Global Minimum of the Adenine···Thymine Base Pair Corresponds Neither to Watson−Crick Nor to Hoogsteen Structures. Molecular Dynamic/Quenching/AMBER and ab Initio beyond Hartree−Fock Studies

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Abstract: Computational analysis of complete gas-phase potential energy and free energy surfaces of the adenine···thymine base pair has been carried out. The study utilizes a combination of molecular dynamics simulations performed with Cornell et al. empirical force field and quenching technique. Twenty seven energy minima have been located at the potential energy surface of the adenine···thymine base pair: nine of them are H-bonded structures, eight are T-shaped dimers, and the remaining nine correspond to various stacked arrangements. H-bonded structures are the most stable while stacked and T-shaped structures are by more than 4 kcal/mol less stable than the global minimum. The global minimum and the first two local minima utilize N_9−H and N_3 groups of adenine for the binding, i.e., the amino group N_6, and ring N_1 and N_7 adenine positions are not involved in the base pairing. The most stable H-bonding patterns cannot occur in nucleic acids since the N_9 position is blocked by the attached sugar ring. Hoogsteen and Watson−Crick type structures (third and fourth local minima) are by about 3 kcal/mol less stable than the global minimum. Energetic preferences of the global minimum and first two local minima were confirmed by correlated MP2 ab initio calculations with 6-31G** and 6-311G(2d,p) basis sets. Relative population of various structures (a quantity proportional to ΔG of base pair formation) was determined by molecular dynamics simulations in the NVE microcanonical ensemble. Although the stability order of the global and first two local minima is unaffected by including the entropy contribution, the stability order of the remaining structures is altered rather significantly in favor of stacked and T-shaped structures. The simulations further show that the population of the global minimum is about 35% and it means that experimental gas-phase studies are likely to detect a vast number of mutually coexisting structures.

1. Introduction

The double-helical DNA stores and transfers the genetic information. The large fidelity of DNA replication is due to the high specificity of nucleic acid (NA)−base pairing in DNA. Guanine (G) binds to cytosine (C) and adenine (A) to thymine (T). High specificity of base pairing is ensured by H-bonding between the bases; the GC pair possesses three and the AT pair two strong H-bonds. These structures are known as the Watson−Crick (WC) structures. In DNA double helix only the GC WC and AT WC base pairs are observed; however, in RNA, triplexes, parallel stranded duplexes, and nucleic acid architectures other than AT (or AU) base pairing patterns were found.[1] These are called Hoogsteen (H), reverse Hoogsteen (RH), and reverse Watson−Crick (RWC) base pairs.

Electronic distributions of both adenine and thymine are characterized by a rather low polarity.[2] This explains why stability of all four AT structures observed in nucleic acids is essentially identical (in contrast to many other dimers such as GG showing very variable strength of base pairing).[2] Ab initio calculations including the electron correlation effects using the second-order Møller−Plesset perturbation method (MP2) with 6-31G*(0.25) basis sets yield the following stabilization energies of the AT base pairs (in kcal/mol): AT H 12.7, AT RH 12.6, AT WC 11.8 and AT RWC 11.7.[2] Experimental stabilization enthalpies[3] for association of methylated bases (purines at position N_9, pyrimidines at position N_1) were compared with theoretical values and very good agreement was found for the Hoogsteen AT structure.[4] This has been considered as indirect evidence that this structure corresponds to the global minimum observed in the experiment. Nevertheless, due to the small differences among the four structures one can assume all of them being populated simultaneously.[5] The calculations were done for non-methylated bases while excluding from the analysis those structures directly involving the H_9(A) and H_1(T) positions in the H-bonding, blocked in the experiment by the methyl groups.[4] The obvious question of whether in the non-methylated adenine···thymine base pair the Hoogsteen structure corresponds to the global minimum remained unanswered. This question is now very important since gas-phase experimental studies of NA

