Influence of the Sequence Dependent Ionization Potentials of Guanines on the Luminescence Quenching of Ru-Labeled Oligonucleotides: A Theoretical and Experimental Study

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The luminescence quenching by electron transfer of a nonintercalating $[Ru(TAP)_2DIP]^{2+}$ complex, which is covalently linked to different guanine containing oligonucleotide duplexes, is compared to the sequence dependent ionization potentials of guanines as estimated from Hartree−Fock calculations on regular B-DNA by means of Koopmans theorem. From these experimental and theoretical results, it is demonstrated that the ionization potential of the guanines has a major influence on the efficiency of hole injection by the groove binding $[Ru(TAP)_2DIP]^{2+}$ complex in a type I photooxidation of DNA. Because the photoelectron transfer between the complex and DNA takes place on a nanosecond time scale, nanosecond molecular dynamics simulations were performed for two of the sequences in order to check for the possible influence of the dynamical fluctuations in the DNA structure on the ionization potential. IP fluctuations up to 0.5 eV were observed on a picosecond time scale along the molecular dynamics trajectories. However, these fluctuations scatter statistically around the value calculated for regular B-DNA and are thus not relevant for the slow photoelectron transfer with a nonintercalating $[Ru(TAP)_2DIP]^{2+}$complex.

Introduction

Oxidative damage to DNA, which plays a key role in mutagenesis and carcinogenesis, has attracted considerable interest recently.[1−3] It is well-known that guanine (G) is the most easily oxidized base,[4−16] and oxidative damage of G residues in DNA have been studied in different experiments by direct photoionization[17] or reaction with photooxidizing agents.[3,14−17,19−30]

The photooxidation of guanines in DNA can take place either by direct electron transfer to the excited state of an acceptor molecule (type I) or via the photosensitization of singlet oxygen (type II).[3,31] In PAGE (polyacrylamide gel electrophoresis) experiments, after hot piperidine treatment, both type I and type II reactions cause cleavage of DNA predominantly at the level of the guanines. For type II photooxidation, the piperidine induces strand breaks that show little variation in cleavage yield at the various G sites,[3,32] whereas clear preference for certain guanines (e.g. 5′−G in 5′-GG-3′ steps) is observed in type I reactions.[3,14−16,18−21] This selectivity for 5′-Gs has been attributed to the sequence dependent ionization potentials (IP) of guanines in DNA that were estimated from Hartree−Fock calculations by Koopmans theorem.[12−16] IPs particularly low were calculated for GG and GGG stacks. This could explain the preferential cleavage of these sites in experiments using sequences that contain several guanines in different stacking environments. It has been proposed by Prat et al.[12] that the sequence dependence of the IPs is mainly due to the electrostatic interaction between the stacked bases.

Once a base radical cation is formed by a one-electron oxidation, it can migrate through DNA until it is trapped at a low IP site, and the hole-trapping efficiency of GG and GGG steps has been utilized in the study of long-range charge transfer in DNA.[33−40] Because of this charge transfer through DNA, PAGE experiments can yield valuable information on hole-trapping but not necessarily on the efficiency of hole injection.

The fact that the hole, once injected into DNA, can travel until it is trapped at a low IP site suggests that it is not necessary for the photooxidizing agent to be bound directly to a guanine to induce strand cleavage. That such a DNA-mediated photooxidation is indeed possible has been demonstrated recently by Wan et al.,[29] using covalently linked ethidium as the electron acceptor and 7-deazaguanine as the electron donor. In their work, electron transfer from 7-deazaguanine to the intercalated excited ethidium was observed even though the dye was not able to reach 7-deazaguanine directly because of the limiting length of the linker. Because it has been demonstrated[41] that ethidium is not able to photooxidize guanine or adenine but only 7-deazaguanine, it can be concluded that DNA-mediated photooxidation is possible for an intercalated donor/acceptor pair where the electron transfer will take place *parallel* to the helical axis. However, DNA-mediated photooxidation cannot necessarily be expected between a groove binding complex and a DNA base where a transfer *perpendicular* to the helical axis occurs. We were able to demonstrate this recently by studying the luminescence quenching of a nonintercalating type I photooxidizing $[Ru(TAP)_2DIP]^{2+}$ complex (DIP = 4,7,-diphenyl-1,10-phenan-

