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Anions of the hydrogen-bonded thymine dimer: ab initio study

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Abstract

Theoretical calculations have been performed to determine the ability of the hydrogen-bonded thymine dimer to form stable anions. The major conclusions of this work are: (i) three of the hydrogen-bonded conformers of the thymine dimer can form stable dipole-bound anions with excess electrons; (ii) thymine dimer can form a covalent anion that has a structure dissimilar from the structures of the neutral dimer; (iii) in the covalent thymine-dimer anion the excess electron is localized in a π-orbital on one of the thymine molecules and this molecule shows an out-of-plane distortion; (iv) the covalent thymine-dimer anion is stable with respect to an adiabatic electron detachment. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

Electrons released from solar-irradiated molecules in the cell environment can attach to biomolecules causing carcinogenesis in mammalian cells [1–4]. It is now widely accepted that DNA is the central target for such sunlight-induced lethality and that pyrimidine base dimers are believed to play a key role. The adjacent bases of thymine in a DNA strand tend to be particularly susceptible to the damage resulting from attack of radical products of the primary reactions induced by ultraviolet (UV) light [1–6], including free or hydrated electrons.

The theoretical and experimental determination of the electron affinities (EA) of nucleic acid bases (NAB) has proven to be a challenging problem. The experimental work on this subject has proceeded along two different directions. Bowen and coworkers [7] used photoelectron spectroscopy (PES) to study EA of NAB in the gas phase. Photodetachment–photoelectron spectroscopy was also recently used by Schiedt et al. [8] to study EA of the pyrimidine NABs and their complexes with water. A gas-phase study of NAB anions was also carried out by Schermann and coworkers [9,10] using Rydberg electron transfer (RET) and field-detachment spectroscopy. This technique was also used in detection of the thymine-dimer (T–T) anions by Schermann and coworkers [11]. (The mass-spectra recording in that work for different values of the principle Rydberg quantum number (n) of the relative rate constant for the formation of the T–T anion showed that both dipole-bound (DB) and covalent electron attachment occur for this system.) The coexistence of these two types of anions is an interesting phenomenon, and the purpose of this work is to provide its analysis based on theoretical calculations.

The gas-phase studies of NAB anions have been directly related to theoretically calculated EA,

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including the values obtained by our group [12–15] using various quantum-mechanical ab initio methods, because both the theoretical calculations and the experimental measurements concerned NAB molecules in isolation. Based on the calculations done by the authors and others [16], and based on the recent experiments in the gas phase of Schermann and coworkers [9,10], Bowen and coworkers [7] and Schiedt et al. [8], it can be concluded that, while the DB anions of NAB are quite well understood, the question of the existence of covalent anions of NAB in isolation remains open. There are some conflicting reports on this issue. For example, two recent experimental findings related to electron attachment to uracil (U) in the the gas phase provided somewhat different accounts of the nature of the gas-phase uracil anion [7,10]. While in the PES experiment of Bowen and coworkers [7], only the DB uracil anion was observed, while in the RET studies of Schermann and coworkers [10] they identified what they claim to be the covalent uracil anion. The two experiments seem to suggest that both DB and covalent uracil anions can be generated in the gas phase and, depending on the procedure for the anion production and the experimental conditions, one or the other is more abundant.

In our recent work [17] we investigated the anions of the $U \cdot (H_2O)_3$ complex and the anions of the uracil-dimer (U–U) [18] and the uracil–thymine (U–T) dimer [19]. Both these studies showed that hydrogen bonding with a solvating molecule stabilize the excess electron in the covalent state. This is in agreement with the suggestion made earlier by Sevilla et al. regarding this phenomenon [16]. Our calculations showed that rearrangement of the H-bonds in the uracil–water and uracil-dimer complexes occur when an excess electron attaches, causing stabilization. Also, while the DB states of the excess electron in NAB are σ-states, in the covalent anions the excess electron occupies a π-orbital. In both U–U and $[U \cdot (H_2O)_3]^-$ anions the ring of the uracil molecule, where the excess electron is located, is noticeably puckered. A similar effect was noticed before in density functional theory (DFT) calculations of U^- by Schermann and coworkers [10]. The out-of-plane distortion of the ring seems to provide additional stabilization

to the anion. We anticipate that a similar feature is present in solvated anions of thymine, including the hydrogen-bonded thymine dimer which is studied in the present work.