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base pairs and related systems become topical in many laboratories.[6] Important advantage of gas-phase techniques is that they allow pure interactions to be extracted and eliminate the complex solvent effects often dominating the interactions in the condensed phase.[7] Despite the progress in various experimental techniques, direct detection (simultaneous with the thermodynamics properties) of the structures of the global and local minima of the NA base pair is rather unlikely. Hence the structures should be deduced in another way[8] and computational procedures combining correlated ab initio calculations with computer simulations represent a very powerful technique. Because experiments are usually performed at non-zero temperatures theoretical investigation should go beyond the energy concept, i.e., it is vital to include the entropy.[8,9]

The aim of the present study is to investigate the complete potential energy surface (PES) and free energy surface (FES) of the adenine···thymine base pair. The PES of the pair is too complicated and it is thus not possible to localize all the stationary points by using just our chemical intuition and experience. Some quantitative and reliable method should be applied. The quenching technique, which is a combination of molecular dynamics (MD) and molecular mechanics, was recently shown to be very efficient.[9] Similar technique will be applied in the present study for investigation of the adenine··· thymine pairing.

2. Strategy of Calculations

The potential energy surface of the AT pair was first investigated by the molecular dynamics/quenching/AMBER simulations. AMBER potential with Cornell et al. force field[10] was used because it was shown to reproduce best (among various empirical potentials) the ab initio stabilization energies of H-bonded and stacked base pairs.[11] Proper sampling of the whole PES was ensured by varying the temperature (kinetic energy) and the length of the quench. After localization of all energy minima at the PES, the calculated AMBER stabilization energies of the global minimum and first two local minima (these novel structures were not considered in the previous ab initio studies) were verified by a comparison with stabilization energies obtained from correlated ab initio calculations (see below). Finally, populations of various structures (which are proportional to ΔG of dimer formation) were determined in the NVE ensemble.

3. Calculations

Quantum Chemical Calculations. Geometries of H-bonded base pairs were optimized at the Hartree−Fock (HF) level with the 6-31G** basis set using the standard gradient optimization method. The use of the HF level for optimizations of H-bonded base pairs is justified because these complexes are stabilized mainly by electrostatic interactions that are included within the HF approximation.[2] The HF method does not include, however, the dispersion attraction and is not sufficient for evaluation of accurate stabilization energies. The stabilization energies were calculated by using the second-order Møller−Plesset perturbation theory (MP2) with the 6-31G*(0.25) basis set. The polarization d-functions in the basis set used are more diffuse (α = 0.25) than those in the standard 6-31G* basis set.[12] The diffuse polarization functions improve the description of the dispersion energy. The stabilization energy was corrected for the basis set superposition error using the Boys−Bernardi counterpoise correction[13] and for deformation energy of monomers.[2] Note that all AT base pairs that can occur in nucleic acids were investigated before with the same method[2] and compared to the corresponding AMBER values.[11] The same or similar computational level was used for characterization of more than 100 H-bonded DNA base pairs, trimers, and complexes of base pairs with metal cations.[14] Moreover, the stabilization energies of hundreds of configurations of stacked DNA base pairs were determined with the same basis set as used here [MP2/6-31G*(0.25) level].[15] This allows extensive and consistent comparison of H-bonded and stacked DNA base pairs and consistent verification of empirical force fields.[11,16]

Accuracy of ab initio results is clearly of topical importance. Results obtained using procedure described above are influenced by two inaccuracies: (i) neglect of correlation energy in geometry optimization and (ii) use of gradient geometry optimization instead of counterpoise (CP)-corrected gradient optimization. The neglect of electron correlation certainly affects the base pair structure and its stability, but these effects are rather small. Structures of two H-bonded base pairs, uracil dimer and cytosine dimer, were optimized at the MP2 level.[16] The distance of two heavy atoms in the X−H···Y H-bonds in both dimers is reduced by about 0.14 Å while the X−H···Y angle is not affected. Stabilization energies of both pairs determined at the MP2//HF and MP2//MP2 levels are similar. To demonstrate the role of CP-corrected optimization we compared[16] standard and CP-corrected HF/6-31G** geometries of three H-bonded base pairs: ATWC, TC2, and GA2. Stabilization energies for all three pairs were very similar and differed by less than 0.1 kcal/mol. Also the geometry changes introduced by the CP corrections were modest. It can be thus concluded that present ab initio results are reliable and can be used for verification of empirical potential data. Obviously the largest inaccuracy of the interaction energies calculated by the ab initio method is caused by underestimation of the dispersion energy due to the size of the basis set. This inaccuracy, however, should have no substantial effect on the relative stability of various structures.