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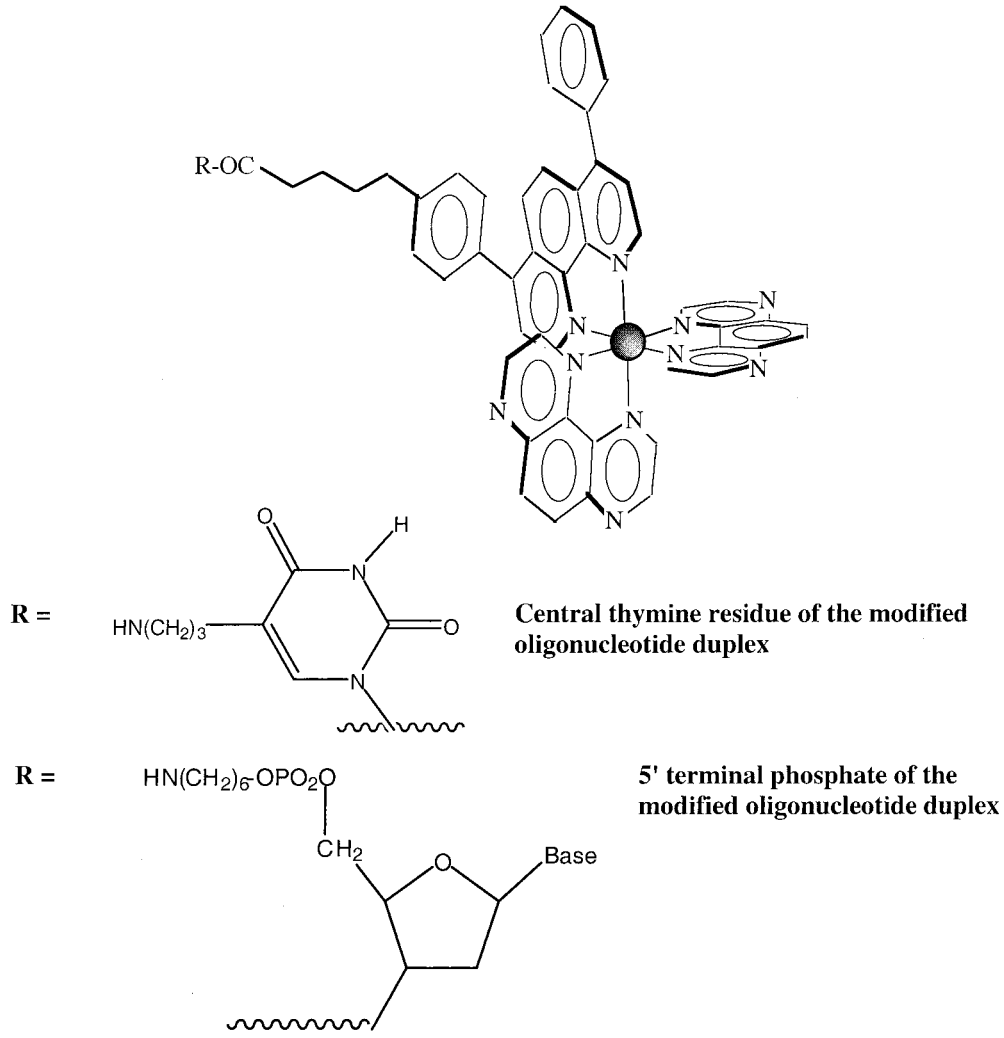

Figure 1. $[Ru(tap)_2(dip)]^{2+}$ complex and modified oligodeoxyribonucleotides with the complex attached in the middle of the strand at position 5 of a thymine and at the end of the strand at the 5′ terminal phosphate group.

throline) that was linked to different guanine containing ds-oligonucleotides.[42] From the fact that the groove binding $[Ru(TAP)_2DIP]^{2+}$ complex was not quenched by distant guanines that could not be reached directly by the complex because of the limitations imposed by the linker, it was concluded that a direct contact between the excited complex and a guanine is a necessary condition for the electron transfer. The difference in hole-injecting behavior for intercalating and nonintercalating electron acceptors should thus be attributed to the intrinsic differences of these two binding modes. The luminescence quenching of an excited Ru(II) complex that is covalently linked to guanine containing ds-oligonucleotides may thus be utilized to study sequence dependent influences on the primary process of hole injection.

Saito et al.[13−16] have demonstrated that ionization potentials estimated from Hartree−Fock (HF) calculations correlate well with the relative hole-trapping efficiencies observed in type I photooxidations of guanine containing oligonucleotides as determined by electrophoresis in a denaturing gel. It is the aim of this paper to show if a similar correlation exists between the sequence dependent IPs and the hole-injecting efficiency as determined by luminescence quenching. For this purpose, several different Ru(II) derivatized 17-mer oligonucleotides have been studied in our group. In these oligonucleotides, a $[Ru(TAP)_2DIP]^{2+}$ complex is covalently linked either to a modified thymine at the central position of the sequence or to the phosphate backbone at the 5′ end of the Ru derivatized strand (Figures 1 and 2). The oligonucleotide duplexes studied in this work differ mainly by the number of guanines and their position relative to the linkage site (Figure 2). The names of the ds-oligos in Figure 2 were chosen to be consistent with those of our previous work.[42] From this first report, sequences have been chosen for which we were able to show that the attached complex can easily reach the guanines. Additional new sequences were selected for the present study to demonstrate the influence of the IP on the quenching (Figure 2).

