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Influence of Microhydration on the Ionization Energy Thresholds of Thymine: Comparisons of Theoretical Calculations with Experimental Values

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In the present study the ionization energy thresholds (IETs) of thymine, and thymine keto−enol tautomers, have been calculated (with the B3LYP, and P3 levels of theory using the standard 6-31++G(d,p) basis set) with 1−3 water molecules placed in the first hydration shell. Calculations show there is a distinct effect of microhydration on the IET of thymine. The first water molecule is seen to decrease the IET by about 0.1 eV, whereas the second and third water molecules cause a further decrease of less than 0.1 eV each. The changes in IET calculated here for thymine with 1−3 waters of hydration are smaller than the experimental values determined by Kim et al. (*J. Phys. Chem.* **1996**, *100*, 7933). Therefore calculations have been performed on the microhydrated keto−enol tautomers of thymine. The calculated results on the keto−enol tautomers are seen to be in better agreement with the experimental results. However, the keto−enol thymine tautomers are considerably higher in energy than the canonical form of thymine, and there is presently no good evidence that these thymine tautomers are actually present in a supersonic jet-cooled experiment.

Introduction

This is the fourth article in a series aimed at calculating accurate ionization energy thresholds (IETs) of the DNA and RNA bases in the gas phase and in aqueous medium. Article III in this series contained gas-phase calculations on microhydrated thymine.[1] The calculated results were compared with the experimental ionization energies of hydrated thymine reported by Kim et al.[2] in a cluster beam study. In the experimental studies a single water molecule is seen to reduce the IET of thymine by 0.3 eV. Additional waters decrease the IET in 0.2−0.1 eV steps. These results are at odds with the calculated IETs of microhydrated thymine reported in article III.[1] Addition of one water of hydration is seen to decrease the IET by 0.1 eV (for the most stable structure T1). Addition of a second water of hydration is seen to decrease the IET of thymine by 0.07 eV. The third water of hydration is seen to further decrease the IET by another 0.08 eV.

It was suggested in article III that the results reported by Kim et al.[2] may be explained by the presence of thymine tautomers in the jet-cooled stream. The aim of the present study is to carefully examine the various hydrated thymine tautomers, and then to consider whether there is a set of microhydrated thymine tautomers that could be used to explain the experimental results presented by Kim et al.[2] Agreement between these theoretical and experimental results would be important for benchmarking calculations of other hydrated systems.

It is first necessary to show that computational methods presently available are accurate enough to reproduce experimental IETs. Article I in this series presented Møller−Plesset (MP2) IET calculations[3] at the PMP2/6-31++G(d,p) level that produced vertical IETs that differed from the experimental values by ca. 0.1 eV, and adiabatic IETs that differed by 0.07 eV. Article II presented similar IET calculations using density functional theory. Calculations at the B3LYP/6-31++G(d,p) level presented vertical IET calculations on the bases that differed from the experimental values by an average of 0.1 eV.[4] This article also contained a summary of more accurate electron propagator calculations (P3) on the bases that were shown to deviate from the experimental values of IETs by an average of only 0.05 eV. All three calculations (DFT, PMP2, and P3) show the order of the IETs of the DNA bases as U > T > C > A > G.

Review of the Literature on Thymine + $n(H_2O)$ Structures

A paper by Chandra et al. describes three thymine monohydrates.[5] The geometries are very similar to those of the uracil monohydrates discussed in article III. A paper by van Mourik et al. shows the positions of four different thymine monohydrates.[6] The numbering scheme they used for the placements of the waters is the same as in Figure 1. A second paper by van Mourik discusses uracil + $(H_2O)_n$ with $n = 2-4$.[7] This paper is helpful in finding the positions of waters around thymine.

