Theoretical Studies of GG-Specific Photocleavage of DNA via Electron Transfer: Significant Lowering of Ionization Potential and 5′-Localization of HOMO of Stacked GG Bases in B-Form DNA

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Abstract: *Ab initio* molecular orbital calculations of stacked DNA bases were performed at the 3-21G(*) and 6-31G* levels to elucidate the origin of the 5′-GG-3′ sequence specificity for the photocleavage of DNA in the presence of electron-accepting photosensitizers. Ionization potentials (IP) were estimated as Koopman's theorem values for 16 sets of two stacked nucleobases and seven sets of stacked nucleobase pair systems in a B-form geometry. It was found that the GG/CC system is the lowest among the 10 possible stacked nucleobase pairs and that approximately 70% of the HOMO is localized on the 5′-G of 5′-GG-3′. These calculations indicate that the 5′-G of 5′-GG-3′ is the most electron donating site in B DNA and suggest that one-electron transfer from DNA to an electron acceptor occurs most effectively at 5′-GG-3′ sites which are fully consistent with the experimental data. In order to know the fate of the cation radical, the vertical IPs were estimated for seven stacked nucleobase pairs. It was found that the GG/CC system possesses the smallest vertical IP and that the cation radical is localized on the 5′-G of 5′-GG-3′. These results imply that the 5′-G of 5′-GG-3′ is a sink in "hole" migration through DNA, *i.e.*, an electron-loss center created in a B-form DNA will end up predominantly on the 5′-G of 5′-GG-3′, and suggest that not only the base specificity for initial photoionization but also subsequent energetically favored hole migration to the lowest 5′-GG-3′ site are the origin of the 5′-GG-3′ specific cleavage. Calculations of stacked GGs with various geometries including orientations of A- and Z-form DNA were also examined.

Introduction

Considerable interest has recently arisen in the one-electron oxidations occurring in DNA in connection with DNA damage caused by ionizing radiation, oxidizing agents, and photoirradiation using endogenous photosensitizers.[1] The site of oxidative damage along the DNA strand has been extensively investigated at the base sequence level using high-resolution polyacrylamide gel electrophoresis. Recently, our laboratories have demonstrated that photocleaving amino acid (PCA, **1**) selectively generates piperidine-sensitive alkaline-labile sites at the 5′-guanine (G) of the 5′-GG-3′ sequence with a lower frequency at the 5′-G of the 5′-GA-3′ sequence upon 366 nm photoirradiation.[2] A similar 5′-GG-3′ specificity for the formation of alkaline-labile sites has been observed for photoirradiation in the presence of various types of photocleaving molecules shown in Figure 1, including nitroaromatic compound **2**,[3] $Co(NH_3)_6^{3+}$,[3,4] naphthalimide **3**,[5] riboflavin **4**,[6] benzophenone derivative **5**,[7] and anthraquinone derivative **6**,[8] as well as for the direct irradiation of DNA with a 193 nm excimer laser.[9]

The common pattern of these 5′-GG-3′ specific DNA cleavages strongly suggests that such specificity is not determined by the binding orientation of the photocleaving molecules but must originate from a common intrinsic chemical property of DNA itself, which has not been well recognized. We have recently reported direct evidence for one-electron transfer from a guanine base in duplex DNA to triplet excited PCA by time-resolved laser flash photolysis and demonstrated that the 5′-G of the 5′-GG-3′ sequence is the most readily oxidizable site in B-form DNA.[2b] Recent investigations suggest that alkaline-labile sites which are cleaved upon piperidine treatment of one-electron-oxidized DNA are 2,2-diaminooxazolone or its precursor 2-aminoimidazol-4-one.[10] Since the guanine base is known to have the lowest ionization potential (IP) among the four DNA nucleobases,[11] the stacking interaction of two consecutive guanine bases would create a site having an extremely low IP in duplex DNA. In this study we have performed high-level *ab initio* molecular orbital calculations of stacked DNA bases with various orientations in order to

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(1) (a) For reviews, see: Cadet, J.; Vigny, P. *Bioorganic Photochemistry: Photochemistry and the Nucleic Acids*; Morrison, H., Ed.; John Wiley and Sons: New York, 1990; Vol. 1, pp 1−272. (b) Bernhard, W. A. *Adv. Radiat. Biol.* **1981**, *9*, 199. (c) von Sontag, C.; Schuchmann, H.-P. *Int. J. Radiat. Biol.* **1986**, *49*, 1. (d) Steenken, S. *Chem. Rev.* **1989**, *89*, 503. (e) Johnston, D. H.; Glasgow, K. C.; Thorp, H. H. *J. Am. Chem. Soc.* **1995**, *117*, 8933.