Recent studies[6,12−16] used base pairs arranged in a regular B-DNA structure to calculate the IP. However, it is known that in aqueous solution DNA can adopt a variety of different structures. Because of the high flexibility of the backbone, rapid changes in the local structure can be expected.[43,44] When this dynamical behavior of DNA is taken into account, it is not obvious whether a static model can be used to estimate the ionization potential. To assess the reliability of this static model, we performed ns-molecular dynamics (MD) simulations for two of our sequences containing a single GG or G step respectively and calculated the IP for several structures along the MD trajectories.

Experimental Section

The synthesis and purification of the complex $[Ru(TAP)_2(DIP)]^{2+}$, the preparation of the oligonucleotides, and the

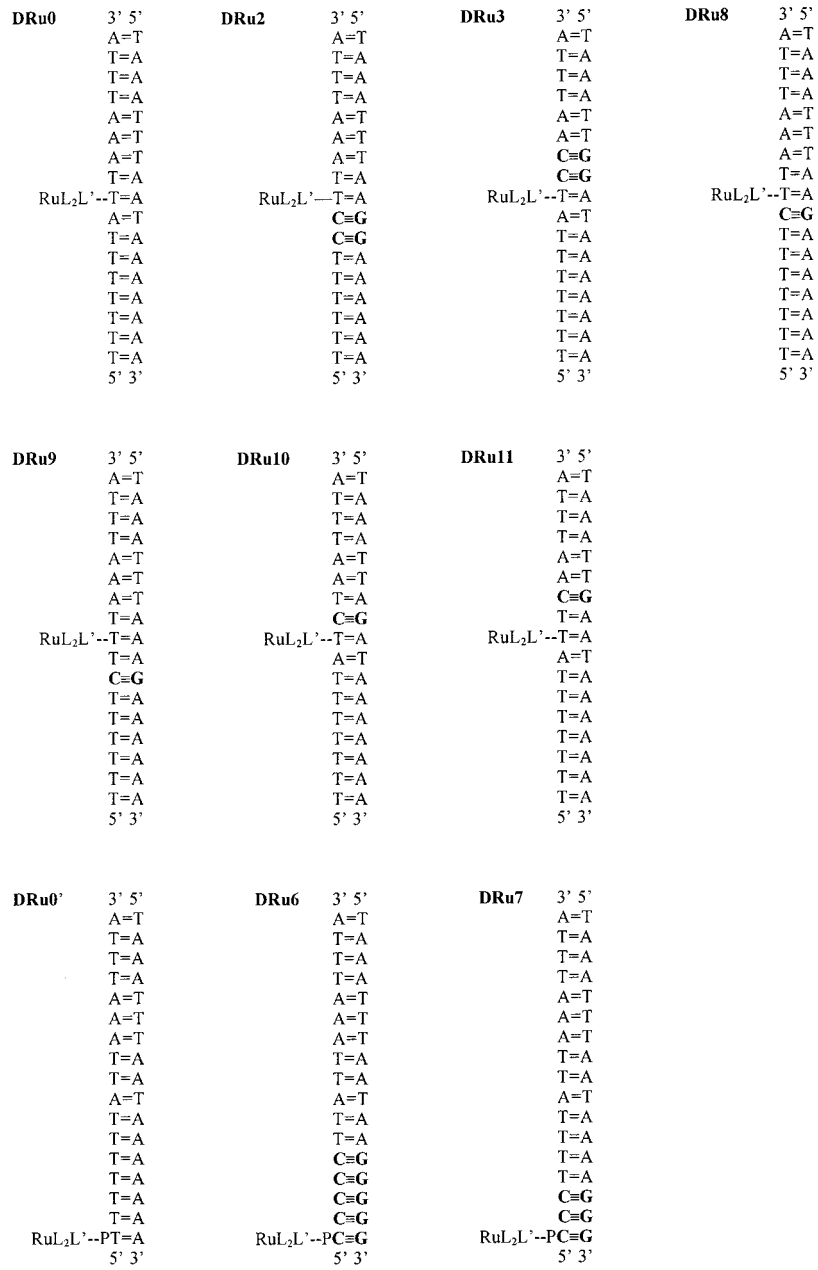

Figure 2. Different duplex sequences. $RuL_2L' = [Ru(tap)_2(dip)]^{2+}$; A, adenine, T, thymine, G, guanine, C, cytosine; P, 5′-terminal phosphate group.

coupling procedure between the oligonucleotide and the Ru(II) complex have been reported previously.[42,45]

The percentages of luminescence quenching have been calculated from the experimentally determined luminescence intensities and weighted average lifetimes with respect to sequences that do not contain a guanine, i.e., DRu0 and DRu0′, according to the type of tethering. The average lifetimes are calculated according to the following equation:

$$\tau_M = \sum(B_i\tau_i)/\sum(B_i)$$

where B_i are the corresponding preexponential factors and τ_i are the discrete lifetime components of the multiexponential fitting.