Recently, two new papers on hydrated uracil and thymine have appeared. Hu et al. present theoretical studies on numerous uracil tautomers and include the energetics of proton transfer in a microhydrated environment.[8] The coordinates of all the tautomers are available and make good starting points for thymine + nH_2O optimizations. A second recent paper by Rejnek et al. calculates thirteen keto and enol tautomers of

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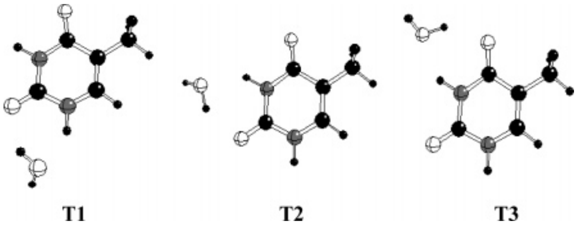


Figure 1. Structures of the three thymine monohydrates.

thymine in the gas phase, in a microhydrated environment (nH_2O, $n = 1, 2$), and in a water environment (CPCM).[9]

It was suggested in article III[1] that the results reported by Kim et al.[2] may be explained by the presence of thymine tautomers in the jet-cooled stream. Therefore, the emphasis in the present study is to examine the IETs of the various thymine tautomers. There is an important experimental paper by Tsuchiya et al. that reports the observation of a small amount of a thymine keto−enol tautomer in a jet-cooled experiment.[10] These results are discussed later on in the present study.

Computational Methods

The procedure followed herein is to take the best geometries available in the literature for thymine with various waters of hydration as input parameters, and to re-optimize the structures at both the B3LYP/6-31++G(d,p) and P3/6-31++G(d,p) levels.[11] Calculations to obtain vertical ionization energies were performed at the B3LYP, and P3 levels of theory using the standard 6-31++G(d,p) basis set. B3LYP frequency calculations were performed with the same basis set to verify stationary points. Calculations were performed on the Gaussian 98 suite of programs.[12]

The process for building a complex of thymine with water molecules is to determine the structure of all possible monohydrated complexes. The complex with the lowest energy is chosen. A second water molecule is added, and the dihydrated complex with the lowest energy is selected. A third water molecule is added and the process repeated.

There do not seem to be good starting geometries in the literature for the radical cations of thymine with waters of hydration. Attempts to compute these optimized geometries turned out to be very time-consuming. The present work therefore considers only vertical ionization energies for the thymine−water complexes. Thus the vertical ionization energies calculated herein involve the difference in total energy between the cation radical and the neutral base in the geometry of the neutral species (optimizations of the neutral molecule using the 6-31++G(d,p) basis set followed by a single point calculation on the cation).

Solvent effects were studied by performing self-consistent reaction field (SCRF) calculations using the polarizable continuum model (PCM)[13] with the integral equation formalism (SCRF=IEFPCM)[14] on the gas-phase optimized geometries of each thymine + nH_2O species. The results of the optimization on the thymine + nH_2O species in the PCM cavity were previously shown to have hardly any effect on the IET calculations.[1] A rational for this observation is presented below, by considering the correlation between the changes in dipole moments and the influence of microhydration.

Although the B3LYP/6-31++G(d,p) level was used successfully to calculated IETs, in previous studies, a question arose about the suitability of this level for the calculations involving microhydration. Truhlar and co-workers have recently looked at a variety of density functionals that are said to be "broadly accurate for nonbonded interactions".[15] Their MPWB1K hybrid functionals method was used here for calculating the IETs of several thymine + nH_2O tautomers. The influence of the basis set superposition error (BSSE) was evaluated by comparing B3LYP/6-31++G(d,p) calculations with calculations performed with Dunning's correlation consistent basis set (aug-cc-pvdz).[16] The counterpoise method was used for these calculations.[17] Both sets of calculations were performed on the G03 suite of programs.[18]