(2) (a) Saito, I.; Takayama, M.; Kawanishi, S. *J. Am. Chem. Soc.* **1995**, *117*, 5590. (b) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K.; Tsuchida, A.; Yamamoto, M. *J. Am. Chem. Soc.* **1995**, *117*, 6406.

(3) Iverson, B. L. Ph.D. Thesis, California Institute of Technology, 1988.

(4) Fleisher, M. B.; Mei, H.-Y.; Barton, J. K. *Nucleic Acids and Molecular Biology*; Eckstein, F., Lilley, M. J., Eds.; Springer-Verlag: Berlin, 1988; Vol. 2, pp 65−84.

(5) (a) Matsugo, S.; Kawanishi, S.; Yamamoto, K.; Sugiyama, H.; Matsuura, T.; Saito, I. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1351. (b) Saito, I. *Pure Appl. Chem.* **1992**, *64*, 1305.

(6) Ito, K.; Inoue, S.; Yamamoto, K.; Kawanishi, S. *J. Biol. Chem.* **1993**, *268*, 13221.

(7) Takayama, M. Ph.D. Thesis, Kyoto University, 1995.

(8) Breslin, D. T.; Schuster, G. B. *J. Am. Chem. Soc.* **1996**, *118*, 2311.

(9) Melvin, T.; Plumb, M. A.; Botchway, S. W.; O'Neill, P.; Parker, A. W. *Photochem. Photobiol.* **1995**, *61*, 584.

(10) (a) Cadet, J.; Berger, M.; Buchko, G. W.; Joshi, P. C.; Raoul, S.; Ravanat, J.-L. *J. Am. Chem. Soc.* **1994**, *116*, 7403. (b) Ravanat, J.-L.; Berger, M.; Buchko, G. W.; Joshi, P. C.; Morin, B.; Weinfeld, M.; Cadet, J. *J. Chem. Soc., Perkin Trans. 2* **1996**, 371. (c) Cullis, P. M.; Malone, M. E.; Merson-Davies, L. A. *J. Am. Chem. Soc.* **1996**, *118*, 2775.

(11) (a) Orlov, V. M.; Smirnov, A. N.; Varshavsky, Y. M. *Tetrahedron Lett.* **1976**, *48*, 4377. (b) Jovanovic, S. V.; Simic, M. G. *J. Phys. Chem.* **1986**, *90*, 974.

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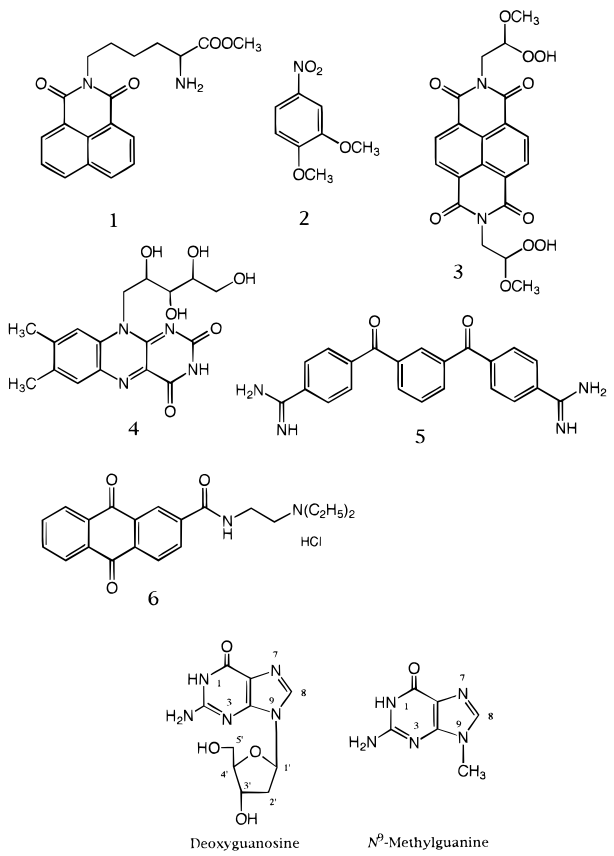

Figure 1. Compounds which induce 5′-GG-3′ specific cleavage by photoirradiation and piperidine treatment.

elucidate the origin of the remarkable 5′-GG-3′ specificity for photocleavage. Present calculations indicate that stacking of two guanine bases significantly lowers the IP and that the HOMO of the stacked 5′-GG-3′ is localized mainly on the 5′-G in B-form DNA. The present theoretical studies would explain why 5′-GG-3′ specific photocleavage occurs in B-form DNA by one-electron oxidation.[2b] The present results also suggest that the 5′-side G of contiguous guanines ($-G_n-$) is extremely important in HOMO−LUMO interactions of B-form DNA with electron-accepting molecules.