Quenching data for sequences DRu2-DRu7 have been reported in a previous work.[42] Emission lifetimes were measured by using the single-photon counting (SPC) technique with an Edinburgh Instruments FL900 spectrometer (Edinburgh, UK) equipped with a hypobaric nitrogen discharge lamp and a Hamamatsu R928 red-sensitive photomultiplier tube. The excitation wavelength was 379 nm, and the scattered light was removed with a 420 nm cutoff filter, Coherent-Ealing 26−4267 (Auburn CA). The emission monochromator was positioned at the maximum luminescence wavelength of each sample (640−650 nm). A total of 10^4 counts were collected in the peak channel. The temperature of the cell holder was thermostated at 25.0 ± 2.0 °C with a Haake NB22 temperature controller (Berlin, Germany). Emission profiles were analyzed with deconvolution of the instrumental response by using the original Edinburgh Instruments software. The decays were fitted from the peak channel to the baseline of the experimental decay. An increasing number of exponentials was used until the fit was statistically acceptable as judged by the χ^2 test (value near 1), the appearance of the weighted residuals plot, the value of the Durbin-Watson parameter, the % of weighted residuals < 3 standard deviations, and the autocorrelation plot. Because the

TABLE 1: Experimentally Determined Percentage of Quenching (%Q) for Sequences DRu2−DRu11 and Ionization Potentials Calculated for 6-mer Model Systems

seq.	model seq.	%Q	calc. IP [eV] B-DNA HF/3-21G(d)	calc. IP [eV] B-DNA HF/6-31G(d)
DRu6	5′-CCCCCT-3′ 3′-GGGGGA-5′	87%[a]	6.03	6.17
DRu7	5′-CCCTTT-3′ 3′-GGGAAA-5′	81%[a]	6.17	6.26
DRu2	5′-TTCCTT-3′ 3′-AAGGAA-5′	60%[a]	6.25	6.32
DRu3	5′-ATCCAA-3′ 3′-TAGGTT-5′	49%[a]	6.34	6.42
DRu8	5′-TTTCTT-5′ 3′-AAAGAA-5′	38%	6.50	6.55
DRu9	same as Ru8	30%	6.50	6.55
DRu10	5′-TATCTA-3′ 3′-ATAGAT-5′	31%	6.54	6.60
DRu11	5′-ATTCAA-3′ 3′-TAAGTT-5′	23%	6.60	6.65

[a] Taken from ref 42.

values for the ratios of intensities and weighted average lifetimes were the same, static quenching was excluded. The resulting percentage of quenching (%Q) is given in Table 1.

Computational Methods

All HF calculations mentioned in this work were performed with Gaussian 98,[46] employing the standard 3-21G and 6-31G-(d) basis sets. The IPs were estimated from the energy of the HOMOs (Koopmans theorem). Because HF calculations on 17-mer ds-oligonucleotides are beyond the possibilities of our computational resources, the calculations were performed on 6-mer model sequences centered on the guanines (Table 1). The stacked base pairs were arranged in a regular B-DNA structure (rise, 3.38 Å; twist, 36°; tilt, 1°) using JUMNA,[47] and the backbone was substituted by methyl groups using MOLDEN.[48] The geometry of the nucleobases and methyl groups was taken as provided by JUMNA and MOLDEN, respectively.

The MD simulations were done starting with the canonical B-DNA structures of sequences DRu2 and DRu9 optimized using JUMNA and were carried out using CHARMM program.[49] These two sequences were chosen as model systems for oligonucleotides that contain a stack of two guanines and a single guanine, respectively. The CHARMM 27 all-hydrogen parameters for nucleic acids[50] was used to calculate the interactions between the ds-oligonucleotides and the solvent. Bonds connecting hydrogens were constrained using the SHAKE[51] algorithm which permitted the use of an integration time step of 2 fs, employed in a leapfrog integration scheme. The simulation consisted of a system defined by the oligonucleotide plus about 3000 water molecules in a periodic volume with dimensions (70/36/36 Å). Thirty-two sodium counterions were added yielding a neutral electrical system. Long-range interactions were treated with the particle mesh Ewald method.[52]

The systems were subjected to 40 ps of dynamics at increasing temperature up to 300 K with oligonucleotide atoms subjected to 5 kcal mol^{-1} $Å^{-1}$ harmonic constraints to relax the solvent and the counterions. Then the whole system was slowly brought up to 300 K and equilibrated for 500 ps, after which 2 and 1.5 ns of production run was carried out, for DRu2 and DRu9, respectively, under constant number, pressure, and temperature (NPT).