TABLE 1: B3LYP Energies for Thymine + nH_2O Structures

T + nH_2O	E(B3LYP) (Hartrees)	vert IET (eV)		dipole moments[a] (Debyes)
			PCM	
thymine	−454.1703735	9.01	6.60	4.61:6.24
T1	−530.6219551	8.91	6.63	3.78:5.10
T2	−530.6186056	8.98	6.63	4.96:6.92
T3	−530.6192396	8.96	6.61	4.73:6.27
T11	−607.0765173	8.84	6.64	3.42:4.65
T13	−607.071109	8.88	6.68	3.90:5.24
T33	−607.0720118	8.88	6.59	4.30:5.81
T111	−683.5274157	8.76	6.61	3.37:4.57
T113	−683.5259002	8.81	6.65	4.46:4.15
T133	−683.5239915	8.81	6.64	3.17:4.36
T233	−683.5231344	9.02	6.65	6.04:7.82

[a] Notation here is (neutral complex):(neutral complex in PCM cavity).

Results and Analysis

Thymine + $(H_2O)_n$. A complete analysis of the influence of waters of hydration on the IET of thymine has been undertaken and presented in Table 1. One notes that the calculated value of the IET of thymine is close to the experimental value of 9.1 eV for the B3LYP calculations. The dipole moments for neutral thymine are given in the last column. There is a correlation between the changes in dipole moments and the influence of microhydration. One notes that for each complex (T + nH_2O, $n = 1, 3$), the smallest IET represents the energy minimum of the set and shows the smallest dipole moment. For the complexes where the change in IET with hydration is very small, the changes in dipole moments are quite large. Thus, in the cases where the water molecules are placed so as to quench the dipole moment of thymine, there is a significant lowering of the IET.

A second observation is that for the case of T + nH_2O, $n = 1, 3$, the results of placing the microhydrated structures of thymine in a PCM cavity was seen to make very little difference in the IET when compared to the IET of ordinary thymine in a PCM cavity. The implications are that accurate calculations of the IETs of thymine can be obtained by simply considering long-range solvation effects. This holds for the present case because for the thymine cations + nH_2O there is very little delocalization of charge or spin on the waters. Of course this situation would not occur if there were some delocalization of charge onto the waters.

Addition of one water of hydration is seen to decrease the IET by −0.1 eV (for the most stable structure). Addition of a second water of hydration is seen to decrease the IET of thymine by 0.07 eV (for T11-T1). The third water of hydration is seen to decrease the IET of thymine by 0.08 eV (for T111-T11). As discussed in article III,[1] these results are at odds with the experimental IETs of microhydrated thymine reported by Kim et al.[2] In the experimental work a single water molecule is seen to reduce the IET of thymine by 0.3 eV. Additional waters decrease the IET in 0.2−0.1 eV steps. The relative accuracy in these experimental measurements is said to be ±0.05 eV.

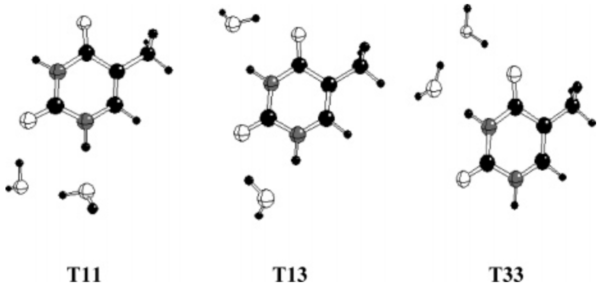


Figure 2. Structures of the three thymine dihydrates.

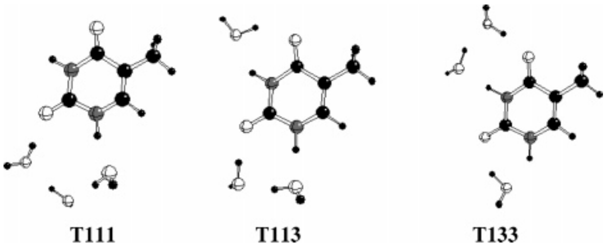


Figure 3. Structures of the three thymine trihydrates.

