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Hoogsteen and Stacked Structures of the 9-Methyladenine···1-Methylthymine Pair Are Populated Equally at Experimental Conditions: Ab Initio and Molecular Dynamics Study

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The potential energy surface (PES) of the 9-methyladenine···1-methylthymine (mA···mT) nucleic acid base pair was investigated using molecular dynamics/quenching method utilizing the Cornell et al. (Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M., Jr.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179) empirical force field. Altogether 16 energy minima were found, 4 of them were planar, hydrogen-bonded, and 9 stacked. The accuracy of the stabilization energies evaluated with Cornell et al. empirical force field was verified by comparing them with correlated ab initio stabilization energies and good agreement was found for hydrogen-bonded as well as stacked pairs. NVE and NVT free energy surfaces were estimated by means of computer simulations. In the NVE simulations stacked structures prevail, while in the NVT calculations an equal mixture of planar hydrogen-bonded reversed Hoogsteen and Hoogsteen structures, and two stacked structures was found. The averaged stabilization enthalpy considering all the dimer structures (NVT simulations) is 11 kcal/mol and agrees well with the experimental stabilization enthalpy for the formation of mA···mT pair (13 kcal/mol) obtained from field ionization mass spectrometry measurements at conditions comparable to the NVT simulations.

Introduction

Nucleic acid (NA) bases form three different types of complexes: planar hydrogen-bonded, stacked, and T-shaped. The first two structural types exist in DNA and RNA and contribute to their stability. Hydrogen-bonding is stronger than base stacking or interaction in the T-shaped structure. Hydrogen-bonding is mainly stabilized by electrostatic and charge-transfer interactions while the base stacking is mainly due to London dispersion forces. Stabilization of T-shaped structures stems from electrostatic, charge-transfer, and dispersion contributions.

Structure of the gas-phase NA base pairs (and of any molecular complexes) is determined, however, not only by the interaction energy. The change of entropy should be also taken into account. It is necessary to pass from the potential energy surface (PES) to the free energy surface (FES). The surfaces might differ and it is even quite common that the global minimum on the PES differs from the global minimum on the FES. This problem becomes topical when comparing characteristics of a complex determined by quantum chemical calculations and by experiments. The former data clearly correspond to the 0 K (PES) while experiments are done at nonzero temperatures (FES).

Recently we have investigated in our laboratory potential energy and free energy surfaces of the uracil dimer,[1] the adenine···2,4-difluorotoluene dimer,[2] methylated uracil dimers,[3] and the adenine···thymine pair.[4] Calculations have demonstrated that the PES for each pair contains more than a dozen energy minima. For their localization it is necessary to use some effective searching method, which scans the whole PES. For this purpose we have adopted the molecular dynamics/quenching (MD/Q) method. The method has been shown[1−4] to be very efficient for NA base pairs. In some cases an unexpected structure corresponding to the global minimum at the PES was found, for example the global minimum for the adenine···thymine NA base pair is neither of Hoogsteen nor of Watson−Crick type.[4] It would be difficult to discover this structure without the MD/Q method. We have further shown that the PES and the FES of the investigated complexes differ. The most significant difference was found in the case of 1-methyluracil dimer where the global minima at the PES and FES differ.[3] While the former minimum is planar hydrogen-bonded, the latter one is stacked.

Experimental data on nucleic acid base pairs in a vacuum are rare.[5−9] Such studies are especially important since they allow determination of structure and properties of base pairs without the influence of a solvent. Among experimental studies mentioned, only two deal with energetical and/or thermodynamical characteristics for the base pair formation. The supersonic beam experiments[6,7] yield unreliable results. The only experimental data (sometimes considered as only indicative) were obtained from field ionization mass spectrometry measurements.[5] Interaction enthalpies for the NA base pair formation were determined on the basis of measurements of the temperature dependence of the equilibrium constant. The following values (in kcal/mol) were obtained for 9- and 1-methylated NA base pairs: GC −21.0, CC −16.0, AT −13.0, TT −9.0. Average temperatures were equal to 381, 381, 323, and 323 K, respectively. Experimental interaction enthalpies were later used for verification of various theoretical estimates and fair agreement was obtained if correlated ab initio values were adopted.[10−14] This agreement was important since it has represented the key argument supporting the use of medium-level correlated ab initio calculations (MP2/DZ+P level). However, experimental conditions did not allow determination of the base pair structure. Experimental interaction enthalpies were therefore compared with theoretical characteristics evaluated for classical planar

