PHOTODYNAMICS OF THE ADENINE MODEL 4-AMINOPYRIMIDINE EMBEDDED WITHIN DOUBLE STRAND OF DNA

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Dedicated to Dr. Zdeněk Havlas on the occasion of his 60th birthday.

On-the-fly surface hopping nonadiabatic photodynamical simulations using hybrid quantum mechanical/molecular mechanical approach of 4-aminopyrimidine were performed to model the relaxation mechanism of adenine within DNA double strand. The surrounding bases do not affect the overall ring-puckering relaxation mechanisms significantly, however, interesting hydrogen-bond dynamics is observed. First, formation of intra-strand hydrogen bonds is found. It is shown that this effect speeds up the decay process. In addition, the Watson–Crick structure is altered by breaking one of the inter-strand hydrogen bonds also leading to a decrease of the life time.

Keywords: *Ab initio* calculations; Excited states; Nucleic acids; Photodynamics; QM/MM method.

The nature of the excited states of DNA/RNA nucleic acids induced by UV radiation has been studied extensively over the last decades. To understand the photochemistry of these compounds, the excited state behavior of individual bases[1–19], base pairs[20–24] and nucleic acid strands[25–31] has been studied both experimentally and theoretically. The ultrafast relaxation of excited states of isolated species, on the time scale of a few picoseconds has been the subject of several computational studies performed by means of nonadiabatic dynamics simulations[11–15,32,33]. It is now generally accepted that nucleobases relax into the ground state through nonadiabatic transi-

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tions via conical intersections characterized by strongly puckered ring structures[7,10,17,18,34,35]. Surrounding nucleobases on the same strand or on the complementary strand can influence the relaxation mechanism through stacking interactions or hydrogen bonding, respectively. Sterical effects of the embedding nucleobases in both strands can alter the probability to reach the puckered structures typical for conical intersections.

Evidences obtained from experiments with nucleic acids fragments indicate that the excited state behavior of nucleobases is altered by surrounding bases. For example, the influence of the interstrand hydrogen bonding on the photodynamics has been documented by a significantly shorter excited state lifetime of a single Watson–Crick pair of guanine and cytosine as compared to non-Watson–Crick arrangements[24]. This observation was explained recently by the existence of a charge-transfer state stabilized by proton transfer between the bases resulting in a conical intersection with the ground state[22,36–38]. Concerning intrastrand stacking interactions in DNA oligomers, the features observed in the time-resolved spectra[25,27–31,39] demonstrate changes in the photophysical behavior as compared to individual nucleobases, which were explained by the presence of excimers.

Theoretical studies of interacting nucleic acid bases are considerably more challenging as compared to calculations on isolated species. Several static calculations[40–49] and only few nonadiabatic dynamics simulations[23,50–52] were reported until today. Dynamics studies of such interacting bases are a natural extension to provide more realistic description of photodynamics of nucleic acids species.

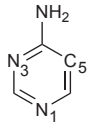
Recently, we have performed nonadiabatic photodynamical simulations of 4-aminopyrimidine (4-APY), used as a model for adenine, embedded between two stacking methyl-guanine bases[50]. For these studies we have used hybrid quantum mechanics/molecular mechanics (QM/MM) description[53]. This model accounts for possible sterical constraints during the relaxation via conical intersections. Since the QM treatment is limited to 4-APY only, the model is unable to describe photodynamics of the system which results from processes such as e.g. charge transfer between the individual bases or excimer formation mentioned above. Comparison with results of dynamics simulations on isolated 4-APY [54,55] shows an overall small elongation of the excited state lifetime due to the embedding. In addition the strongly puckered structures allow for a frequent formation of intra-strand hydrogen bonds which speed up the relaxation process. In this contribution, we extend the model to provide more reliable description of DNA molecule by considering also the complementary strand in the embedding scheme. This approach allows investigating the effect of hydrogen bonds in a Watson–

Crick arrangement as these bonds can decrease the flexibility of 4-APY during the course of dynamics and thus make the formation of puckered conical intersections much less likely.