Empirical Potential. The Cornell et al. force field in the original parametrization was used.[10] The atomic charges of adenine and thymine were determined consistently with the original force field, i.e., using the restrained electrostatic potential fitting procedure (RESP) at the

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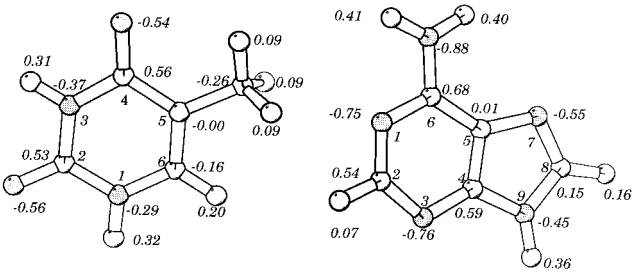

Figure 1. Structures of thymine and adenine with RESP/6-31G* atomic charges.

HF/6-31G* level. The charges and atom numbering of adenine and thymine are given in Figure 1.

Molecular Dynamics/Quenching/AMBER Calculations. Constant-energy molecular dynamics simulations were performed with assuming rigid monomers (quaternion formalism). The respective code uses fifth-order predictor-corrector formalism.[17] A 0.4 fs integration time step was used. The total energy of a pair was conserved within 1.4×10^{-2} kcal/mol during the MD run; this fluctuation is due to the numerical method used.

PES was scanned at 300 K. For a description of PES, i.e., for localization of all energy minima, rarther short MD simulations are required while calculation of relative populations from quenching requires long MD simulations. In the present study, quenches were made after 5.36 ps, and we made about 50 000 time steps (270 ns). A more detailed description of the procedure used can be found in ref 9.

4. Results and Discussion

4. 1. Potential Energy Surface. The MD/quenching/AMBER investigation of the PES reveals 27 energy minima. Nine of them are planar H-bonded base pairs, nine are stacked dimers, and eight are T-shaped complexes. Structures of 17 energy minima populated by more than 1% (see later) are visualized in Figure 2 and their stabilization energies are presented in Table 1.

Let us explain the abbreviations used in Figure 2 for various H-bonded dimers, since the nomenclature is different from that used so far. In our first ab initio paper on H-bonded NA base pairs[18] we investigated all DNA base pairs that can occur in nucleic acids and we introduced straightforward abbreviations such as GG1, AC2, ... The capital letters label a NA base while the numbers indicate the stabilization energy order for the particular base pair at the HF/MINI-1 level of theory.[18] This abbreviation scheme has been subsequently adopted in many other studies as the standard abbreviation. Nevertheless, recent calculations with inclusion of electron correlation effects changed somewhat the original stabilization energy order compared to the earlier evaluations.[2] Thus the numbers in the abbreviations lost their original meaning. Further, and more important, in case of the MD/quenching studies we need to describe a substantially larger number of H-bonded structures. In the case of uracil dimer[9] we localized seven H-bonded energy minima and in the present case of the AT pair even nine H-bonded pairs were found. This has forced us to seek another and less arbitrary system for labeling of NA base pair structures, independent of the stability order of the pairs. The system proposed is based on standard atom numbering of NA bases used in biochemical literature (for A and T see Figure 1). The capital letters again label a NA base pair while the numbers attached indicate those heavy atoms directly involved in H-bonding: the first and second numbers label the heavy atoms from the first and second NA base forming a H-bond. The