2. Calculations and discussion

The NAB pairs play an essential role in conveying genetic information, justifying the interest in how electron attachment may affect the structures of these systems. It has been determined in numerous studies that the thymine dimer is particularly damage-prone [20]. Irradiation of DNA with UV-B light causes the formation of mutagenic DNA lesions such as *cis–syn* and *trans–syn* cyclobutane pyrimidine dimers. These lesions are usually effectively repaired by DNA photolyases which are flavin-dependent repair enzymes and which directly revert the mutagenic *cis–syn* pyrimidine dimers into the corresponding monomers by a light-facilitated repair reaction [21–23]. Another lesion generating factor in the cell environment are free radicals and among them, free electrons or hydrated electron are most potent. One source of free electrons (or hydrated electrons) in biological environments are secondary reactions which follow the UV-induced radiolysis of water. The damaging effect on free electrons on the DNA and RNA structures are directly related to the outcome of the electron attachment to the NAB pairs. The present work on electron adducts of the hydrogen-bonded thymine dimer is motivated by the biological relevance of this system, as well as by the interesting duality of the covalent-DB anion interplay as displayed by the RET experiment of Schermann and coworkers [11].

Our work on the uracil-dimer anion [18] showed that in this system the excess electron can either be dipole-bound or can covalently attach. In the covalent uracil-dimer anion, the excess electron is exclusively located at one of the uracil molecules and the second uracil molecule only plays the role of "spectator". However, due to the almost perpendicular relative orientation of the two uracils, the anion acquires considerable stability due to the solvation effect which the spectator uracil molecule has on the molecule carrying the excess electron.

Another notable structural feature of the covalent uracil dimer anion is a puckering deformation of the ring of the uracil molecule where the excess electron is located.

In the present study we extend the investigation of the electron attachment of NAB pairs and examine anions of the thymine dimer. The questions which we attempt to answer in this work are:

- Can hydrogen-bonded thymine dimers form DB anions with excess electrons and, if they do, what are the electron binding energies in these systems?
- Can thymine dimer form a covalent anion and, if it does, what is the structure of this system? Is the electron localized on one of the thymine molecules, like in the uracil dimer, or is it delocalized over the whole system? Is the covalent thymine-dimer anion a stable system with respect to the electron detachment?

In order to provide answers to the above questions, a series of calculations were performed with the use of the GAUSSIAN94 quantum-chemical program package [24]. The results are described below.

The purpose of the first set of calculations was to determine equilibrium configurations of the hydrogen-bonded neutral dimer of thymine, which may have sufficient dipole moments to form DB states with an excess electron. For the uracil dimer, we identified four configurations with non-zero dipole moments. Due to the structural similarity between uracil and thymine, the search for high-dipole configurations in the present was limited to configurations which are analogical to the four found in the uracil dimer. We specifically searched for those configurations with dipole moments exceeding the value of 2.5 Debye. This dipole value

is the experimentally determined threshold for a molecular system to form a stable DB anion with an excess electron [25]. In the present calculations, the optimizations of the dimer structures have been performed at the RHF/6-31++G**(6d) level of theory and the final energy of each of the four dimer structures found was determined at the MP2/6-31++G**(6d) level. The energy and dipole moment results are presented in Table 1 and the dimer structures are shown in Fig. 1. The structures are numbered **1–4**. The results compiled in Table 1 indicate that three out of four dimer configurations have sufficiently high dipole moments to form DB anions (structures **1**, **2** and **3**). The total MP2 energies of configurations **1** and **2** are very close and the energy of configuration **3** is 2.8 kcal/mol above the energy of **1**.