We used CURVES[53] to analyze the MD trajectories on conformations collected every 50 ps. The HF calculations were performed on 6-mer subsystems using the stacking geometries as obtained from the MD simulation. Because the internal structural parameters for the nucleobases (i.e., bond length, bond angles, and dihedrals) are different in the sampled MD conformations and JUMNA, CURVES was used to fit the nucleobase structures from the JUMNA library to the stacking geometry obtained from the MD simulation. By this procedure, we could make sure that the same bond lengths and angles were used in the IP calculations on regular B-DNA and the MD conformations.

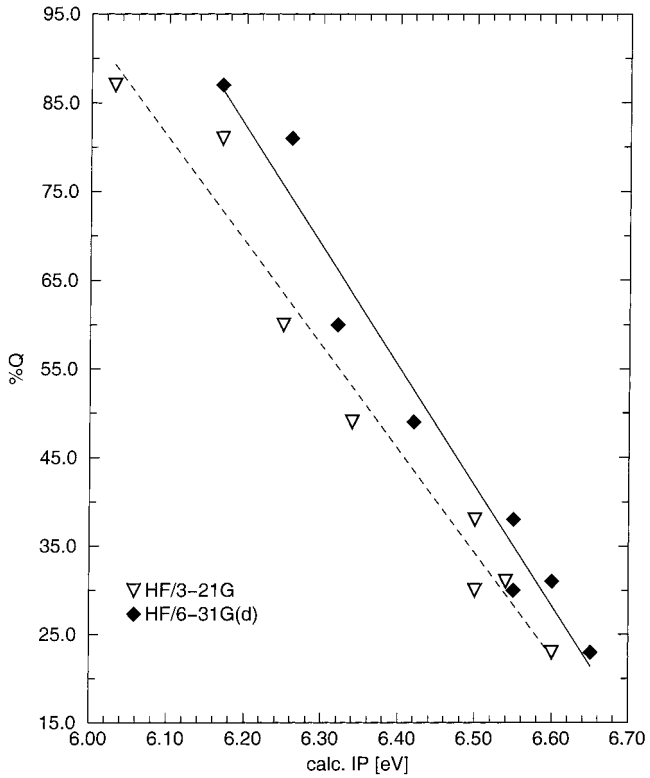

Figure 3. Plot of the experimentally determined percentage of quenching (%Q) versus the calculated ionization potentials (IP). The %Q and IP values for the different sequences are listed in Table 1. Correlation coefficients of −0.9834 and −0.9825 were determined at the HF/3-21G and HF/6-31G(d) level of theory, respectively.

Results and Discussion

The photoelectron transfer from a guanine residue of the respective oligonucleotide duplex to the $[Ru(TAP)_2DIP]^{2+}$ complex may depend mainly on its exergonicity. In such a case, a strong influence of the IP of the donor on the luminescence quenching is expected.

The experimentally determined percentage of quenching (%Q) for the nine different Ru(II) derivatized ds-oligonucleotides (Figure 2) is compared to the calculated IPs in Table 1. A plot of %Q versus the calculated IPs is shown in Figure 3. The percentage of quenching rises linearly with decreasing IP in this plot. Correlation coefficients of −0.9834 and −0.9825 were determined at the HF/3-21G and HF/6-31G(d) level of theory, respectively. These results indicate a strong influence of the IP on the efficiency of the direct electron transfer from a guanine residue to the excited groove binding $[Ru(TAP)_2DIP]^{2+}$ complex. The correlation in Figure 3 indicates also that for these small oligonucleotides sequences with a stack of several guanines the IP seems to play a more important role than the number of G's (statistical factor). This implies that the stacks of guanines reachable by the complex behave like a single

reactive center and not like individual guanine molecules. Nevertheless, it should be noted that a different %Q was found for sequence DRu8 and DRu9 even though their IPs are identical because their 6-mer model system is the same. This discrepancy can be explained by a different accessibility of the guanine in sequence DRu8 and DRu9 because of the sterical hindrance imposed by the linker. The lower %Q was observed for DRu9, where the guanine is further away from the linkage site than in DRu8. Thus, in the systems studied, the accessibility of a certain guanine might decrease with the distance from the linkage site.