METHODS

On-the-fly surface hopping nonadiabatic dynamics simulations of 4-aminopyrimidine embedded in the structure of DNA are performed using a combined quantum mechanical/molecular mechanical (QM/MM) approach[53,56,57]. The model was constructed by replacing adenine with 4-APY in the (Gua-Ade-Gua).(Cyt-Thy-Cyt) sequence cut out from the dodecamer crystal structure (PDB_ID: 196D)[58]. Methyl groups were used to terminate the N-glycosidic bonds in the guanine molecules in one strand and in cytosine and thymine in the complementary strand. The electronic excitations are confined to 4-APY which is treated quantum mechanically, whereas all other bases are treated at the MM level. During the dynamics simulations the methylated bases were constrained to keep their position and orientation as they appear in real DNA double strand by fixing hydrogen atom positions of the methyl groups. The 4-APY movement was constrained by fixing the hydrogen atoms of C_5 and C_6 atoms (replacing the imidazole ring of adenine) in the Cartesian space (4-APY-F) (for numbering see Scheme 1).

The 4-APY-F (QM part) was calculated at the state-averaged complete active space self-consistent field (SA-CASSCF) level. The state-averaging procedure includes three states. The active space was constructed from two lone pair orbitals localized on the nitrogen atoms of the pyrimidine ring and three π orbitals and three π* ones, i.e. 10 electrons in 8 orbitals (CAS(10,8)). The 6-31G* basis set[59,60] was used throughout the calculations. The surrounding environment (MM part) which includes the Gua bases on the same strand as 4-APY-F and the complementary strand composed of Cyt-Thy-Cyt bases were treated using the empirical Amber Parm99 potential[61,62]. Atomic point charges were determined with the ChelpG [63] method as implemented in Gaussian program package[64] employing the HF/6-31G*



SCHEME 1
Numbering scheme of 4-aminopyrimidine

level. The electrostatic potential of these charges was included in the Hamiltonian of the QM system within the framework of electrostatic embedding scheme (for more details on the employed QM/MM scheme see ref.[53]). The initial conditions for dynamics simulations were generated separately for QM and MM regions. Hundred initial structures and atomic velocities for the QM region were generated using a Wigner distribution of the quantum mechanical oscillator in the ground state as described in ref.[65]. For each geometry the vertical electronic excitation into the S_1 state and the corresponding Einstein absorption coefficient B was computed. From these data a semi-classical simulation of the UV spectrum was performed (for details see ref.[65]) using a Lorentzian line-shape with the phenomenological broadening of 0.05 eV. The initial structures for the MM region were taken from MM ground state dynamics simulations with frozen QM core.

On-the-fly *ab initio* dynamics simulations were performed using Tully's surface hopping approach[66] by solving Newton's equations for the nuclear motion with a 0.5 fs time step, using the Velocity–Verlet algorithm[67]. For the integration of the time-dependent Schroedinger equation the 5th-order Butcher algorithm[68] was used. Surface hopping probabilities between electronic states were calculated by means of Tully's fewest switches algorithm[66]. Analytic energy gradients and nonadiabatic coupling vectors necessary for the dynamics were calculated as described in refs[69–72]. An empirical decoherence correction with a decay parameter of 0.1 Hartree was included as described in ref.[73].

The dynamics simulations of 4-APY-F embedded in the DNA double strand (4-APY-DS) have been performed for a total of 100 trajectories and a maximum simulation time of 7 ps. These results were compared to the dynamics study of isolated 4-APY-F obtained for a total number of 70 trajectories using the same constraints as for 4-APY-DS. During the whole course of the dynamics the structures of both systems were analyzed and characterized by means of the Cremer–Pople parameters[74] and the Boeyens classification scheme[75]. Additionally, the formation of hydrogen bonds within one chain or breaking of hydrogen bonds with the bases of the complementary chain was monitored. The selected hydrogen bonds are indicated in Fig. 1. The distances D1–D6 correspond to the hydrogen bonds formed between 4-APY-F and methylated guanines in stacking interactions (intra-strand hydrogen bonding). The distances D7 and D8 monitor the inter-strand hydrogen bonds which correspond to Watson–Crick base pairing. D7 corresponds to the hydrogen bonds formed between hydrogen atoms of the NH_2 group of 4-APY and the oxygen of CO group of thymine on the complementary

strand and D8 is defined as hydrogen bond formed between hydrogen bound to nitrogen of the pyrimidine ring of thymine and N atom on the pyrimidine ring of 4-APY.

The QM/MM dynamics studies were performed with the program package NEWTON-X [56,57] extended by a module which includes the QM/MM approach[53] in combination with COLUMBUS program system[76–78] used for the QM part.