TABLE 2: B3LYP and P3 Energies for Thymine Tautomers

thymine tautomers	E(B3LYP) (Hartrees)	vert IET (eV) DFT	vert IET (eV) P3	dipole moment (Debye)
thymine	−454.1703735	9.01	9.14	4.61
TN1HC2Ec	−454.1040284	8.86	8.93	6.29
TH1HC2Et	−454.1231655	8.99	8.97	9.04
TN1HC4Ec	−454.1500631	8.71	8.80	5.49
TN1HC4Et	−454.1377396	8.79	8.83	8.44
TN3HC4Ec	−454.1290907	8.50	8.47	6.47
TN3HC4Et	−454.1335433	8.56	8.55	7.80
TN3HC2Ec	−454.1396929	8.68	8.77	1.85
TN3HC2Et	−454.1529866	8.67	8.80	2.89

It is necessary to look at the actual experimental results reported by Kim et al.[2] In their cluster beam experiments, the vertical ionization energy of unhydrated thymine was not actually measured.[19] The experimental values had to be scaled to match experimental IETs reported by Hush and Cheung.[20] For the changes in IETs reported for hydrated thymine, one cannot rule out the possibility that those measurements involve the vertical IET of an enol tautomer of thymine complexed to a water molecule due to the relatively high water pressure and temperatures used in these experiments.[1]

Thymine Tautomers. The next step is to systematically examine the tautomers of thymine in the gas phase and in a microhydrated environment. The notation will be to list the rotomers of the C2 and C4 OHs as being cis (c) or trans (t) to N3, and to use the same numbering scheme for the placements of the waters as in Figures 1−3.

Table 2 shows first the keto−enol tautomers of thymine. There are several calculations in the DFT column that have rather low IETs. The dienols studied here all had rather high IETs compared to the keto−enol tautomers.[21] Therefore the present study deals mainly with the keto−enol tautomers.

The results in Table 2 show calculations on various thymine enol-keto tautomers. Because Kim et al.[2] were looking at hydrated thymines, the importance of this table is more for establishing a baseline of the energetics of the various tautomers in the vacuum state, and to have an idea of their dipole moments. For the entries in this table the spread of energies from the most stable to least stable (compared to thymine) is 10.9−25.9 kcal/mol.

In Table 3 there are various monohydrated thymine keto−enol derivatives. Some of the entries in Table 3 have rather small IETs. Two tautomers of interest are the ones labeled TN3HC2Et1 and -3. The difference in IETs of these and the canonical form of thymine is 0.38 and 0.34 eV, very closed to the stabilization energy reported by Kim et al. (0.30 eV).[2] Structures TN1HC4Ec1 and -3 are also of interest. Here the difference in IETs is nearly what Kim et al. report, 0.30 eV. These structures are shown in Figure 4.

TABLE 3: B3LYP and P3 Energies for Thymine Monohydrated Tautomers

$T + nH_2O$	E(B3LYP) (Hartrees)	vert IET (eV) DFT	vert IET (eV) P3	dipole moment (Debye)
thymine	−454.1703735	9.01 {9.01}[a]	9.14	4.61
TN1HC2Ec1	−530.5874586	8.86	8.69	7.53
TH1HC2Ec2	−530.5969602	8.81	8.94	5.15
TN1HC2Ec4	−530.5866210	8.92	9.18	6.96
TN1HC2Et3	−530.5699459	8.99	9.44	12.33
TN1HC4Ec1	−530.6033441	8.74 {8.73}	8.82	4.22
TN1HC4Ec3	−530.6043921	8.71	8.82	4.63
TN1HC4Et1	−530.5914521	8.79	8.87	7.09
TN1HC4Et4	−530.5840943	8.39	8.45	8.92
TN3HC2Et1	−530.6068629	8.63 {8.68}	8.76	3.78
TN3HC2Et3	−530.6044435	8.67 {8.67}	8.79	1.99
TN3HC2Et4	−530.5977026	8.81	9.03	4.47
TN3HC4Et1	−530.5802782	8.75	8.77	8.44
TN3HC4Et2	−530.5852012	8.50	8.49	5.15
TN3HC4Et2	−530.5864343	8.56	8.57	6.64
TN3HC4Et4	−530.5823132	8.10	8.07	11.23

[a] Numbers in braces are IETs computed at MPWB1K/6-31++G(d,p).