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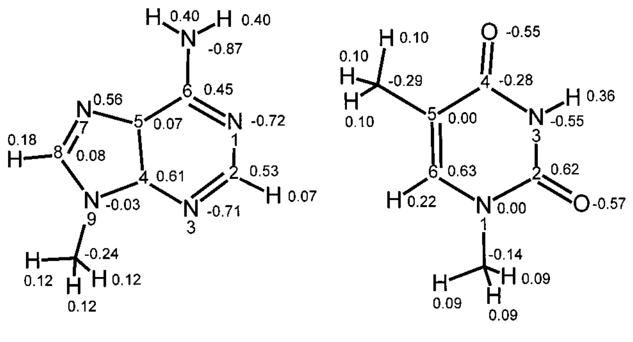
hydrogen-bonded structures occurring in NA. In the case of guanine···cytosine (GC) and adenine···thymine (AT) base pairs the Watson−Crick (WC) and Hoogsteen (H) structures were considered.[10−14] (Bramel et al.[13] took into account four planar hydrogen-bonded structures of the WC and H types, but did not consider existence of other structures of the pair.) Such a treatment is, however, incorrect since the experiment temperature is rather high and other than classical planar hydrogen-bonded structures might be significantly populated. Care should be paid first to the description of the PES (all structures should be localized) and later to the proper sampling of the PES. Experimental data should be compared with theoretical characteristics evaluated for all structures, which are significantly populated in the experiment. Consideration of these structures can lead to deterioration of the above-mentioned agreement between theoretical and experimental stabilization enthalpies that could have serious consequences to all theoretical calculations for interaction of NA bases. Let us remind readers that the quality of all empirical potentials used for interaction of NA bases was tested[15] on the above-mentioned medium-level correlated ab initio calculations.

In this paper we analyze the PES of the 9-methyladenine···1-methylthymine (mA···mT) base pair using various methods. The aim of the study is 3-fold: (i) to describe the PES and localize all energy minima, (ii) to describe the FES, (iii) to determine the interaction enthalpies at 323 K and compare them with the experimental values.

Strategy of Calculations. First of all, the PES of the mA···mT pair was investigated by MD/Q simulations. AMBER[16] with Cornell et al. force field[17] was used because it was shown to best reproduce (among various empirical potentials) the ab initio stabilization energies of hydrogen-bonded and stacked base pairs.[18] After localization of all energy minima on the PES, AMBER stabilization energies of the canonical hydrogen-bonded structures (WC, WCr, H, Hr where r stands for reversed) and one stacked structure were verified by comparing them with correlated ab initio stabilization energies. Care should be paid to the type of the computer simulations performed. Simulations in the NVE microcanonical ensemble (number of particles (N), volume (V), and energy (E) are conserved during the simulations) describe an isolated system while simulations in the NVT canonical ensemble (temperature (T) is conserved rather than energy) describe a system which is in thermal equilibrium with a bath. Experimental conditions of the Yanson et al. study[5] clearly correspond to the NVT constant temperature simulations. NVE constant energy simulations on the other hand should be compared with (not yet realized) supersonic beam experiments.