Results and Discussion

1. IPs of Stacked DNA Bases. Although theoretical calculations of DNA bases have been extensively investigated,[12] no *ab initio* calculation of stacked nucleobases has been reported. Recently, Colson *et al*. performed *ab initio* calculations of monomeric DNA nucleobases and reported that the Koopman theorem values,[13] the HOMO energies of the molecules, calculated at the 3-21G(*) and 6-31G* levels, are well correlated to the experimental vertical IPs.[14] We thus examined estimating the IPs of stacked DNA bases from the HOMO energies of stacked N-methylated nucleobases as a model by means of high-level *ab initio* calculations. The atomic coordinates and the stacked geometrical orientations of nucleobases are taken from standard parameters for DNAs which have been optimized by X-ray crystallographic analysis of relevant monomers and X-ray diffraction data of the polymers.[15] In order to maintain a stacked geometry, all calculations were performed without geometry optimization. Since the IP values obtained for four monomeric N-methylated nucleobases (Table 1) at *ab initio* HF/3-21G(*) and HF/6-31G* levels were in good agreement with the experimental IP values,[11a,16] as well as with the previously reported *ab initio* calculation data[14] using geometry optimization, single-point calculations for stacked nucleobase systems would be considered to be sufficient to provide reasonable and acceptable IP values.

Table 1. Ionization Potential of N-Methylated Nucleobase Monomers and Stacked Nucleobases (eV)[a]

	3′-base			
5′-base	G	A	C	T
	7.75	8.24	8.87	9.14
G	7.28	7.51	7.68	7.69
A	7.51	7.97	8.20	8.19
C	7.24	7.75	8.36	8.69
T	7.67	8.15	8.79	8.97

[a] Ionization potentials were estimated by Koopmans' theorem. The values are the HOMO energies of 6-31G* single-point calculations. G = 9-methylguanine, A = 9-methyladenine, C = 1-methylcytosine, T = 1-methylthymine.

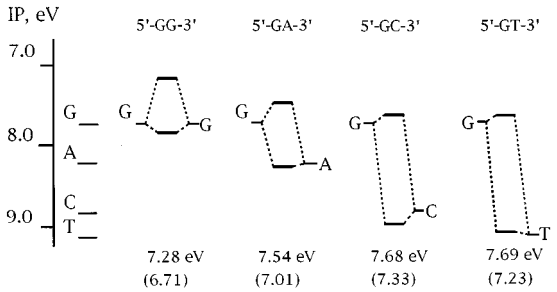

Figure 2. Energy levels of four sets of stacked nucleobases, GG, GA, GC, and GT, showing the effect of stacking interaction on the Koopmans IPs. The values in parentheses are IP values obtained for stacked base paired dinucleotides (Table 3).

IPs of four DNA base monomers and 16 sets of nearest neighbor stacked nucleobases in a B-form geometry were calculated at the 6-31G* level (Table 1). It was revealed that, in all cases, the HOMO is predominantly localized on the base of lowest IP. When the same two bases are stacked on each other in a B-form geometry, the HOMO is localized on the base at the 5′ side. In some cases, significant lowering of IPs (0.5−0.2 eV) due to the stacking interaction compared to those for unstacked N-methylated bases has been observed. Figure 2 shows the energy levels of four sets of stacked nucleobases, GG, GA, GC, and GT, showing the effect of stacking interaction on the Koopman's IPs. These results demonstrated that the interaction between the two HOMOs induces an energy gap between stabilized and unstabilized orbitals to result in a significant lowering of IPs as depicted in Figure 2.

This type of IP lowering by stacking interaction was further confirmed for three and four consecutive stacked nucleobases, in which IPs gradually dropped with increasing number (n) of stacked $-G_n-$. Table 2 summarizes the calculated IPs for the stacked contiguous $-G_n-$ at the 6-31G* level. In accord with this theoretical result, efficient DNA cleavage was observed at the 5′-GGG-3′ sequence compared to that expected from a simple assumption of the cleavage for two GGs.[2b] Interestingly,

(12) (a) Scanlan, M. J.; Hillier, I. H. *J. Am. Chem. Soc*. **1984**, *106*, 3737. Aida, M.; Yamane, K.; Nagata, C. *Mutat. Res*. **1986**, *173*, 49. (b) Cieplak, P.; Bash, P.; Singh, U. C.; Koolman, P. A. *J. Am. Chem. Soc*. **1987**, *109*, 6283. (c) Colson, A.-D.; Basler, B.; Sevilla, M. D. *J. Phys. Chem*. **1993**, *97*, 13852. (d) Oyler, N.; Adamowicz, L. *Chem. Phys. Lett*. **1994**, *219*, 223. (e) Sponer, J.; Hobza, P. *J. Am. Chem. Soc*. **1994**, *116*, 709. (f) Sevilla, M. D.; Basler, B.; Colson, A.-O. *J. Phys. Chem*. **1995**, *99*, 1060.