Table 1. Interaction Energy (ΔE) and Relative Population for Various Structures of the Adenine···Thymine Complex, Calculated by Amber Empirical Potential

structures[a]	(3192)	(3392)	(3394)	(6273)	(1162)	(6473)
ΔE/kcal/mol	−15.58	−14.10	−13.92	−12.70	−12.58	−12.57
population/%[b]	35.0	11.0	13.9	5.1	5.2	5.6
structures[a]	(6271)	(1362)	(1364)	(S1)	(T1)	(S2)
ΔE/kcal/mol	−12.44	−12.04	−11.92	−11.39	−11.12	−11.10
population/%[b]	1.2	2.0	1.0	5.4	5.6	5.9
structures[a]	(T2)	(S3)	(S4)	(T3)	(T4)	
ΔE/kcal/mol	−10.70	−10.56	−10.48	−10.14	−9.80	
population/%[b]	2.6	2.1	1.0	0.3	1.4	

[a] Cf. Figure 2. [b] Quenching/MD results.

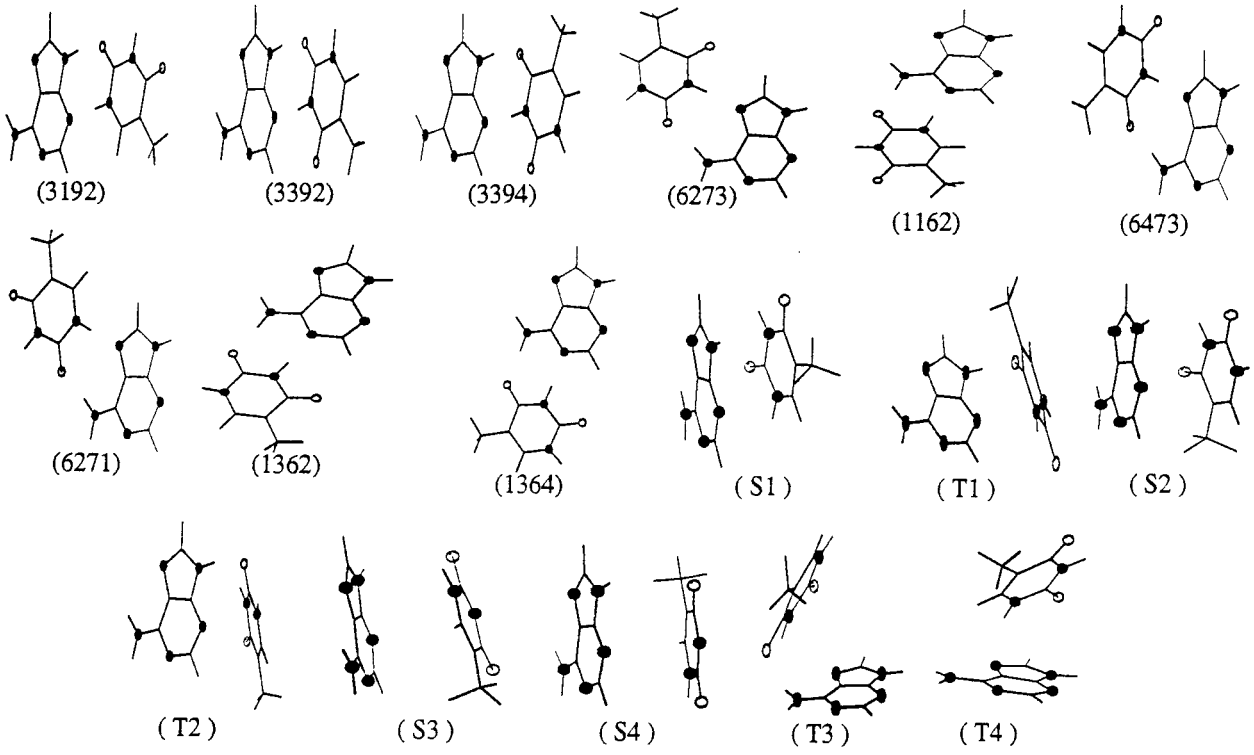

Figure 2. Structures of the adenine···thymine pair; numbers indicate the H-bonds, T and S indicate T-shaped and stacked structures.

second (third) pair of numbers indicate the second (third) H-bond. For example, the AT WC pair is designated as AT1364. This means N_1 of adenine binds to (H)N_3 of thymine, etc. There is a rule that the numbers are ordered from lower to higher and the letters are used alphabetically, to make the abbreviations unambiguous, i.e. AT1364 and not AT6413, TA3146, etc. stands for the ATWC.