Calculations. *Quantum Chemical Calculations*. Geometries of mA and mT (Figure 1) as well as of planar hydrogen-bonded structures were determined by gradient optimization at the HF/6-31G** level. Interaction energies were evaluated at the MP2/6-31G*(0.25) level. Polarization functions in this basis set are more diffuse ($\alpha = 0.25$), with the aim to describe correctly the interaction correlation energy, than in the standard 6-31G* basis ($\alpha = 0.8$). Deformation energies and BSSEs were included in the calculation of final interaction energies; for more details see our previous study.[4] The necessity to use this level of calculation is assumed from the fact that it will be used for hydrogen-bonded as well as stacked NA base pairs. Quality of MP2/6-31G*(0.25) hydrogen-bonding and stacking energies of NA base pairs can be deduced from recently published benchmark calculations (hydrogen-bonded systems,[19] stacked systems).[20] From these papers it follows that the present theoretical level yields very good estimates of stabilization energies for hydrogen-bonded and stacked NA base pairs. Let us finally emphasize again that all the DFT methods fail completely for stacked NA base pairs[18] and thus are not suitable for the present purpose.



9-methyladenine (mA) **1-methylthymine (mT)**

Figure 1. Geometry and AMBER charges (e^-) of 1-methyladenine and 9-methylthymine.

Stabilization of stacked structures stems from intersystem correlation (dispersion) energy and it would be desirable to optimize these structures at the correlated level. Such an optimization is, however, very tedious and has only been done for a few smallest NA base pairs.[18] The conformational space of the stacked structures was scanned by a set of single-point MP2/6-31G*(0.25) calculations in the same way as we have done previously for other stacked base pairs.[21,22] The subsystems were kept rigid at HF/6-31G** geometries and were placed in parallel planes with a vertical distance of 3.4 Å (which is the average distance of base pairs in DNA). First, the dependence of stacking energy on rotation angle was investigated for an undisplaced dimer. In an undisplaced structure, the centers of mass of mA and mT are placed on the z-axis of the coordinate system while that of mT is shifted from mA by 3.4 Å along the z-axis. For description of the rotation angle we used two vectors: **vec1** (N9 of the mA−mA center of mass) and **vec2** (N1 of the mT−mT center of mass). The rotation angle is a clockwise rotation of the mT in the stacked dimer around the z-axis passing through the centers of mass of both subsystems. In the second step, the dependence of stacking energy on vertical distance was studied. Both molecules were again placed into the coordinate system as in the previous case, and the angle between vectors **vec1** and **vec2** was set to 60°. Finally, we investigated the dependence of stacking energy on displacement. Molecules were placed into the coordinate system as defined previously; vertical distance was set at optimal value 3.3 Å and the angle between vectors **vec1** and **vec2** was set to 60°, these values correspond to the lowest interaction energy obtained from previous scanning. Methylated thymine was then shifted in 0.75 Å steps in the xy-plane, facing the x- or y-axis, to further compare empirical and ab initio potential.

Empirical Potential. The original parametrization of the Cornell et al. empirical force field[17] was used. The atomic charges of mA and mT were determined consistently with the AMBER force field, i.e., using the restrained electrostatic potential (RESP) fitting procedure[23] at the HF/6-31G* level (this level gives charges 10−20% higher in order to mimic a missing polarization term). The charges are presented in Figure 1. No additional parameters were required.

NVE Microcanonical Ensemble MD/Q Simulations. MD simulations were performed at constant total energies of −38.30 and −13.69 kcal/mol (corresponding average temperature were

323 and 450 K). The MD simulations (integration step 0.5 fs) were stopped at equidistant intervals (every 0.5 ps) and followed by energy minimization. Each minimized structure was stored, compared with previously founded structures, and was considered in the overall statistics. Total simulations time was 125 ns. Convergence of the populations was checked by splitting the whole simulations into 3 parts. The standard deviation of populations was calculated for each part and at the end of the simulations the convergence was better than 1%. Vibrational analysis demonstrated that all structures found by MD/Q procedure correspond to energy minima.