(13) Koopmans, T. A. *Physica* **1933**, *1*, 104.

(14) Colson, A.-O.; Besler, B.; Colse, D. M.; Sevilla, M. D. *J. Phys. Chem*. **1992**, *96*, 661.

(15) (a) Arnott, S.; Hukins, D. W. L. *Biochem. Biophys. Res. Commun*. **1972**, *47*, 1504. (b) Arnott, S.; Selsing, E. *J. Mol. Biol*. **1974**, *88*, 509.

(16) Hush, N. S.; Cheung, A. S. *Chem. Phys. Lett*. **1975**, *34*, 11.

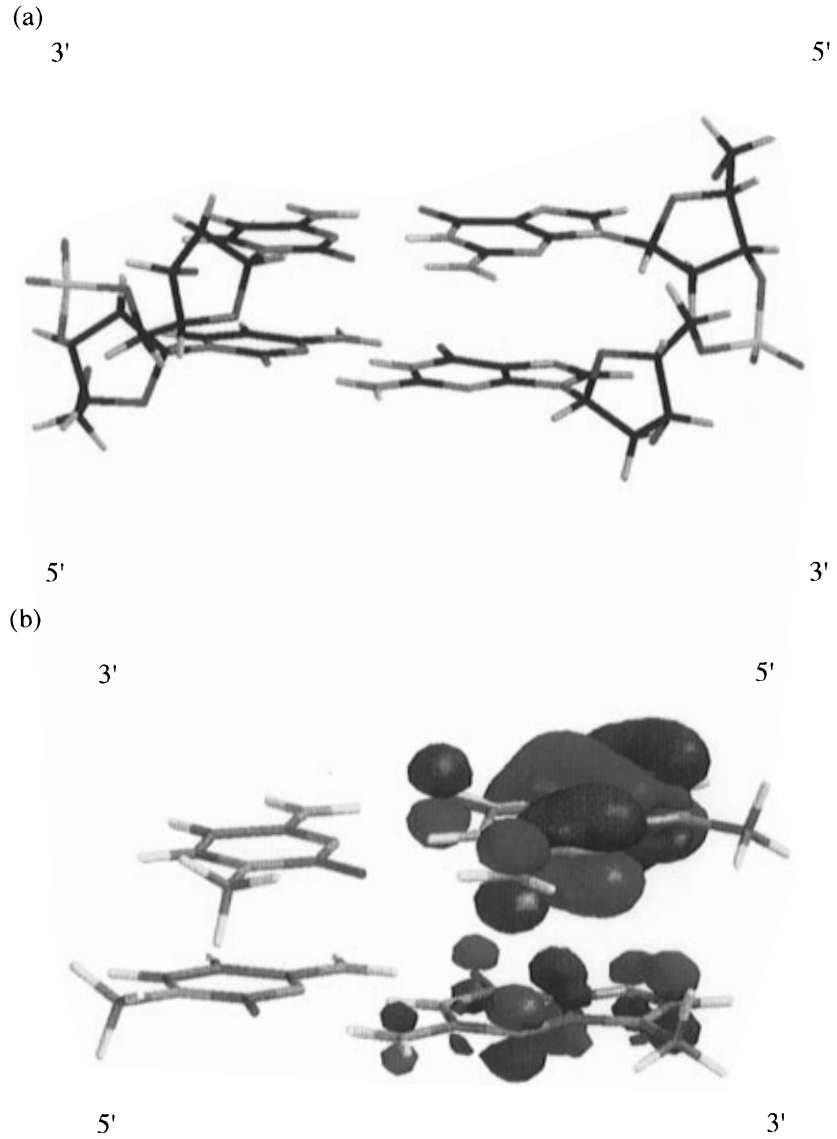

Figure 3. (a) Model of the 5′-GG-3′ sequence of B-form DNA and (b) HOMO of the stacked N-methylated GG/CC system calculated at the 3-21G(*) level.

Table 2. Ionization Potentials of Stacked Contiguous Guanines[a]

base	IP (eV)
G	7.75
GG	7.28 (0.47)[b]
GGG	7.07 (0.68)
GGGG	6.98 (0.77)

[a] Ionization potentials were estimated by Koopmans' theorem. The values are the HOMO energies of 6-31G* single-point calculations. [b] The value in parentheses is the difference from the IP of nonstacked guanine.

an analogous enhancement of the reactivity of consecutive $-G_n-$ is observed for guanine N7 alkylation of DNA by cationic alkylating agents.[17] Pullman and Pullman showed by *ab initio* SCF calculation that the 5′-GG-3′ sequence has the largest molecular electrostatic potential among the stacked nucleobases.[18]