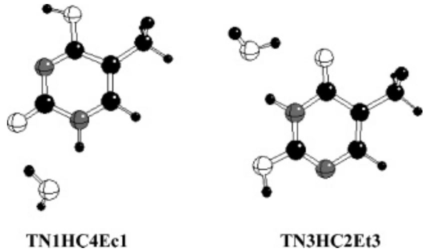


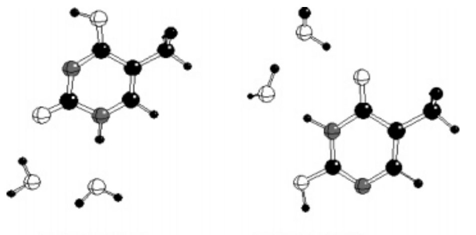
Figure 4.

TABLE 4: B3LYP and P3 Energies for Thymine Dihydrated Tautomers

$T + nH_2O$	E(B3LYP) (Hartrees)	vert IET (eV) DFT	vert IET (eV) P3	dipole moment (Debye)
thymine	−454.1703735	9.01 {9.01}[a]	9.14	4.61
TN1HC2Ec12	−607.0449381	8.59	8.73	6.18
TN1HC2Ec23	−607.0534115	8.76	8.95	4.66
TN1HC2Ec24	−607.0431738	8.89	9.18	5.69
TN1HC4Ec11	−607.0581957	8.68 {8.67}	8.75	3.56
TN1HC4Ec13	−607.0575480	8.73	8.84	3.00
TN1HC4Ec34	−607.0452041	8.77	8.95	2.78
TN1HC4Et14	−607.0379315	8.44	9.00	8.16
TN3HC2Et11	−607.0611300	8.59 {8.59}	8.74	3.52
TN3HC2Et13	−607.0578878	8.62	8.76	2.65
TN3HC2Et33	−607.0582005	8.60 {8.60}	8.71	1.42
TN3HC2Et14	−607.0517427	8.73	8.96	4.71
TN3HC4Et22	−607.0393528	8.46	8.46	6.40
TN3HC4Et23	−607.0403639	8.48	8.47	6.24
TN3HC4Et12	−607.0330515	8.73	8.70	6.96
TN3HC4Et14	−607.0299575	8.34	8.51	12.55
TN3HC4Ec12	−607.0315818	8.69	8.96	6.86

[a] Numbers in braces are IETs computed at MPWB1K/6-31++G(d,p).

Table 4 contains the dihydrated thymine tautomers. According to Kim et al., the IETs of the dihydrates are approximately 0.2 eV below that of the monohydrates.[2] Several entries have IETs in this range. The two entries with the lowest energy, TN1HC4Ec11 (or TN1HC4Ec13) and TN3HC2Et11 (or TN3HC-



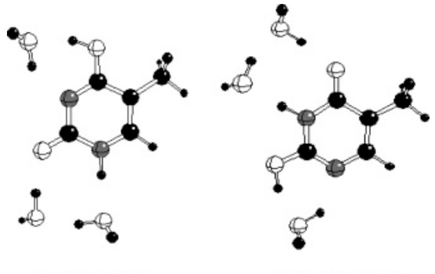
TN1HC4Ec11 **TN3HC2Et33**

Figure 5.