NVT Canonical Ensemble. Populations for various conformations of NA base dimers depend on the ensemble type used.[1,3] We have shown that the simulations in NVE and NVT ensembles give different results for a double well potential, where the wells have different depths.[3] Since the number of intermolecular degrees of freedom considered is six, it is more convenient to calculate the configuration integral numerically rather than to perform a Monte Carlo simulations. In the NVT ensemble the energy can always be expressed as a sum of kinetic and potential energy contributions; i.e., the partition function factorizes into a product of kinetic and potential parts.[24] The kinetic part will be equal for all conformations, and only the configuration integral (Z) has to be calculated. The configuration integral is given by

$$Z_{\mathrm{NVT}} = \int \mathrm{e}^{(-V(r)/k_{\mathrm{b}}T)}\mathrm{d}r \qquad (1)$$

where V(r) and k_{b} mean the potential energy and the Boltzmann constant, respectively. The configuration integral is formally not bound. However, it is made convergent by discarding all points that have a potential energy above a certain threshold. The energy threshold must be smaller than the dissociation energy. All configurations with higher potential energy are considered nonbonded and discarded. In the calculations presented here a threshold of -1.0 kcal/mol was used. In practice for each point ρ, ϕ, α, β, γ (ρ and ϕ are spherical coordinates, α, β, γ Euler angles) in the grid, gradient optimization is performed to determine which minimum the point belongs to.

$$Z_{\mathrm{NVT}} = \int_0^R \int_0^{\pi} \int_0^{2\pi} \int_0^{2\pi} \int_0^{\pi} \int_0^{2\pi} \mathrm{e}^{(-V(r)/k_{\mathrm{b}}T)} r^2 \times \sin(\theta)\,\sin(\beta)\,\mathrm{d}r\,\mathrm{d}\theta\,\mathrm{d}\phi\,\mathrm{d}\alpha\,\mathrm{d}\beta\,\mathrm{d}\gamma \qquad (2)$$

The step length in the numerical integration was 0.125−0.250 Å depending on where on the PES the point was situated. The enthalpy of formation (ΔH) was estimated from the following equation:

$$\Delta H = \int V(r)\mathrm{e}^{(-V(r)/k_{\mathrm{b}}T)}\mathrm{d}r - RT \qquad (3)$$

All calculations were performed with rigid monomers at 323 K.