The calculated IPs of the stacked nucleobases are in the following order: 5′-CG-3′ (7.24 eV) < 5′-GG-3′ (7.28 eV) < 5′-GA-3′ (7.51 eV) = 5′-AG-3′ (7.51 eV). Some of these calculated IP values are in agreement with the experimentally observed reactivities of 5′-GG-3′ and 5′-GA-3′.[2] However, the IP values for 5′-CG-3′ and 5′-AG-3′ are not in agreement with the experimental data, *i.e.*, 5′-AG-3′ is not a major cleavage site like 5′-GG-3′ or 5′-GA-3′, and almost no cleavage is observed at 5′-CG-3′.[2a] We assumed that such a partial

Table 3. Ionization Potentials[a] of Stacked Base Paired Dinucleotides

dinucleotide base pairs	IP (eV) 3-21G(*)	6-31G*	vertical 3-21G(*)[b]
(GG)/(CC)	6.71 (0.63)[c]	6.73	5.41
(CG)/(CG)	7.00 (0.34)	7.00	5.67
(GA)/(TC)	7.01 (0.33)	7.00	5.82
(AG)/(CT)	7.06 (0.28)	7.06	5.92
(TG)/(CA)	7.12 (0.22)	7.12	5.68
(GT)/(AC)	7.23 (0.11)	7.23	6.19
(GC)/(GC)	7.33 (0.01)	7.31	5.65
(G)/(C)	7.34	7.34	6.05
(A)/(T)	8.10	7.99	6.62

[a] Ionization potentials were estimated by Koopmans' theorem. The values are the HOMO energies of 6-31* and 3-21G(*) single-point calculations. [b] The vertical IPs were calculated by computing the difference in energies between the neutral base pair system and the cation radical with the same geometries as those for the neutral system. [c] The value in parentheses is the difference from the IP of (G)/(C).

(17) Hartley, J. A. *Molecular Basis of Specificity in Nucleic Acid-Drug Interaction*; Pullman, B., Jortner, J., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1990; pp 513−530.

(18) Pullman, A.; Pullman, B. *Q. Rev. Biophys.* **1981**, *14*, 289.

discrepancy between the calculated IPs and the experimental data is probably due to the lack of hydrogen bonding with the complementary base in our stacked nucleobase models. Therefore, we next examined the calculations of the stacked base pair systems. Calculated energies of seven stacked base pairs together with 5′-GC-3′ and 5′-AT-3′ base pairs at the 6-31G* and 3-21G(*) levels are listed in Table 3. In all cases, significant IP lowering was observed due to the base stacking as well as to the base pair formation. Interestingly, the GG/CC system has now the lowest IP among the seven possible guanine-containing stacked base pairs, which is entirely consistent with the experimental data. Figure 3 shows the model of the 5′-GG-3′ sequence of B-form DNA (a) and the HOMO of the stacked N-methylated 5′-GG-3′ (b). In this case, approximately 70% of the HOMO is localized on the 5′-G of 5′-GG-3′.

These calculations indicate that the 5′-G of 5′-GG-3′ is the most electron donating site in B DNA and suggest that electron transfer from DNA to an electron acceptor occurs most effectively at the 5′-GG-3′ site. In order to know the fate of the cation radical, the vertical IPs were estimated for seven stacked base pairs. The vertical IPs were calculated by computing the difference in energies between the neutral base and the cation radical. The same geometries for neutral base pair systems were used in order to calculate the energies of the corresponding cation radicals. The calculations indicate that the GG/CC system possesses the smallest vertical IP and that the cation radical is localized exclusively on the 5′-G of 5′-GG-3′, just the same as obtained for the HOMO of the neutral base pair system. These results suggest that the 5′-G of 5′-GG-3′ is a sink in "hole" migration through DNA, *i.e.*, an electron-loss center created in a B-form DNA will end up predominantly on the 5′-G of 5′-GG-3′.[2b]

Recently, Melvin *et al*. investigated the photoionization of DNA by irradiation with a 193 nm excimer laser.[9] They showed that 50−75% of the photoionization occurs at the guanine base in DNA and subsequent hole migration from initially formed radical cation sites to guanine base is completed within 5 μs. These results suggest that not only the base specificity for the initial photoionization but also subsequent energetically favored hole migration to the 5′-GG-3′ site are the origin of the 5′-GG-3′ specificity in the high-energy photoionization of duplex DNA.