RESULTS AND DISCUSSION

The Cremer–Pople classification of the structures occuring at the hopping event is shown in Fig. 2. These structures can be divided into three regions with respect to their twisted bonds: (i) twist around the N_1C_6 and C_5C_6 bonds and the puckering of the N_1 and C_6 atoms (region A), (ii) twist around the N_3C_4 bond and puckering at the N_3 and C_4 atoms (region B) and (iii) twist around the N_1C_2 and C_2N_3 bonds and puckering at N_1, C_2 and N_3 atoms (region C). Figure 3 shows the Cremer–Pople characterization of the hopping structures for 4-APY-F and 4-APY-DS. The relative occurence of the conical intersections within particular regions is given in Table I for

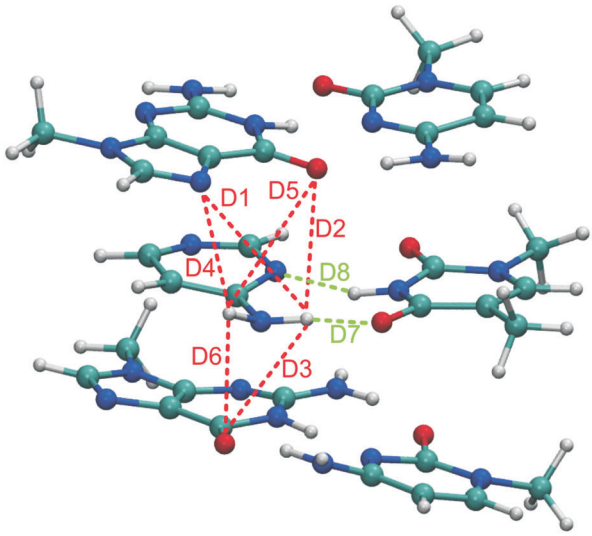


FIG. 1
The double-strand structure with inserted 4-APY. The intra-strand distances (D1–D6) are drawn by red dashed lines and inter-strand distances (D7, D8) by green dashed lines. They were used for monitoring of possibility of hydrogen bonds formation

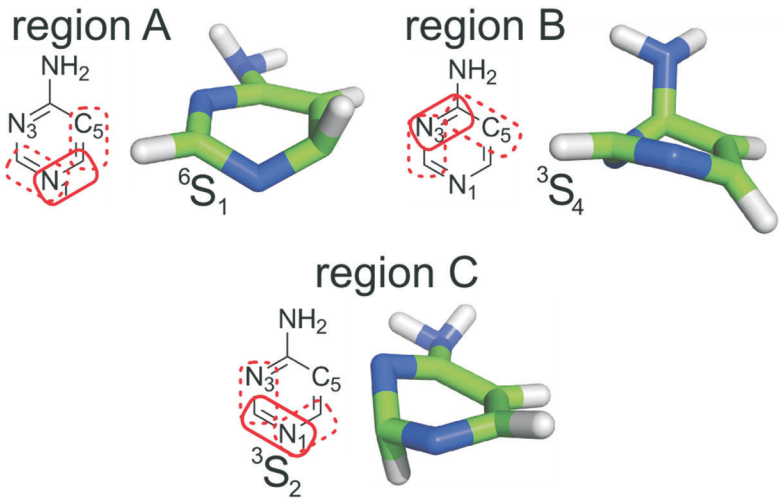


FIG. 2
The conical intersection structures with different ring puckering character divided into three regions by the Cremer–Pople parameters

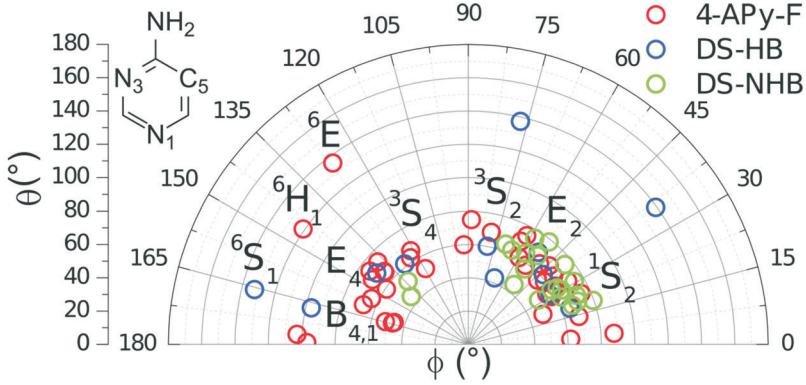


FIG. 3
The Cremer–Pople parameters of isolated 4-APy-F hopping structures and 4-APY embedded within double-strand (4-APY-DS): double-strand hopping structures with (DS-HB) and without (DS-NHB) hydrogen bonds between 4-APY and other bases