Results and Discussion

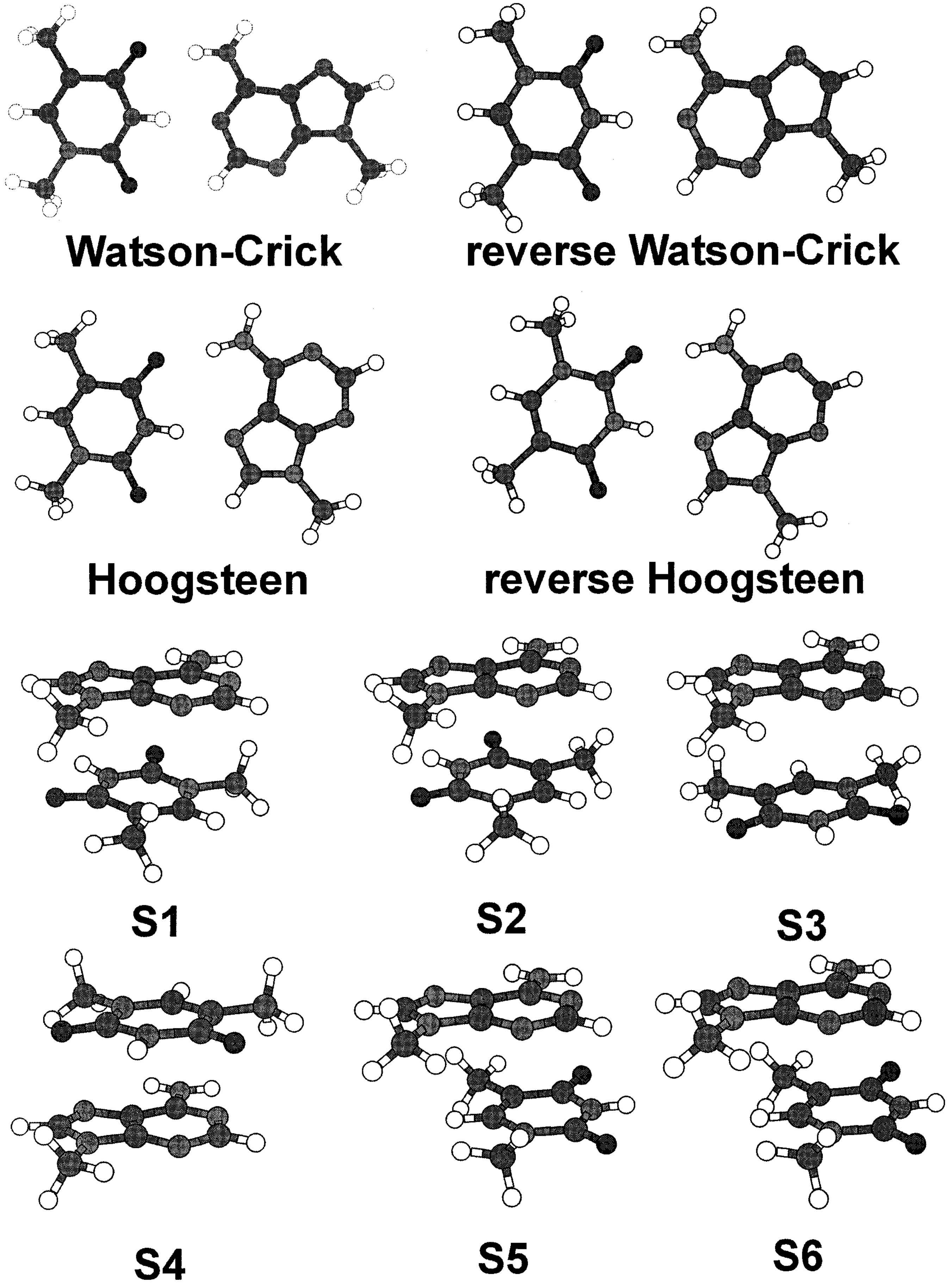
Potential Energy Surface. In the MD/Q calculations we found 16 energy minima: 4 planar hydrogen-bonded structures with two hydrogen-bonds (H, WC), 9 stacked structures (S), 2 T-shaped structures (T), and 1 planar structure (P) with just one hydrogen-bond. Structures of 10 most stable isomers (4 planar hydrogen-bonded, 6 stacked, and 3 T-shaped) are shown in Figure 2. Stabilization energies of these isomers evaluated using the Cornell et al.[17] empirical force field are depicted in the lower part of Figure 3 (cf. also Table 1). The global minimum on the PES corresponds to the reversed Hoogsteen structure, which is, however, only slightly more stable than two stacked structures S1 and S2. The third local minimum corresponds to the Hoogsteen structure, which is followed by stacked structure S3, reversed Watson−Crick structure, another stacked structure S4, and the Watson−Crick structure. The remaining structures are stacked or T-shaped, and there is also one planar structure with one hydrogen-bond. It should be mentioned that the stabilization energies of the first eight structures are similar and the difference between the global minimum and the seventh local minimum is only 1.2 kcal/mol.