2. Origin of IP Lowering in Base Stacking in B-Form DNA. In order to know the nature of the HOMO and the HOMO energy distribution in a stacked geometry of the duplex, we carried out calculations of stacked GG with different twist angles. The calculated HOMO and HOMO1 energies are shown in Figure 4. Within a wide range of twist angles (−180° to 180°), a significant energy splitting of HOMO and HOMO1 and, as a result, a lowering of the HOMO energies are observed. This implies that base stacking is an important factor for the lowering of HOMO energy but the lowering of HOMO energy is not always linearly related to the overlapping area of the two nucleobases. For example, the overlapping area at a 90° twist angle is significantly smaller than that for the vertical overlapping (Figure 5), but these two systems exhibited the same extent of IP lowering. It is important to note here that below −18° more than 95% of the HOMO is localized on the upper G (5′-side in B DNA) and above 18° more than 95% of the HOMO is localized on the lower G (3′-side), and between +9° and −9°, the HOMO is distributed equally on the two bases. The HOMOs of GG for different twist angles from −45° to 45° are illustrated in Figure 6. Within a normal range of twist angles for B DNA (−25° to −45°), IP values of GG are between 7.2 and 7.4 eV and the HOMO is predominantly localized on 5′-G. This implies that in B-form DNA the 5′ side G of the 5′-GG-3′ sequence is the most strongly interacting site with electron-accepting molecules in a charge transfer type interaction. This principle may be very important in understanding a wide range of charge transfer type interactions involving DNA such as DNA−drug and DNA−protein interactions.[19]

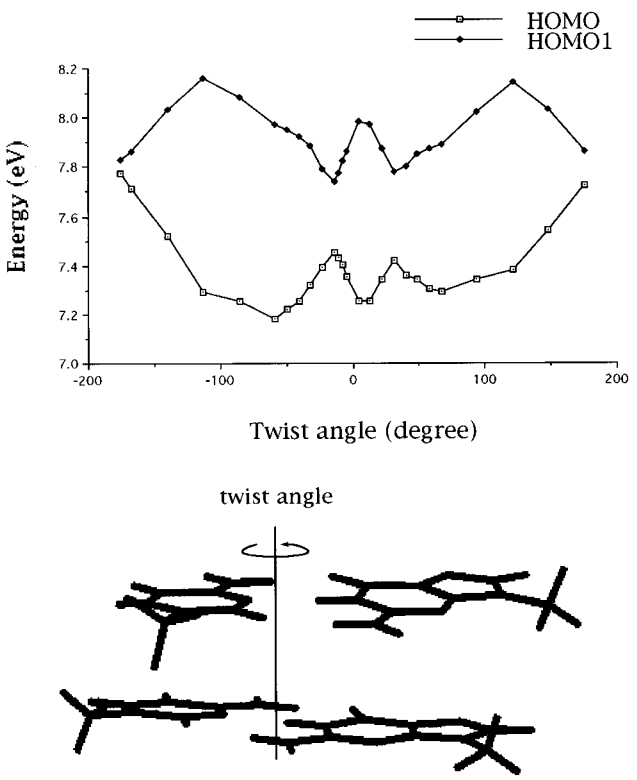

Figure 4. HOMO and HOMO1 energies of stacked 5′-GG-3′ with different twist angles.

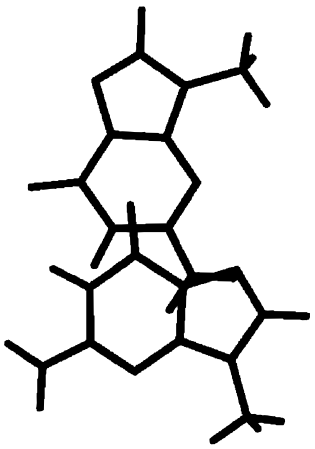

Figure 5. View of stacked *N*-methylguanines with 90° twist angle from the axis.

In order to elucidate the origins of HOMO localization and the lowering of IP in a B-form DNA stacking mode, we examined the molecular orbital calculations of the stacked GG in four different geometries which roughly represent the component of the stacked geometry in the B form; these include completely vertical overlapping (**7**), a −36.0° rotation of the upper base (**8**), a 1.00 Å slide of the upper base toward the long axis (**9**), and a 1.95 Å slide of the upper base toward the short axis (**10**) as represented in Figure 7. In all geometries, almost the same extent of IP lowering was observed. However, the distribution of HOMO dramatically depends upon the

(19) For example: Puglisi, J. D.; Chen, L.; Blanchard, S.; Frankel, A. D. *Science* **1995**, *270*, 1200.