In the next series of calculations we considered DB electron attachment to thymine dimers **1–3**. As in the case of our previous calculations on DB anions of molecular clusters, a standard basis set was augmented with a set of diffuse functions centered on the atom in the cluster that is positioned closest to the positive direction of the cluster dipole and is most distant from the center of the cluster. The basis set used in the present calculations consisted of the standard 6-31++ G**(5d) basis set augmented with six sp-shells with exponents 0.01, 0.002, 0.0004, 0.00008, 0.000016 and 0.0000032. This additional diffused set will be denoted as ''X'' in further discussion. First the geometries of the three DB anions were optimized at the UHF/6-31++G**(5d)X level of theory and the total energy was calculated at the UMP2/6-31++G**(5d)X level. The results are presented in Table 2. In the next step, starting from the equilibrium structures of the DB anions, the geometries of neutral clusters were optimized at the RHF/6-31++G**(5d)X level of theory to

Table 1

Total and relative MP2/6-31++G** energies and dipole moments of four conformations of the thymine dimers[a]

	1	**2**	**3**	**4**
MP2 (hartree)	−905.781913	−905.781025	−905.777419	−905.779382
Dipole (Debye)	8.5	8.9	6.1	1.2
ΔMP2 (kcal/mol)	0.0	0.6	2.8	1.6

[a] Geometries of the dimers optimized at the RHF/6-31++G** level of theory.
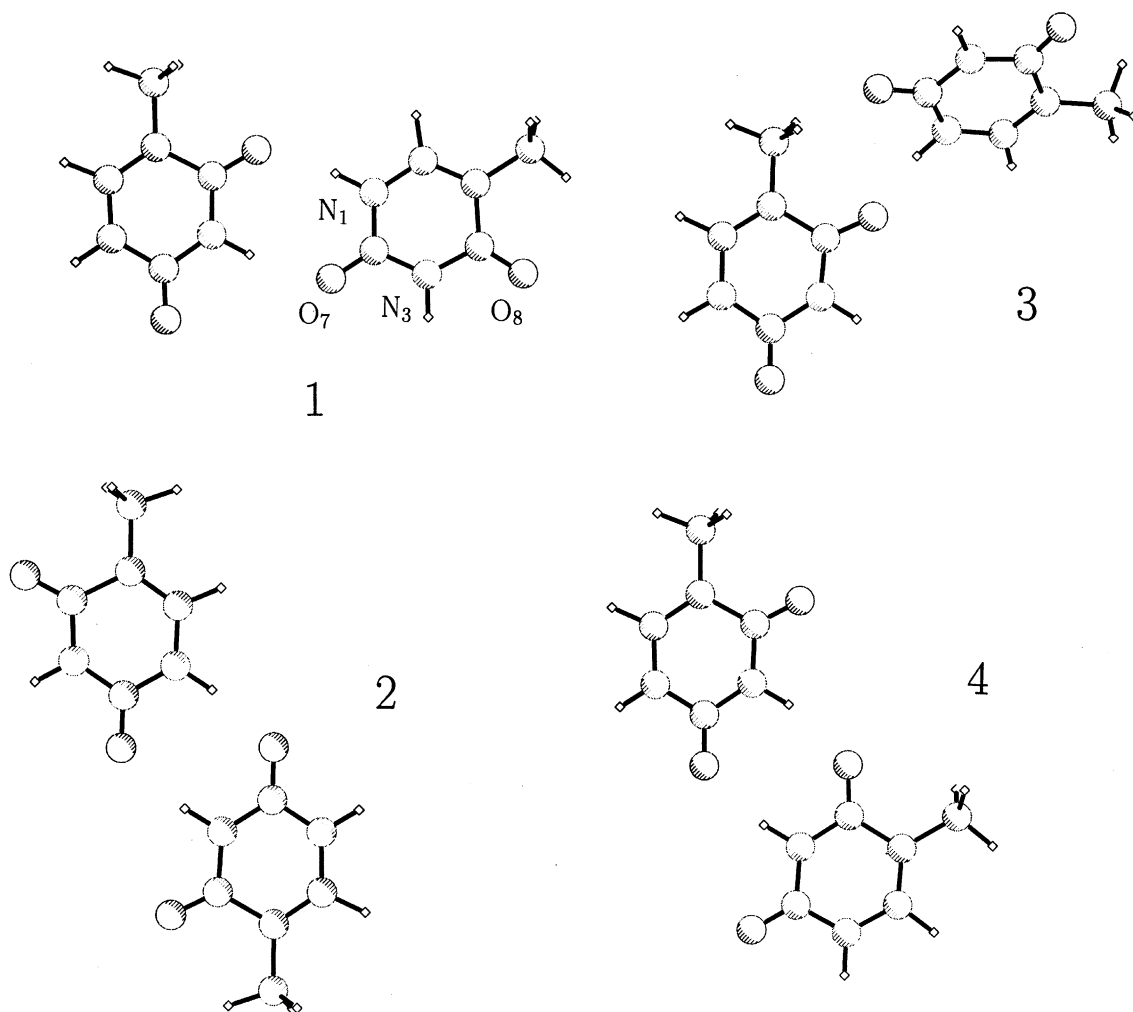
Fig. 1. Four thymine-dimer configurations investigated for their abilities to form DB anions with an excess electron.