How accurate are the stabilization energies determined with the Cornell et al.[17] force field? The only way to answer this question is to evaluate the stabilization energy for each structure at nonempirical correlated ab initio level. Table 2 shows ab initio and empirical stabilization energies determined for four planar hydrogen-bonded structures as well as for the stacked structure.[25] Agreement between ab initio and empirical stabilization energies in the case of planar hydrogen-bonded structures is satisfactory, and the difference between ab initio and empirical stabilization energy is not larger than 1.2 kcal/mol. Ab initio stabilization energy for stacked structure S agrees also well with the respective empirical stabilization energy. Figures 4 and 5, showing dependencies of empirical and ab initio interaction energies on rotation angle and vertical distance, give other evidence of the reliability of the empirical stabilization energies. It needs to be mentioned here that similarly good agreement between Cornell et al. and correlated ab initio stabilization energies was found also for other hydrogen-bonded and stacked NA base pairs.[22]

Free Energy Surface. *NVE Microcanonical Ensemble*. Populations of various energy minima determined by long MD runs are depicted in the upper part of Figure 3. It is evident that the reversed Hoogsteen structure, which corresponds to the global minimum at the PES, is only slightly populated. On the other hand, stacking structures S1, S2, and S3 are populated more and it is the stacked structure S3 that is the most populated among all structures (∼29%). From Figure 3 it is further evident that all the stacked structures are populated much more than the hydrogen-bonded structures. Total populations of the former and latter structures amount to 79 and 19%, respectively. Highest populations among the hydrogen-bonded structures belong to the Hoogsteen structures (H ∼4%, Hr ∼3%) while both the Watson−Crick structures are negligibly populated (∼1%).

From the Figure 3 obtained from NVE simulations it is thus evident that at the experimental conditions corresponding to the microcanonical simulations (e.g., supersonic beams) planar hydrogen-bonded structures will not be significantly populated and mostly the stacked structures will be detected. Increasing the total energies (with corresponding average temperature) the overall picture is not changed and populations of single structures was changed by less than 1%. This finding is of key importance for future (not yet realized) gas-phase supersonic beam experiments.

NVT Canonical Ensemble. Results of the NVT ensemble calculations are also depicted in the upper part of Figure 3. It is clear that the stacked and hydrogen-bonded structures are almost equally populated. Total population of the stacked structures is 56% and of hydrogen-bonded structures 43%. T-shaped structures are populated negligibly. The populations of two Hoogsteen hydrogen-bonded structures and the populations of stacked structures S1 and S2 range from 18 to 19% each. Total population of hydrogen-bonded WC structures is smaller and amounts to about 6%. Significant NVT populations



Watson-Crick

reverse Watson-Crick

Hoogsteen

reverse Hoogsteen

S1

S2

S3

S4

S5

S6

Figure 2. Selected planar and stacked structures of 1-methyladenine and 9-methylthymine pair.

TABLE 1: Interaction Energies, Free Energies, and Populations of Various Structures of the mA···mT Base Pair Determined with the Empirical AMBER[16] Cornell et al. Force Field[17]

structure[a]	$\Delta E^{\mathrm{AMBER}}$ [kcal/mol]	population NVE [%]	population NVT [%]	ΔG NVT[b] [kcal/mol]	RR-HO-IG[c] $\Delta G^{\mathrm{AMBER}}$ [kcal/mol]	RR-HO-IG[c] $\Delta G^{\mathrm{ab\ initio}}$ [kcal/mol]
Hr	−13.11	3.4	19.4	−0.6	−0.6	0.8
S1	−13.08	19.2	19.4	−0.6	0.8	
S2	−12.95	29.0	17.6	−0.5	1.0	
H	−12.94	3.8	17.8	−0.5	−0.6	0.8
S3	−12.25	9.5	8.1	−0.1	1.2	
WCr	−12.00	1.3	2.5	0.6	0.5	0.8
S4	−11.93	12.0	6.6	0.0	1.1	
WC	−11.92	1.4	2.6	0.6	0.5	0.5
S5	−10.32	4.7	2.7	0.6	2.6	
S6	−10.06	4.0	1.7	0.8	2.8	
T1	−9.59	1.4	0.6	1.5	2.0	
T2	−8.96	1.6	0.3	1.9	2.2	
P	−7.61	1.8	0.1	2.5	2.3	

[a] cf. Figure 2. [b] Relative values from the NVT analysis using AMBER potential; for comparison with RR-HO-IG/AMBER analysis the ΔG° of Hr was set to −0.6 kcal/mol. [c] Rigid rotor-harmonic oscillator-ideal gas approximation.