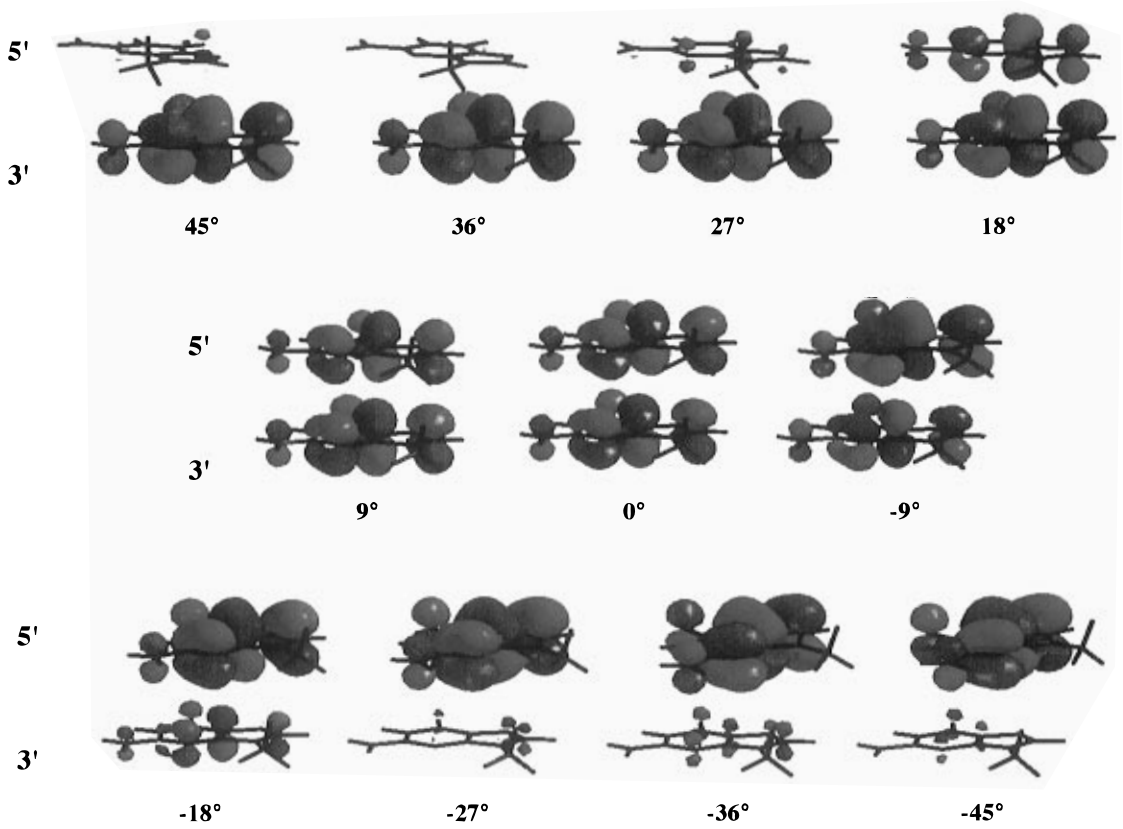

Figure 6. HOMOs of 5′-GG-3′ with different twist angles from −45° to 45°.

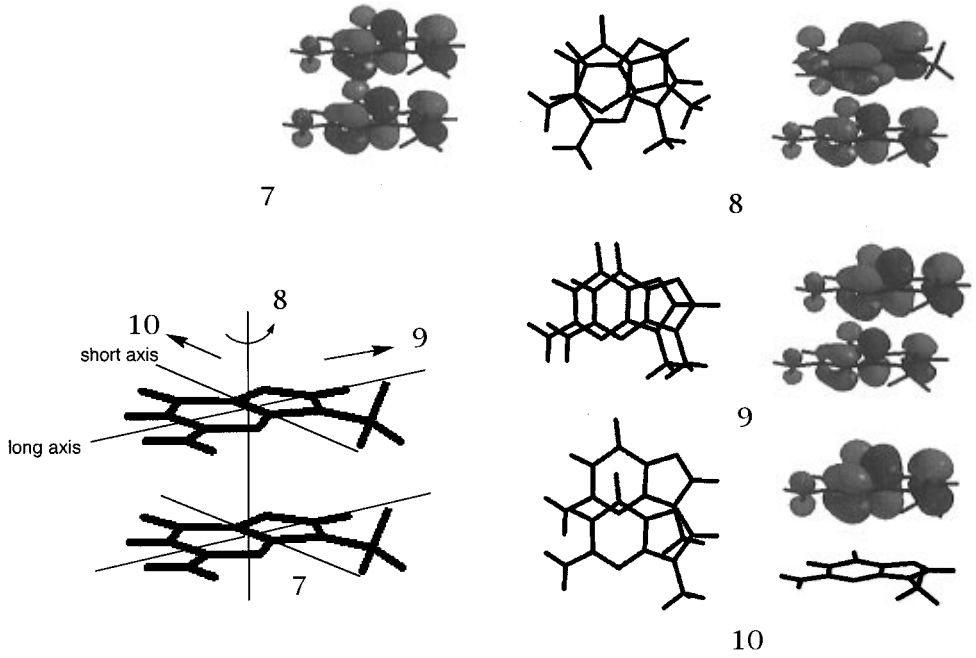

Figure 7. HOMO localization of the stacked 5′-GG-3′ in four different geometries representing the component of the stacked geometry in the B form; vertical overlapping (**7**), a −36.0° rotation of the upper base (**8**), a 1.00 Å slide of the upper base toward the long axis (**9**), and a 1.95 Å slide of the upper base toward the short axis (**10**).