TABLE 5: B3LYP and P3 Energies for Thymine Trihydrated Tautomers

T + nH$_2$O	*E*(B3LYP) (Hartrees)	vert IET (eV)		dipole moment (Debye)
		DFT	P3	
thymine	−454.1703735	9.01 {9.01}[a]	9.14	4.61
TN1HC2Ec123	−683.5014472	8.56	8.72	5.37
TN1HC2Ec134	−683.4915312	8.69	8.91	7.20
TN1HC4Ec123	−683.5120400	8.72	8.89	2.71
TN1HC4Ec113	−683.5123447	8.67 {8.65}	8.89	2.91
TN1HC4Et134	−683.4913821	8.69	8.91	2.78
TN3HC2Et133	−683.5114167	8.52 {8.55}	8.71	2.73
TN3HC2Et134	−683.5017925	8.73	8.92	4.12
TN3HC4Et124	−683.4824239	8.39	8.15	11.90
TN3HC4Et122	−683.486171	8.66	8.74	7.00
TN3HC4Et224	−683.4886129	8.09	8.15	9.56

[a] Numbers in braces are IETs computed at MPWB1K/6-31++G(d,p).



TN1HC4Ec113 **TN3HC2Et133**

Figure 6.

2Et33) are trivial to form. Both involve a simple proton transfer from an N−H to an adjacent C═O, and are shown in Figure 5.

Table 5 contains various trihydrated thymine tautomers. According to Kim et al., the IETs of the trihydrates are approximately 0.1 eV below that of the dihydrates.[2] Several entries have IETs in this range. Both involve a simple proton transferred from an N−H to an adjacent C═O, and are shown in Figure 6.

Criteria for Selecting Tautomers. A problem now is to find a consistent set of microhydrated tautomers to be use in explaining the experimental results of Kim et al.[2] One could simply choose the most stable calculated geometries in Tables 3−5. However, it is not only the energy of the product that is of concern but also the energy necessary to create the various tautomers. Information on how the tautomers are created is not available from the experiments. The jet-cooled experiments deal with the products observed downstream and are not able to access the upstream processes of formation. Certainly there is energy available to produce a variety of tautomers, but one really needs to know which ones will actually be produced in sufficient quantity to be observed?

There are two types of experiments that could provide information on which of the many thymine tautomers are actually formed in a supersonic jet expansion. First there are supersonic jet-cooled expansion experiments on the DNA bases that provide a wealth of information in the form of sharp vibronic features from various microhydrated tautomers. For example, two photon ionization spectra of laser desorbed, jet-cooled, cytosine have been reported by Nir et al.[22] They observe both the keto and enol forms of cytosine.[23] Also of interest is the report of proton transfer between cytosine bases at high rates both with and without photoexcitation. This proton exchange is believed to take place either in the desorption stage or in the multiple collisions occurring in the high-pressure region of the supersonic expansion. Unfortunately, the same experiments on thymine produce only broad and diffuse vibronic bands indicating either interactions between multiple excited states or a large geometry change between ground and excited states.

A second type of experiment involves fluorescence excitation and dispersed fluorescence of jet-cooled thymine. These experiments, by Tsuchiya et al.[10] report the observation of a small amount of the thymine keto−enol (but not the dienol) tautomer of thymine. These experiments could not distinguish between the C2 enol-C4 keto and the C2 keto-C4 enol tautomers, and so herein it is assumed that both are possibly present in experiments performed by Kim et al.[2]

The results of Tsuchiya et al.[10] were called into question by Brady et al.[24] Basically they repeated the experiments of Tsuchiya et al. with the additional feature of mass detection. They obtain a similar fluorescence excitation spectrum from a mass 120 product which obviously cannot be from uracil. This species appears to be an unidentified impurity formed from uracil in the heating prior to injection into the nozzle.

On one hand, it may seem as if the paper by Brady et al.[24] presents a problem in the present analysis of the results of Kim et al.[2] because this paper is often quoted to say that there are no keto−enol tautomers present in supersonic jets of uracil (or indeed thymine). However, the work of Brady et al.[24] (and their comments on Tsuchiya et al.[10]) are for thymine in the gas phase. The work of Kim et al.[2] is on hydrated thymine, and there are several reports of keto−enol tautomers of thymine in aqueous medium.[25] Furthermore, in the experiments of Kim et al.[2] mass spectroscopy was used to sort the different hydrated thymines. So it is assumed herein that Kim et al.[2] were indeed observing thymine + nH$_2$O species, and not some different mass impurity.