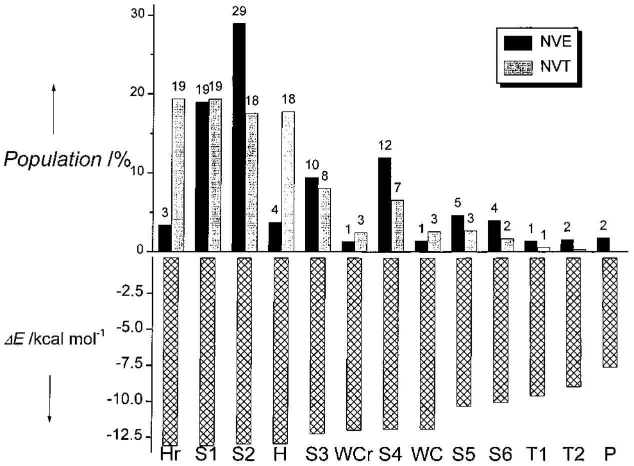


Figure 3. Populations and stabilization energies of 13 energy minima corresponding to the hydrogen-bonded and stacked structures (cf. Figure 2). The MD simulation was 125 ns and maximal convergence error was approximately 1%. Total number of interconversions between the isomers was 213701 (from 250000 quenches).

TABLE 2: Interaction Energies of Various Hydrogen-Bonded and Stacked Structures of the mA···mT Base Pair Determined at Nonempirical ab Initio (MP2/6-31G*(0.25)//HF/6-31G) and Empirical AMBER[16] (Cornell et al. Force Field)[17] Levels**

structure[a]	$\Delta E^{\mathrm{ab\ initio}}$ [kcal/mol]	$\Delta E^{\mathrm{AMBER}}$ [kcal/mol]
Watson−Crick (planar)	−12.18	−11.93
Watson−Crick reverse (planar)	−10.98	−12.00
Hoogsteen (planar)	−12.64	−12.94
Hoogsteen reverse (planar)	−11.96	−13.11
stacked[b]	−11.30	−11.90

[a] cf. Figure 2. [b] Step-by-step calculations with rigid subsystems were used in the case of ab initio and empirical potential calculations, and the following energy minimum parameters were found: rotation angle 60.0° (MP2 and AMBER); displacement 0 Å (MP2 and AMBER); vertical distance 3.3 Å (MP2), 3.5 Å (AMBER).

were observed for structures with large stabilization energy. This is not surprising since the Boltzmann factor discriminates minima with smaller stabilization energies. It should be emphasized that results of the NVT ensemble calculations are very sensitive to the accuracy of the potential used. A small shift in the interaction energy difference leads to a large change in the relative populations of respective energy minima.

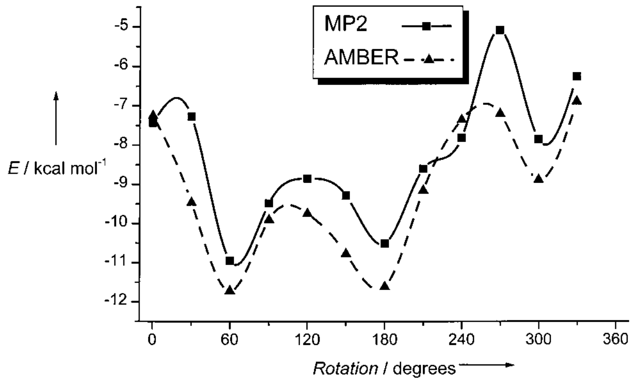


Figure 4. Dependence of interaction energy (Δ*E*) on the rotation angles between **vec1** and **vec2** (see text for more information).