It has been demonstrated by Sugiyama et al.[13] that the ionization potential of guanines in DNA is not only sequence dependent, but it also depends on the secondary structure and especially on the relative orientation of the adjacent base pairs. Because of the high flexibility of the backbone, rapid changes in the local structure of DNA in aqueous solution can be expected. Thus, it is not obvious that a static model can be used to estimate the ionization potential. To assess the reliability of this static model we studied the influence of the dynamical behavior of DNA on the calculated IPs. This is indeed needed because the electron-transfer rate for our systems has been estimated to be in the ns time range,[42] which is rather slow compared to the picosecond time scale observed for intercalating donor/acceptor pairs.[29] Starting from a B-DNA like structure, we thus performed 2 and 1.5 ns molecular dynamics simulations for sequence DRu2 and DRu9, respectively, including explicit water molecules and counterions. The analysis of these trajectories revealed that no transitions to a bent or otherwise distorted structure take place within the time scale considered. The fluctuations in the most important internal structural parameters such as twist, roll, slide, and sugar puckering were all within the range that could be expected for B-DNA.[54−56] It is especially important to note that even for the GG step in DRu2 no transition to a local A-DNA structure could be observed in this simulation. IPs were determined at the HF/3-21G level of theory for the 6-mer model systems given in Table 1 using stacking geometries taken every 50 ps from the trajectory. The fluctuations of the estimated IPs along the trajectories are depicted in Figures 4 and 5. The difference between the highest and lowest IP is 0.5 eV; however, the values scatter statistically around an average value of 6.23 and 6.46 eV for DRu2 and DRu9, respectively. These values are nearly identical to the value of 6.25 and 6.50 eV determined for a regular B-DNA structure. No tendency toward a permanently distorted structure with a lower or higher IP was observed.

Because the luminescence lifetime of the attached $[Ru(TAP)_2DIP]^{2+}$ complex is of the order of several 100 ns and the rate for electron transfer is rather slow (nanoseconds),[42] it seems in this case to be valid to take the values calculated for regular B-DNA as a measure for the average IP calculated along the trajectories, which can be viewed as an effective IP for the photoreaction. Because the 17-mers in our study differ only by a few base pairs, one may assume that a similar dynamic behavior would be observed for the other sequences as well. However, it should be noted that the IP fluctuations observed in our simulations might be important for the electron transfer between intercalated donor/acceptor pairs, which usually takes place in the picosecond timerange.[29]

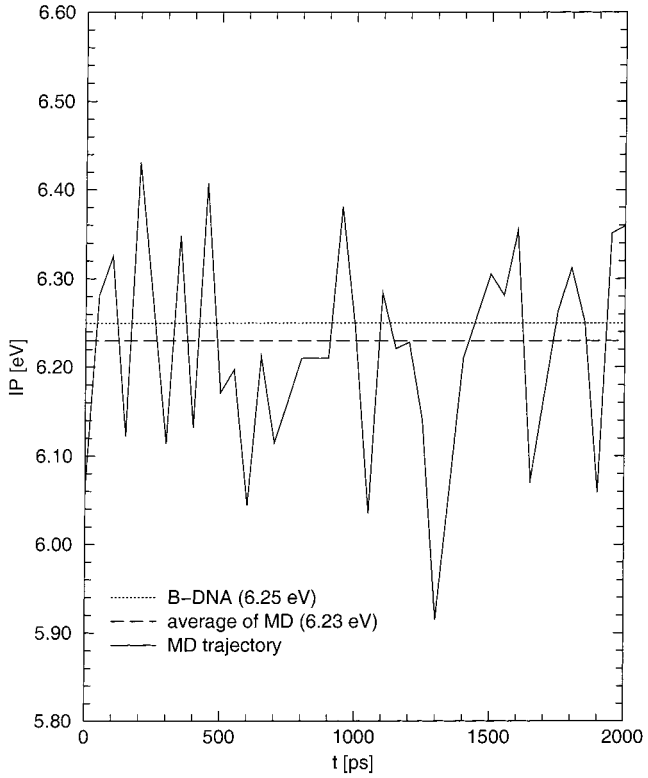

Figure 4. Change in the ionization potential of DRu2 conformations sampled along a 2 ns molecular dynamics trajectory. Sequence DRu2 contains a single GG stack. The IPs are calculated at the HF/3-21G level of theory.

