic beam, see Table 1.

There is a substantial difference between the PES spectra of UF^- and TF^- (see Figure 2). The maximum for TF^- at 1.1 eV is even broader than for UF^- . A significant intensity in the 0.5–2.0 eV range suggests that the beam of TF^- species contains both complexes in which intact T^- is stabilized through hydrogen bonding with F as well as those in which a proton is transferred from the carboxylic group of F to T^- . Thus, the experimental results suggest that T might be less susceptible to hydrogenation than U , at least in anionic complexes with formic acid.

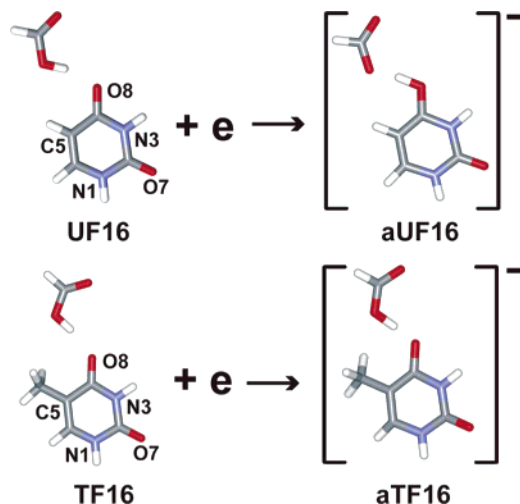


Figure 4. At the B3LYP and MP2 levels, barrier-free proton transfer upon an excess electron attachment occurs for the UF16 structure (top) but it does not for the TF16 structure (bottom).

An explanation of the difference between the PES spectra of UF^- and TF^- is challenging for the B3LYP/6-31++G** theoretical method and the harmonic oscillator/rigid rotor approximation to thermodynamic functions. The reason is that the stability of the aXF2 and aXF16 structures is very similar, but both structures undergo BFPT in the case of U but only one (aTF2) in the case of T. The Gibbs free energies of aXF2 and aXF16, obtained at the B3LYP/6-31++G** level, differ by only 0.02 and 0.07 eV for U and T, respectively, which is beyond a typical range of accuracy of the current exchange correlation functionals. Thus, the B3LYP/6-31++G** method is inconclusive as to the relative population of the most stable anionic structures, though it provides an indication that the methyl group of thymine could make a difference in the intermolecular proton transfer. Unfortunately, highly correlated electronic structure methods, such as the coupled cluster method with single, double, and triple excitations, are not yet applicable to open shell systems of the size of TF^- .

With these reservations in mind, we notice that the aTF16 structure does not undergo BFPT whereas aUF16 does (see Table 1 and Figure 4). This finding was corroborated at the MP2/6-31++G** level of theory. The resistance of aTF16 to hydrogenation must result from a steric protection provided by the methyl group of T. The aTF14 structure differs from aTF16 by having F flipped along the N1O8 axis away from the methyl group to the N3 side of O8.⁷ The former does undergo BFPT even though the protonation energy at the O8 site of T^- is smaller by 0.04 eV from the N3 than C5 side.

The most stable, and thus most dominant, structures for the neutral complexes are XF1, XF2, and XF3 (see Table 1), in agreement with earlier studies on the uracil–glycine and uracil–alanine complexes.^{7,8} The relative stability of anionic complexes is different, with aXF2 becoming the most stable and aXF1 and aXF3 the least stable. The preferential stabilization of the XF2, XF4, XF14, and XF16 structures and destabilization of the XF1

and XF3 structures is related with the localization of the excess electron in the O8–C4–C5–C6 region of the nucleic base.

In summary, the anionic complexes of formic acid with uracil and thymine reveal broad photoelectron spectroscopy features centered at 1.7 and 1.1 eV, respectively. The recorded spectra and the results of quantum chemical calculations suggest that both nucleic acid bases undergo barrier-free proton transfer in anionic complexes with formic acid. The difference in experimental spectra of UF^- and TF^- provides an indication that the methyl group of thymine could make a difference in the intermolecular proton transfer.

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