For the present discussion it is expected that for the formation of the tautomers, simple proton transfers from N−H to C═O are favored. In the experiments performed by Kim et al.,[2] these would actually be water-assisted proton transfers. It should be noted that, in considering the various thymine tautomers, one has no idea as to whether the populations of tautomers formed in these jet-cooled experiments are in thermal equilibrium. Furthermore, one notes in Tables 3−5 that the energies of some of these tautomers are rather close together. Also, one must be aware that the difference in excited-state lifetimes may play a role in the selective observation of specific structures.[26]

One also has to recognize that the precision of the calculated relative energies reported here is limited, especially because rotations of the methyl group and C−O−H are not considered. Also the waters of hydration are in general confined to the ring plane. In summary, it seems reasonable to consider some of the low energy tautomers (those that differ in energy by 2.0 kcal/mol) as possibly candidates to be observed under the conditions present in the experiments reported by Kim et al.[2]

Models for Explaining the Microhydrated Thymine IET Data. As discussed above, in the cluster beam experiments reported by Kim et al.[2], the vertical ionization energy of unhydrated thymine was not actually measured.[19] The experimental values had to be scaled to match experimental IETs

TABLE 6: B3LYP and P3 ΔIETs for Hydrated Thymine Tautomers

$T + n\mathrm{H_2O}$	ΔIET (eV)		
		calculated	
	experimental	B3LYP[a]	P3
thymine	(9.15)	(9.01) {8.98}	(9.14)
TN1HC4Ec1	0.30	0.27 {0.26}	0.32
TN1HC4Ec11	0.20	0.06 {0.10}	0.07
TN1HC4Ec113	0.10	0.01 {0.00}	no change
TN3HC2Et3	0.30	0.34 {0.32}	0.35
TN3HC2Et33	0.20	0.07 {0.08}	0.08
TN3HC2Et133	0.10	0.04 {0.01}	0.03
TN1HC4Ec1	0.30	0.27 {0.26}	0.32
TN3HC2Et11	0.20	0.15 {0.12}	0.08
TN3HC2Et133	0.10	0.08 {0.03}	0.03

[a] Numbers on the left are computed using B3LYP/6-31++G(d,p). Numbers in braces are the BSSE (counterpoise) corrected results using B3LYP/aug-cc-pvdz.

reported by Hush and Cheung.[20] So, for example, the IET of TN1HC4Ec1 in Table 3 is computed to be 8.74 eV. The ΔIET for this tautomer is taken to be the difference between the computed IET of canonical thymine (9.01 eV) and 8.74 eV (or 0.27 eV).

Two of the more stable structures among the thymine monohydrates in Table 3 are TN1HC4Ec1 (or TN1HC3Ec3) and TN3HC2Et1 (or TN3HC2Et3) (they differ by ∼2.0 kcal in energy). These tautomers are rated “easy to form” in that their formation involves the transfer of an N−H proton to an adjacent C═O. So if experimental conditions are favorable for the formation of thymine tautomers, both these species are likely to be present.

Table 6 presents three sets of ΔIET data. First are listed the various hydrated states of TN1HC4Ec along with the changes in the experimental and calculated IETs. One sees very good agreement between the experimental and calculated data here for the monohydrate. The agreement is not so good for the dihydrate and the trihydrate.

The second entry is for various hydrated states of TN3HC2Et. Here one sees rather good agreement between the experimental and calculated data for the monohydrate and the trihydate, and not such good agreement for the dihydrate. The third entry is taken by locating the lowest energy states from Tables 2−4 and involves a combination of the low energy TN1HC4Ec and TN3HC2Et tautomers. Here there is rather good agreement between the experimental and calculated B3LYP results for all three stages of hydration.