obtain the reference energies of the neutral clusters for determination of their adiabatic electron affinities (AEA). For all the neutral dimers considered, the RHF/6-31++G**(5d)X geometries are virtually identical to the RHF/6-31++G**(5d) geometries described in the previous section. Also, there are very small differences between the anion geometries and the geometries of the corresponding neutral dimers. We only observed a small decrease in the H-bond distances upon the DB electron attachment. Following the geometry optimizations, we calculated the vibrational IR fre-

quency for the three anions and the corresponding neutral systems using the harmonic approximation. These calculations, which resulted in all real and positive frequency values, provided verification that the dimer geometries obtained in the calculations correspond to true minima on the potential energy surfaces of the thymine-dimer DB anion and the neutral dimer. They also provided zero-point vibrational energy (ZPE) contributions, which are shown in Table 2. The sum of the ZPE and MP2 energies is called E_{TOTAL} in Table 2, and its values were used to determine the AEA and the

Table 2
DB AEA calculations for thymine dimer[a]

	1		2		3	
	Anion	Neutral	Anion	Neutral	Anion	Neutral
HF	−903.090589	−903.089126	−903.091347	−903.088991	−903.085292	−903.083618
MP2	−905.751672	−905.749444	−905.752380	−905.748810	−905.745185	−905.742684
ZPE[b]	0.248888	0.248921	0.248925	0.248949	0.248241	0.248599
E_{TOTAL}[c]	−905.502784	−905.500523	−905.503455	−905.499861	−905.496944	−905.494085
ΔE (kcal/mol)	0.4	0.	0.	0.4	4.1	4.0
AEA (meV)		62		98		78

[a] Calculations performed with the 6-31++G**(5d)X basis set. Geometries of the anionic and neutral complexes done at the HF/6-31++G**(5d)X level of theory.
[b] ZPE, unscaled.
[c] $E_{TOTAL} = MP2 + ZPE$.

relative energies of the DB anions and the neutral dimers.