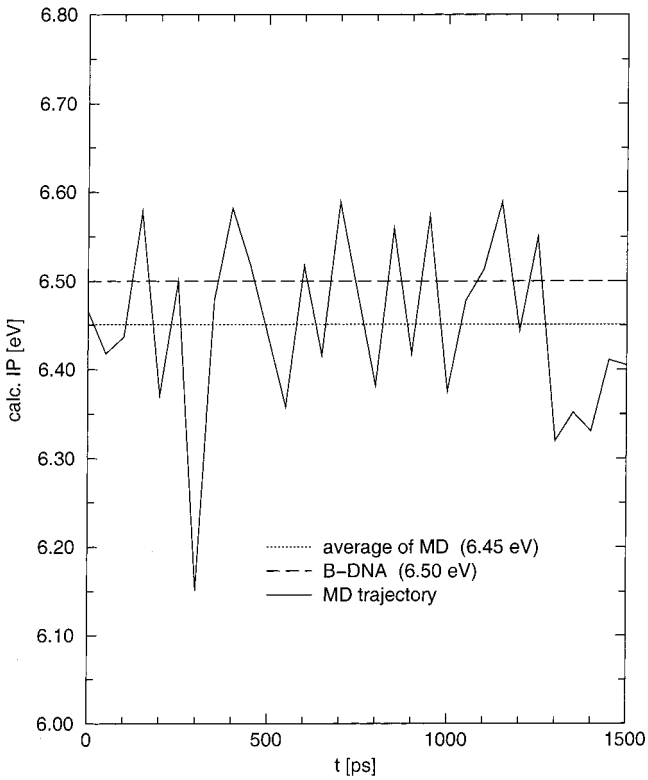

Figure 5. Change in the ionization potential of DRu9 conformations sampled along a 1.5 ns molecular dynamics trajectory. Sequence DRu9 contains a single guanine. The IPs are calculated at the HF/3-21G level of theory.

Conclusions

Our results suggest that the sequence dependent ionization potential of guanine residues in DNA has a major influence on the efficiency of hole injection in a type I photooxidation, provided that the electron transfer takes place directly between the guanine and a nonintercalating complex as photooxidizing agent. Furthermore, it has been demonstrated that the high flexibility of DNA in aqueous solution induces rapid changes

in the local structure of stacked base pairs which can lead to large differences in the ionization potential. However, these fast IP fluctuations do not have a measurable influence on the observed quenching, because of the slow electron transfer and the long excited-state lifetime of $[Ru(TAP)_2DIP]^{2+}$. Thus, for the systems used in this study, B-DNA seems to be a valid model to estimate the effective IP for the photooxidation reaction.