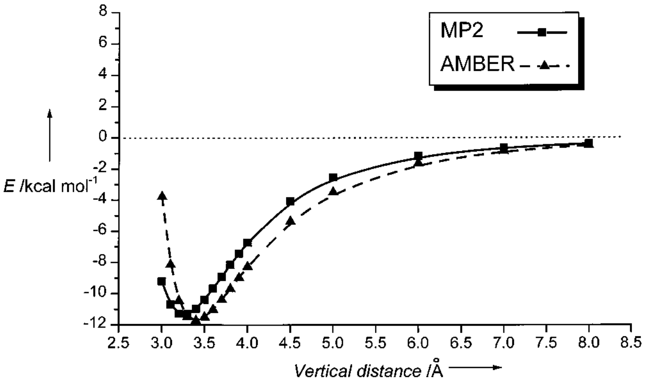


Figure 5. Dependence of interaction energy (Δ*E*) on the vertical distance between molecules.

Comparing rigid rotor−harmonic oscillatior−ideal gas approximation (RR-HO-IG) and NVT ensemble AMBER free energies, we find very good agreement in the case of planar hydrogen-bonded and T-shaped structures while in the case of stacked structures the latter values are systematically larger (from 1.1 to 2 kcal/mol). NVT ensemble free energies for hydrogen-bonded and stacked structures are more uniform than those from RR-HO-IG calculations, and this difference might be due to inclusion of anharmonicity into the NVT ensemble calculation. In case of uracil dimer,[3] we also found larger values of NVT free energies as compared to the RR-HO-IG ones but the respective differences were smaller. This can be interpreted by more anharmonic character of stacked structures of the mA···mT pair compared to the uracil dimer. Ab initio free energies (RR-HO-IG) evaluated for canonical planar hydrogen-bonded pairs are very uniform (differences are within 0.3 kcal/mol) while empirically treated free energies differ more (by 1.2 kcal/mol). The overall agreement between both values is, however, still satisfactory.

Comparison of Experimental and Theoretical Stabilization Enthalpies for the Formation of mA···mT Pair. Conditions of field ionization mass spectrometry experiments of Yanson et al.[5] which yielded stabilization enthalpies of mA···mT corresponded to the NVT constant temperature simulations. Average stabilization enthalpy for the formation of mA···mT pair at 323 K determined using eq 3 amounts to 11.0 and 10.4 kcal/mol, if NVT and RR-HO-IG characteristics were used. In this case all structures of the pair (on the basis of their population) were considered while in the previous comparisons[10−12] of theoretical and experimental data only the global minimum theoretical enthalpies were taken into account. Agreement between the present theoretical NVT enthalpy and experimental value (13 kcal/mol) is good. The underestimation of the theoretical stabilization enthalpy is easily understandable in the light of the fact that present ab initio as well as Cornell et al.[17] empirical potential stabilization energies of hydrogen-bonded and stacked pairs are underestimated: former pairs by 2.0−2.5 kcal/mol[19] and the latter pairs by 1.0−1.5 kcal/mol.[20]

Conclusions

There are 16 energy minima on the PES of the mA···mT. The global minimum is the hydrogen-bonded reversed Hoogsteen structure but stabilization energies of other stacked and hydrogen-bonded structures are similar. AMBER empirical stabilization energies were verified by comparing them with correlated ab initio stabilization energies.

Constant energy NVE ensemble MD simulations gave largest populations for stacked structures and all planar hydrogen-bonded structures were populated negligibly. It means, in the future (not yet realized) gas-phase supersonic beam experiments at 300−500 K only stacked structures will be “visible”. On the other hand the simulations in the NVT ensemble showed equal populations of two planar hydrogen-bonded and two stacked structures. Reasonable agreement between theory and experiment suggest reliability of out theoretical procedure.

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(25) Stacked structure obtained by step-by-step optimization is not identical with stacked structures S1, S2, and S3 generated by the gradient optimization but it is similar to structure S2.