4-APY-F and 4-APY-DS and compared to the previously reported data on isolated 4-APY and that embedded between two stacked methylated guanines (4-APY-S). A significant decrease in the population of the conical intersections of region A (characterized mainly by distorsion of N_1C_6) observed for 4-APY-F and 4-APY-DS compared to 4-APY and 4-APY-S models is a consequence of fixing of hydrogen atoms on C_5 and C_6 atoms.

Embedding within one strand (see data for 4-APY and 4-APY-S in Table I) already changed the relative population of trajectories decaying via conical intersections of region B. The decrease of the population of conical interactions of region B is slightly higher for embedding within double-stranded fragment which indicates that both the sterical hindrance caused by surrounding bases in stacking interactions and the constraints of N_3 and C_4 atoms fixed by inter-strand hydrogen bonds D7 and D8 with the second strand of DNA molecule make this type of conical intersections less accessible. For the model reported here the relaxation mechanism changed and the most efficient channels become those which decay via conical intersection of region C. As mentioned above, such a significant decrease of the relative populations decaying via conical intersections of region A is mainly by too strong fixing of 4-APY-F. Since in the DNA molecule nucleobases are very likely more flexible the changes in the relaxation mechanism obtained with our model are certainly overestimated.

Figure 4 shows the histogram in which the occurrence of intra- and inter-strand hydrogen bonds is monitored. For 4-APY embedded in the single strand we have found that the intra-strand hydrogen bonds are formed mainly by the interactions of hydrogen atoms of the amino group of 4-APY

TABLE I
The relative population (in %) of the hopping structures according to their structure characterization

Structure	A	B	C
Isolated 4-APY[a]	63	20	17
4-APY-S[b]	65	11	24
4-APY-F[c]	5	39	56
4-APY-DS[d]	3	15	82

[a] Isolated 4-APY, ref.[79]; [b] 4-APY embedded with the single strand, ref.[50]; [c] Isolated 4-APY with fixed hydrogen atoms; [d] 4-APY embedded with DNA double-strand with fixed hydrogen atoms.

with the oxygen atom of the carbonyl group of embedding guanine bases (D2 and D5)[50]. As a consequence of inter-strand hydrogen bonds which involve the amino group, the formation of D2 and D5 hydrogen bonds is much less frequent in the case when the complementary strand is present. For example for the hopping structures, these bonds represent only about 22% of the total amount of hydrogen bonds in 4-APY-DS, compared to almost 70% found in the case of 4-APY-S. Nevertheless, among the structures found at the hopping event of 4-APY-DS about 36% form the intra-strand hydrogen bonds, resulting in the same relative population as found for 4-APY-S model. These results indicate that the fixing of the structure by the presence of inter-strand hydrogen bonds does not influence the probability of the intra-strand hydrogen bonds formation.

As shown in the histogram (Fig. 4), inter-strand D8 bond persists during the whole course of dynamics. On the contrary, the out-of-plane displacement of the NH_2 group of 4-APY causes breaking of the D7 bond quite frequently. For 35% of hopping structures we observed breaking of this