The most stable structure (the global minimum) and the first two local minima of the AT dimer (see Figure 2) form H-bonds through N_3 and N_9-H of adenine while N_6-H, N_1, and N_7 positions of adenine characteristic for Watson−Crick and Hoogsteen structures are not involved. Therefore, none of the three most stable structures can occur in DNA (the N_9 position of adenine is blocked by the covalently attached backbone) and were not investigated before. These structures are, however, clearly of primary importance for any gas-phase experiment. All of these structures have two H-bonds of the C═O···H−N and N···H−N type, specifically N_3···H−N_1, N_9−H···O_2; N_3···H−N_3, N_9−H···O_2; and N_3···H−N_3, N_9−H···O_4 for structures 3192, 3392, and 3394, respectively. Preferential binding through N_3 and N_9-H of adenine cannot be explained by larger atomic charges localized at N_3 and H(N_9) as compared to these at N_1 and H(N_6) (see Figure 1) but by more suitable complementarity of the electrostatic potentials of the bases. Note also that the H_1 hydrogen of thymine is involved in the most stable base pairing pattern and this site is also preferentially used for H-bonding in the uracil dimer.[9] Stabilization energy differences between the global minimum and the third and fourth local minima (which are the first structures of the Hoogsteen and Watson−Crick type) are large and equal to 2.9 and 3.0 kcal/mol, respectively.

Nine most stable structures are planar H-bonded pairs with stabilization energy between 15.6 and 11.9 kcal/mol. The remaining structures are stacked and T-shaped dimers with stabilization energies between 11.4 and 8.1 kcal/mol. Worth mentioning is the fact that stacked and T-shaped structures (having one H-bond) are comparably stable (see Table 1).

Despite the fact that the Cornell et al. force field yields very good estimates of H-bonding energies for NA base pairs,[11] we decided to verify the stabilization energy preference of new structures 3192, 3392, and 3394 over WC and H types by ab initio calculations since in these structures the H-bonding involves those sites which were not included in the previous ab initio studies. Stabilization energies determined at the MP2/6-31G*(0.25) level using the HF/6-31G** geometries are 16.76, 14.31, and 14.42 kcal/mol for structures 3192, 3392, and 3394, respectively. It is evident that agreement between MP2 and Cornell et al. values is again very good. The MP2 method yields for structures studied slightly larger stabilization energies, the largest difference (1.2 kcal/mol) being for the global minimum. We have further increased the size of the basis set to 6-311G-(2d,p) but it has only a marginal effect on the stabilization energy of the structures studied (16.65, 14.15, and 14.22 kcal/mol). It again gives us confidence in the quality of results obtained at the MP2/6-31G*(0.25) level. One can conclude that the AMBER empirical potential as well as correlated MP2 ab initio calculations give clear evidence about the preference of H-bonded structures with participation of N_9 and N_3 of adenine; Watson−Crick and Hoogsteen structures forming H-bonds through N_6 and N_1 (N_7) of adenine are less stable.