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References and Notes

(1) Burrows, J. C.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109.
(2) Pogozelski, W. K.; Tullius, T. D.*Chem. Rev.* **1998**, *98*, 1089.
(3) Armitage, B. *Chem. Rev.* **1998**, *98*, 1171.
(4) Orlov, M. V.; Smirnov, A. N.; Varshavsky, Y. M. *Tetrahedron Lett.* **1976**, *48*, 4377.
(5) Hutter, M.; Clark, T. *J. Am. Chem. Soc.* **1996**, *118*, 7574.
(6) Voityuk, A. A.; Jortner, J.; Bixon, M.; Rösch, N. *Chem. Phys. Lett.* **2000**, *324*, 430.
(7) Steenken, S.; Jovanovic, S. *J. Am. Chem. Soc.* **1997**, *11*, 6617.
(8) Russo, N.; Toscano, M.; Grand, A. *J. Comput. Chem.* **2000**, *21*, 1243.
(9) Kim, N. S.; LeBreton, P. R. *J. Am. Chem. Soc.* **1996**, *118*, 3694.
(10) Fernando, H.; Papadantonakis, G. A.; Kim, N. S.; LeBreton, P. R. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5550.
(11) Kim, N. S.; Zhu, Q.; LeBreton, P. R. *J. Am. Chem. Soc.* **1999**, *121*, 11516.
(12) Prat, F.; Houk, K. N.; Foote, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 845.
(13) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063.
(14) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatami, K. *J. Am. Chem. Soc.* **1995**, *117*, 6406.
(15) Saito, I.; Nakamura, T.; Nakatami, K.; Yoshioka, Y.; Yamaguchi, K.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 12686.
(16) Saito, I.; Nakamura, T.; Nakatami, K. *J. Am. Chem. Soc.* **2000**, *122*, 3001.
(17) Saito, I.; Takayama, M.; Shosuke, K. *J. Am. Chem. Soc.* **1995**, *117*, 5590.
(18) Melvin, T.; Plumb, M. A.; Botchway, S. W.; O'Neill, P.; Parker, A. W. *Photochem. Photobiol.* **1995**, *61*, 584.
(19) Ly, D.; Kan, Y.; Armitage, B.; Schuster, G. B. *J. Am. Chem. Soc.* **1996**, *118*, 8747.
(20) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731.
(21) Breslin, D. T.; Schuster, G. B. *J. Am. Chem. Soc.* **1996**, *118*, 2311.
(22) Gicquel, E.; Paillous, N.; Vicendo, P. *Photochem. Photobiol.* **2000**, *75*, 583.
(23) Moucheron, C.; Kirsch-De Mesmaeker, A.; Kelly, J. M. *J. Photochem. Photobiol. B: Biol.* **1997**, *40*, 91.
(24) Lecomte, J.-P.; Kirsch-De Mesmaeker, A.; Feeny, M. M.; Kelly, J. M. *Inorg. Chem.* **1995**, *34*, 6481.
(25) Lecomte, J.-P.; Kirsch-De Mesmaeker, A.; Kelly, J.; Tossi, A.; Gorner, H. *Photochem. Photobiol.* **1992**, *55*, 681.
(26) Lecomte, J.-P.; Kirsch-De Mesmaeker A.; Orellana, G. *J. Phys. Chem.* **1994**, *98*, 5382.
(27) Moucheron, C.; Kirsch-De Mesmaeker, A.; Kelly, J. M. *Struct. Bonding* **1998**, *92*, 163.
(28) Hall, D. B.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 5045.
(29) Wan, C.; Fiebig, T.; Kelly, S. O.; Treadway, C. R.; Barton, J. K.; Zewail, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6014.
(30) Fukui, K.; Tanaka, K.; Fujitsuka, M.; Watanabe, A.; Ito, O.; *J. Photochem. Photobiol. B: Biol.* **1999**, *50*, 18.
(31) Foote, C. S. *Photochem. Photobiol.* **1991**, *54*, 659.
(32) Devasagayam, T. P. A.; Steenken, S.; Obendorf, M. S. W.; Schulz, W. A.; Sies, H. *Biochemistry* **1991**, *30*, 6283.
(33) Grinstaff, M. W. *Angew. Chem., Int. Ed.* **1999**, *38*, 3629.
(34) Armitage, B.; Ly, D.; Koch, T.; Frydenlund, H.; Orum, H.; Batz, H. G.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12320.
(35) Giese, B.; Spichty, M. *ChemPhysChem* **2000**, *1*, 195.
(36) Giese, B.; Wessely, S.; Spormann, M.; Lindemann, U.; Meggers, E.; Michel-Beyerle, M. E. *Angew. Chem., Int. Ed.* **1999**, *38*, 996.
(37) Meggers, E.; Michel-Beyerle, M. E.; Giese, B. *J. Am. Chem. Soc.* **1998**, *120*, 12950.
(38) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631.
(39) Bixon, M.; Giese, B.; Wessely, S.; Langenbacher, T.; Michel-Beyerle, M. E.; Jortner, J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11713.
(40) Gaspar, S. M.; Schuster, G. B. *J. Am. Chem. Soc.* **1997**, *119*, 12762.
(41) Fiebig, T.; Wan, C.; Kelly, S. O.; Barton, J. K.; Zewail, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1187.
(42) García-Fresnadillo, D.; Boutonnet, N.; Schumm, S.; Moucheron, C.; Kirsch-De Mesmaeker, A.; Defrancq, E.; Constant, J.-F.; Lhomme J. *Biophys. J.* In press.
(43) Chetham, T. E., III; Kollman, P. A. *Annu. Rev. Phys. Chem.* **2000**, *51*, 435.
(44) Robinson, B. H.; Mailer, C.; Drobny, G. *Annu. Rev. Biophys. Biomol. Struct.* **1997**, *26*, 629.
(45) Ortmans, I.; Content, S.; Boutonnet, N.; Kirsch-De Mesmaeker, A.; Bannwarth, W.; Constant, J.-F.; Defrancq, E.; Lhomme, J. *Chem. Eur. J.* **1999**, *5*, 2712.
(46) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.7; Gaussian, Inc.: Pittsburgh, PA, 1998.
(47) Lavery, R.; Zakrzewska, K.; H. Sklenar, H. *Comput. Phys. Commun.* **1995**, *91*, 135.
(48) Schaftenaar, G.; Noordik, H. J. *J. Comput.-Aided Mol. Design* **2000**, *14*, 123.
(49) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.
(50) Foloppe, N.; MacKerell, A. D., Jr. *J. Comput. Chem.* **2000**, *21*, 86.
(51) Ryckaert, J.-P.; Cicotti, G.; Berendsen, H. J. *J. Comput. Phys.* **1977**, *23*, 327.
(52) Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G. *J. Chem. Phys.* **1995**, *103*, 8577.
(53) Lavery, R.; Sklenar, H. *Curves*, version 5.1; Laboratoire de Biochimie Théorique: Paris, France, 1997.
(54) Calladine, C. R.; Drew, H. R. *Understanding DNA*, 2nd ed.; Academic Press: San Diego, 1997.
(55) Sinden, R. R, *DNA Structure and Function*; Academic Press: San Diego, 1994.
(56) Hunter, C. A.; Lu, X.-J. *J. Mol. Biol.* **1997**, *265*, 603.