As the results indicate, the most stable anion is formed by dimer **2** and the corresponding AEA is 98 meV. The AEA value for dimer **1** is smaller and equal to 62 meV. Thus, we predict an interesting stability reversal upon a DB electron attachment to the thymine dimer, i.e., configuration **1**, which has lower energy than configuration **2** in the neutral dimer, becomes less stable than **2** in the DB dimer anion. The relative energies of the DB anion and the neutral corresponding to dimer **3** are both positive and are about 4 kcal/mol above the energies of dimers **1** and **2**, indicating that this system should not be observed in the RET experiment. However, in a thermodynamically equilibrated mixture of DB anions of the thymine dimer, both dimers **1** and **2** should be present in nearly equal amounts. Due to the stability reversal, one may notice an increase in the population of dimer **2** with respect to dimer **1** when free electrons are introduced to the sample.

A characteristic feature of the DB anions are very diffuse states of the excess electrons and their localization outside the molecular frame of the system. In Fig. 2 we demonstrate these features for the three DB anions of the thymine dimer. The plots clearly show the DB character of the excess electron in all three systems.

The next series of calculations was performed to determine whether a thymine dimer can form a stable covalent anion. Again, due to the structural similarity of the uracil and thymine, the search for
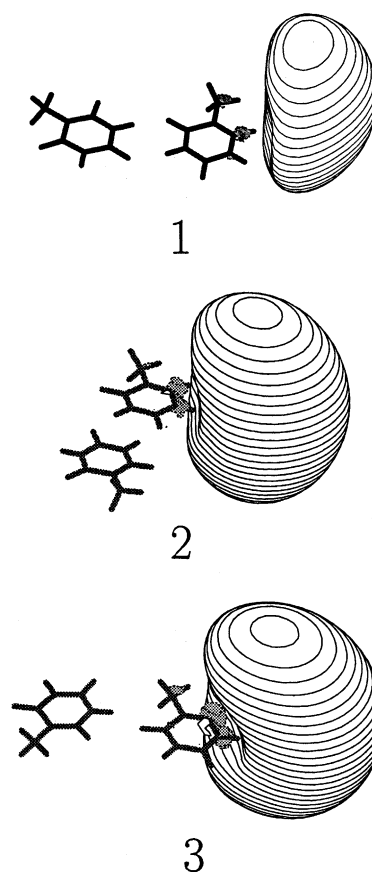

Fig. 2. The orbitals occupied by excess electrons in the three DB anions of the thymine dimer.

the structures of the thymine-dimer covalent anions was inititated with thymine-dimer geometries

similar to the two found for the covalent anions of the uracil–thymine dimer [19]. As in the case for the covalent anions of the uracil–thymine dimer, the ring of one of the two thymines in the starting thymine-dimer geometries in the anion optimizations was deformed from planarity. The optimizations were performed at the UHF/6-31++G**(6d) level of theory and two configurations with very similar structures were found. For both configurations the UMP2/6-31++G**(6d) energy was calculated, and the results are shown in Table 3. In the next step two RHF/6-31++G**(6d) geometry optimizations were performed for the neutral dimer starting with the two equilibrium anion UHF/6-31++G**(6d) geometries. Both optimizations converged to the same geometry. The MP2/6-31++G**(6d) energy calculated at this geometry for the neutral dimer was used to determine the adiabatic electron detachment energies (ADE) for the two thymine-dimer covalent anions found in the calculations. The AEA values shown in Table 3 also include the ZPE contributions calculated using the harmonic IR frequencies. The two structures of the covalent thymine-dimer anion and the structure of the corresponding neutral dimer are shown in Fig. 3. The two thymine molecules in the dimer anion are neither coplanar nor have parallel stacked configuration, but they appear to form a nearly perpendicular conformation. As in the uracil–uracil and uracil–thymine dimers [18,19], this type of configuration seems to provide the most
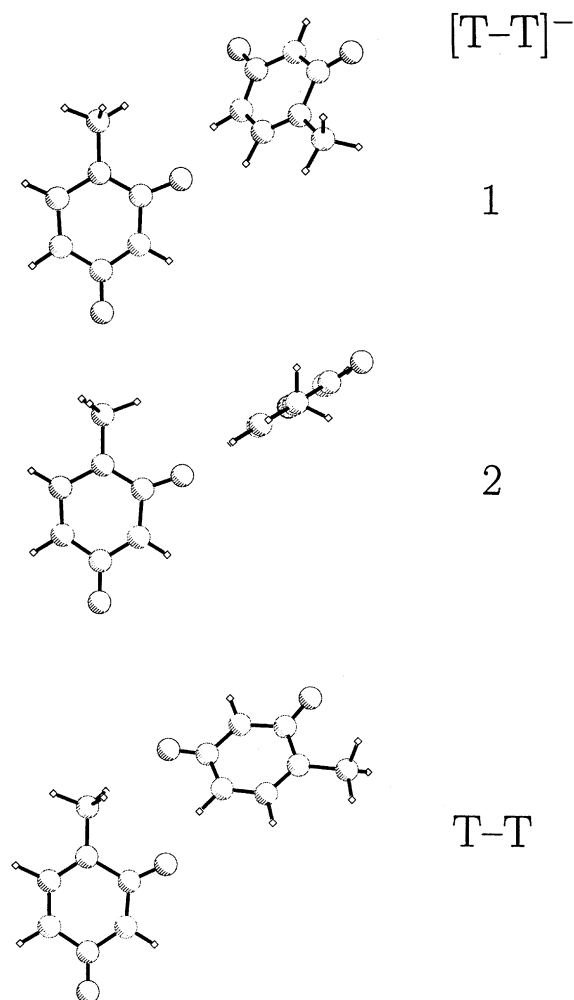