When one obtains rather satisfying agreement between theoretical calculations and experimental results, it is proper to ask if the agreement is only fortuitous. Good results with the use of the 6-31++G(d,p) bases sets were obtained for the IETs of the DNA bases, with both DFT and PMP2 calculations, as shown in articles I[3] and II.[4] One could question whether this is an appropriate basis set to use for the microhydrated bases.

Truhlar and co-workers have recently looked at a variety of density functionals that are said to be “broadly accurate for nonbonded interactions”.[15] Their MPWB1K hybrid functionals method was used as a test on some of the thymine + $n\mathrm{H_2O}$ tautomers listed in Table 6. One obtains essentially the same IETs using these hybrid functionals (see the results in braces in Table 6). Thus the B3LYP hybrid functionals satisfactorily model the nonbonded interactions between thymine and the water molecule complexes.

To explore a different method of calculating IETs, ab initio electron propagator calculations in the partial third-order P3 approximation with the 6-31++G(d,p) basis set have been employed.[11] Tables 3−5 all include columns of vertical IETs labeled P3. Table 6 has the important ΔIET calculations for the lowest energy thymine tautomers. Here one sees hardly any difference between the ΔIET calculate with B3LYP or with the P3 approximation.

One must also consider the influence of the basis set correction error in these calculations. There have been numerous studies on what the BSSE does and does not affect. Of particular note is the conclusion by Gaigeot and Ghomi that, if diffuse functionals are used, BSSE corrections barely affect the energy differences separating various configurations of uracil + $n\mathrm{H_2O}$.[27] For the present study the BSSE correction was explored using the aug-cc-pvdz basis set. The corrected IETs are shown in braces in Tables 3−5. One sees that there is hardly any effect of the BSSE on these calculations. Altogether, our calculations using the B3LYP/6-31++G(d,p) level of theory provide reasonably good accuracy to support the present analysis of the experimental data reported by Kim et al.[2]

Although calculations on hydrated keto−enol tautomers of thymine do seem to fit the experimental data, there is no good evidence that these tautomers are present in jet-cooled experiments. Furthermore, the energies involved in forming tautomers such as TN1HC4Ec or TN3HC2Et are relatively high (>10 kcal/mol). This means that if an ensemble of canonical thymine were at thermal equilibrium, less than 1% of the molecules will be tautomerized.

Conclusions

It has been shown that the methods used to perform the calculations reported here have produced results for the IETs of the gas-phase DNA bases that compare with experimental data to within 0.1 eV.[3,4] It would be desirable to make the same comparisons for the microhydrated DNA bases. The present study has shown that hydration of canonical thymine (diketo form) does not lower the IETs sufficiently to explain the experimental data of Kim et al.[2] (see Table 1).

A second conclusion of the present study is that there may be problems with interpreting the experimental results of Kim et al.[2] There is some uncertainty as to whether thymine tautomers are actually formed in jet-cooled experiments. If thymine tautomers are indeed not present in jet-cooled experiments, then it is necessary to explain problems Kim et al.[2] may have encountered in scaling their data on thymine to the experimental IET data. To this end, it appears that new experimental investigations are necessary.

The present results should aid experimentalists in sorting out the sometimes complicated IR and UV double resonance experiments. In the IR hole-burning experiments used to differentiate between tautomers, it would appear as if TN1HC4Ec and TN3HC2Et would have the same signature (one >N1−H, one >C═O, and one >C−OH). If future experiments are performed on microhydrated thymine, then one should also be able to make use of the energetics and conformations of the various hydrated thymine tautomers presented here.

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Supporting Information Available: Table showing the *xyz* coordinates of the optimized structures of the thymine monohydrated, dihydrated, and trihydrated tautomers listed in Tables 3, 4, and 5, respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

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