4.2. Free Energy Surface. MD simulations yielded again 27 free energy minima of the AT base pair. Ten of them were

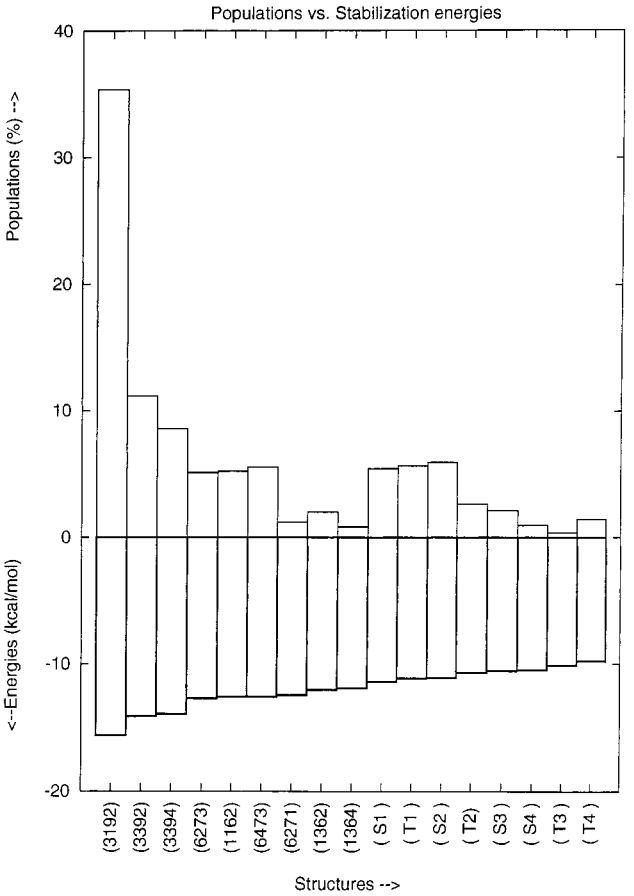

Figure 3. Relative populations and stabilization energies of 17 energy minima obtained from MD/quenching and AMBER/Cornell et al. force field calculations. The maximum convergence error in populations is about 2%. The number of interconversions was 48 949.

populated insignificantly (less than 0.5%). Relative populations of the remaining 17 minima together with their stabilization energies are plotted in Figure 3. The dominant peaks at both potential and free energy surfaces correspond to H-bonded structures 3192, 3392, and 3394, i.e., these structures represent the global and first two local minima at both the PES and FES. A new feature at the FES is the sharply increased population of stacked and T-shaped structures. Particularly, population of the stacked structure S2 is the fourth highest and this structure is populated more than any H-bonded structure relevant to nucleic acids. Population of the T-shaped structure T1 is only slightly lower than that of the stacked structure S2. Evidently, entropy favors stacked and T-shaped structures over the planar H-bonded ones.

5. Conclusions

The present study reveals 27 energy minima on the PES of the adenine···thymine base pair in the gas phase: nine are H-bonded ones, nine are stacked, and eight are T-shaped. The global minimum with stabilization energy of 15.6 kcal/mol corresponds neither to the Watson−Crick nor to the Hoogsteen types of pairing but possesses H-bonds with participation of N_9 and N_3 of adenine. WC and Hoogsteen structures are about 3 kcal/mol less stable. H-bonded structures are more stable than stacked and T-shaped structures. Structures of the global and first two local minima do not change when considering the free energy surface. The fourth most populated structure, however, already represents one of the stacking arrangements. This is beacuse entropy favors stacked and T-shape structures over the

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H-bonded ones. These results clearly indicate that the gas-phase experiments should detect a mixture of structures while WC and Hoogsteen structures are populated rather insignificantly. Their populations at low and high temperatures will be much lower than those of H-bonded structures with participation of N_9 and N_3 of adenine. One can assume that occurrence of WC and Hoogsteen arrangements can be achieved by blocking the N_9 (A) and N_1 (T) positions by methyl groups. However, even this strategy would not guarantee a preferable formation of the desired structures. First, methyl groups attached to the nitrogens in the nucleobase rings could retain a substantial portion of the polarity of the original sites. Thus, unlike the carbon-attached 5-methyl group of thymine, they may be involved in some sort of binding, albeit weaker compared with hydrogens in these positions. Second, considering free energy surface, some stacked and T-shaped structures show populations comparable with AT WC and Hoogsteen pairs. Methylation of the bases is likely to further bolster the dispersion-stabilized stacked structures and the resulting FES surface of the base pair could be very complicated. Our results indicate that the original experimental mass-field spectroscopy study by Yanson et al.[3] also detected a complex mixture of structures.

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