Fig. 3. The structures of the two covalent thymine-dimer anions and the structure of the corresponding neutral dimer found in the HF/6-31++G** calculations.

effective stabilization of the covalently attached excess electron.

Apart from the MP2 + ZPE calculations, we also performed DFT calculations for the covalent anions of the thymine dimer for comparison with the ab initio results. The DFT results are also included in Table 3. In the search for the minima on the DFT PES of the covalent anion, we performed two geometry optimizations starting from the structures obtained in the UHF/6-31++G**(6d) optimizations. Both optimizations converged to the same minimum.

Table 3
Covalent anions of thymine dimer: calculation of the ADE performed with 6-31++G**(6d) basis set (total energies in hartrees; AEA in eVs)

	Anion		Neutral
	1	**2**	
HF	−903.080714	−903.080717	−903.085406
MP2	−905.782107	−905.781684	−905.777421
ZPE[a]	0.242880	0.242941	0.248446
DFT	−908.381080	−908.381080	−908.355710
E_{TOTAL}[b]	−905.539227	−905.538743	−905.528975
ADE (MP2 + ZPE)	0.28	0.27	
ADE (DFT)	0.69	0.69	

[a] ZPE, unscaled.
[b] $E_{TOTAL} = \text{MP2} + \text{ZPE}$.

The first observation one makes upon examining the results is that, at the MP2 and DFT levels of theory, the covalent thymine-dimer anion is stable with respect to an adiabatic electron detachment leading the configuration of the neutral dimer shown in Fig. 3. Before we move to a discussion of the electron affinity values, let us first examine the wave function of the covalent anion. Since the two covalent anions found in the calculations are very similar, in Fig. 4 we present a picture of the orbital (HOMO) occupied by the excess electron in one of them (anion **2**). As expected, the HOMO has π-symmetry and it is exclusively localized on one of the thymine molecules. Structure analysis indicates that this molecule is out-of-plane distorted (see Fig. 5) in a similar way as the molecule carrying the excess electron was in the uracil–uracil and uracil–thymine dimers [18,19]. Thus, we can suggest that, like in the U–U and U–T covalent anions, the stability of the covalent thymine-dimer anion results from the solvation effect that the spectator thymine molecule has on the molecule which carries the excess electron.