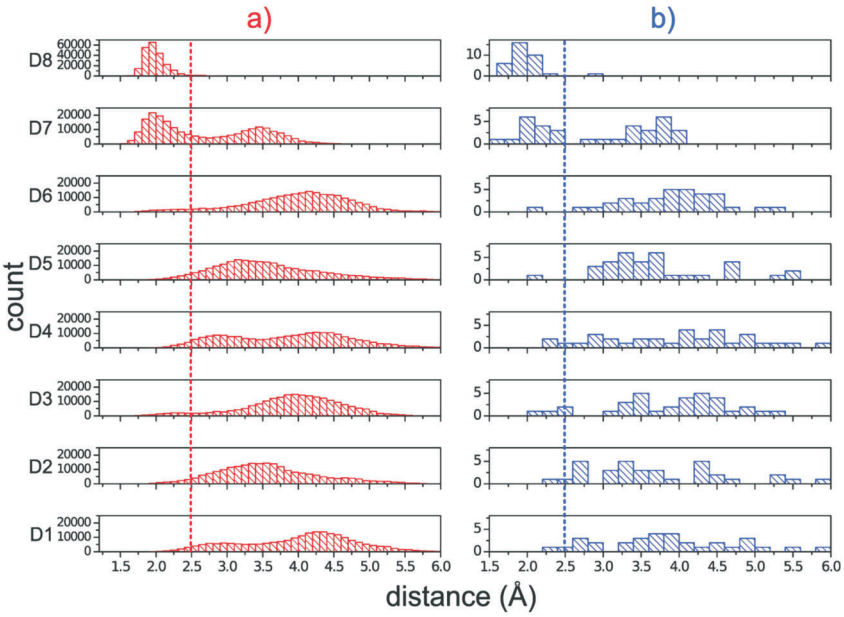


FIG. 4
Histograms of distances D1–D8 over the whole trajectories (a) and for hopping structures (b) of 4-APY-DS. The dashed line at 2.5 Å denotes the distance under which the interactions were considered as hydrogen bonds

hydrogen bond. Also the histogram which collects the structures during the whole course of dynamics (Fig. 4a) shows two well pronounced maxima in D7 curve indicating flexibility of this bond during the dynamics.

The fixing of hydrogen atoms of C_5 and C_6 atoms greatly influences the time course of dynamics of both systems 4-APY-F and 4-APY-DS resulting in a significant elongation of their excited state lifetimes. Since less than 50% of trajectories decay into the ground state within the simulation time-period in both systems, we did not attempt to estimate the total lifetimes. It is however still interesting to analyze the influence of both inter- and intra-strand hydrogen bonds formed at the hopping structures on the resulting lifetimes. These results together with the degree of puckering (Q-parameter) found at the hopping are shown in Table II. As expected, inter-strand hydrogen bonds constrain the hopping structures resulting in lowering of the values of Q-parameters by 0.1 Å. In addition, the trajectories decaying via conical intersections with both hydrogen bonds relax by about 0.5 ps longer as compared to the situation where one inter-strand hydrogen bond is broken. Similarly to the case of 4-APY-S [50] the trajectories in which the intra-strand hydrogen bonds are formed at the hopping structures decay within the time of about 1.1 ps shorter as compared to those which do not form this type of bonds. Surprisingly, for the former type of hopping structures the Q-parameter is smaller.

TABLE II
The lifetimes and averaged Q-parameter estimated for trajectories decaying at the structures forming (HB) and non-forming (NHB) inter- and intra-strand hydrogen bonds at the hopping structures of 4-APY-DS

Structure	Lifetime, fs	Q-parameter, Å
Inter-strand[a]		
HB	3000	0.41
NHB	2524	0.51
Intra-strand		
HB	2097	0.38
NHB	3221	0.48

[a] D7 hydrogen bond.

CONCLUSION

The photodynamics of 4-APY embedded in the DNA fragment (Cyt-Thy-Cyt).(Gua-4-APY-Gua) was studied at the multi-configurational *ab initio* level using the nonadiabatic surface hopping method. This approach accounts for possible sterical constraints during the excited state relaxation via conical intersections while the electronic reactivity between the individual bases is not considered. Comparison of our previous results of an embedding within one strand with the results reported in the current study indicates that the presence of the second strand does not significantly influence the photodynamical behavior of nucleobases:

1) Comparison of the results of dynamics studies of 4-APY fixed in the space (4-APY-F) and that fixed and embedded in the DNA fragment (4-APY-DS) shows that the surrounding bases have a significant influence on the hydrogen bonded structures but do not change the relaxation mechanism of ring puckering strongly.

2) During the course of dynamics the formation of intra-strand hydrogen bond was detected to a similar extent as found for embedding within one strand. Also in the case of embedding within DNA fragment this formation speeds up the decay process.

3) For a significant number of trajectories breaking of inter-strand hydrogen bond D7 was observed contrary to D8 which does not break during the whole course of dynamics. The estimated excited state lifetime for trajectories decaying via conical intersections with broken D7 bond is shorter as compared to those which keep both Watson–Crick hydrogen bonds.

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