Finally, based on the energy results presented in Table 3, we calculated the ADE of the covalent thymine-dimer anion. The ADE values presented in Table 3 were calculated with respect to the neutral dimer that was obtained when the geo-
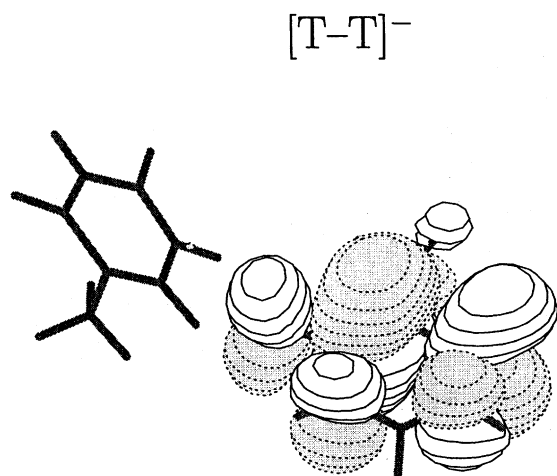
$$[T–T]^-$$

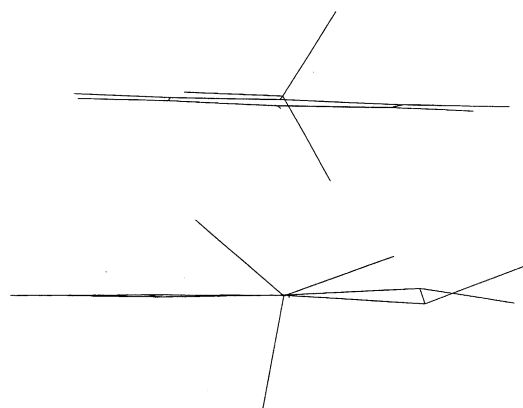Fig. 4. The orbitals occupied by the excess electrons in the covalent anion of the thymine dimer.

Fig. 5. Comparison of the structures of the two thymines in the thymine-dimer covalent anion. The top (slightly puckered) structure corresponds to the molecule where the excess electron is localized and the bottom stucture corresponds to the molecule where no excess-electron density is present.

metry optimization was initiated with the optimal structures of the dimer anions. With respect to this dimer, the MP2 + ZPE ADE value is positive and equal to 0.28 eV (or 0.27 eV for the second anion) indicating that the anion is adiabatically stable. The DFT ADE result is much higher and equal to 0.69 eV. This is accompanied by significantly lower DFT total energies than the MP2 energies for both the anion and the neutral dimer. Although the MP2 + ZPE ADE values are expected to increase when higher-order electron correlation effects are included, it is unlikely that the increase will alleviate the difference between the DFT and MP2 values. Thus, it is likely that the DFT result overestimates the ADE while the MP2 result underestimates it.

3. Conclusions

DB and covalent anions of thymine dimer have been studied using ab initio and DFT theoretical calculations. Based on the calculated results we have made the following predictions:

- There are three configurations of the hydrogen-bonded thymine dimer that possess sufficiently large dipole moment to form DB anions with

an excess electron. Electron attachment reverses the thermodynamic stability of the two most stable high-dipole dimers.

- Two hydrogen-bonded thymine molecules can form a covalent dimer anion with an excess electron. In this dimer the electron is localized on one of the thymine molecules which, as a result, undergoes a ring-puckering deformation. The second thymine molecule "solvates" the first one, and the solvation effect stablizes the excess electron. Similar electron localization at one of the uracil units was predicted by Voityuk and Rösch in their recent theoretical study of the anion of the uracil-dimer C_5–$C_{5'}$, C_6–$C_{6'}$ adduct [26]. In the covalent thymine-dimer anion that we obtained in the calculations, the thymine molecules are oriented nearly perpendicularly to each other.

The above predictions open an interesting challenge for an experimental verification. Particularly interesting is the predicted coexistence of the the covalent and DB anions of thymine dimer in the gas phase, which is consistent with the outcome of the RET experiment of Schermann and coworkers [11].

Acknowledgement

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