stacking geometries as evident from Figure 7. In the interaction modes **7** and **8**, the HOMO is equally distributed in both Gs. For **9**, about 55% of the HOMO is localized on the upper G, whereas for **10** almost 100% of the HOMO is populated on the upper G. These calculations suggest that the major contributor for HOMO localization on 5′-G in a B-form DNA stacking mode would be a sliding of the upper base toward the short axis as in **10**. In order to evaluate energy splitting of HOMO and HOMO1, we carried out the calculations of the stacked GG at various distances. For simplification, vertical overlapping geometry was used. As summarized in Figure 8, the energy gap between HOMO and HOMO1 is highly dependent on the distance, and the effect of energy splitting of the two HOMOs was observed up to 5.5 Å.

3. IP Lowering for GG in Different DNA Conformations. It has been well recognized that DNA structure has a remarkable conformational heterogeneity.[20] In order to know whether a similar IP lowering in other DNA conformations may occur, we carried out molecular orbital calculations of GG/CC in the A form and the Z form. The results are summarized in Table

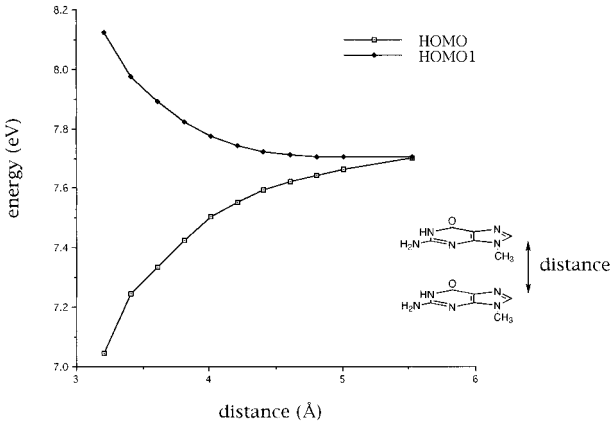

Figure 8. Distance dependency of HOMO and HOMO1 energies of the vertical stacked GG.

Table 4. Ionization Potentials[a] of (GG)/(CC) Base Pairs in Different Conformations

dinucleotide base pairs	conformation	HOMO energy (eV) 5′-G	3′-G
(GG)/(CC)	B	6.71	7.13
(GG)/(CC)	A	6.67	7.27
($G_{anti}G_{syn}$)/(CC)	Z	7.20	7.24
($G_{syn}G_{anti}$)/(CC)	Z	6.64	6.89

[a] Ionization potentials were estimated by Koopmans' theorem. The values are the HOMO energies of 3-21G(*) single-point calculations.

4. In an A-form DNA stacking mode, almost the same IP lowering and HOMO localization as obtained for a B DNA stacking mode were observed, suggesting that 5′-GG-3′ specificity of the photocleavage would be still valid in the A conformation as well. In the Z conformation, alternating syn anti conformation of the nucleoside unit is strictly required.[21] In a crystal structure of 5′-GG-3′ sequence-containing Z-form hexamers, there are two distinct GG stacking modes, 5′-G(syn)G-(anti)-3′ and 5′-G(anti)G(syn)-3′.[22] Interestingly, in the Z-form 5′-G(anti)G(syn)-3′ stacking mode, the localization of the HOMO at the 5′ side seems to have disappeared (Table 4). Since the Z conformation is difficult to attain for oligomers not repeating purine−pyrimidine alternation, to our knowledge, the Z conformation of the oligomer with the 5′-GG-3′ sequence in solution has not yet been reported. Recently, we demonstrated that an oligomer containing the 5′-GG-3′ sequence can be easily converted to the Z form by incorporating 8-methylguanine in place of guanine in the flanking sequence of 5′-GG-3′.[23] Experiments are underway to test this possibility.

Method of Calculations

All calculations were performed at the HF/3-21G(*), HF/6-31G, and HF/6-31G* levels utilizing Gaussian 92 and Spartan (version 3.1) programs on a Silicon Graphics IRIS Indigo R4400. Geometries of stacked methylated nucleobases at N_1 (pyrimidine base) and N_9 (purine base) were constructed as follows. The corresponding dinucleotides were built up using the Insight II program with standard B-form helical parameters (pitch, 3.38 Å; twist, 36°; tilt, 1°). All the sugar backbones of dinucleotides and complementary bases were removed except for the deoxyribose C1′ carbon and C1′ H. Two H atoms were then attached to the C1′ methine to complete stacked N-methylated nucleobases. Geometries of the stacked base pairs in the standard A and B conformations were constructed by means of a builder module in the Insight II program. For the A conformation, the sugar phosphate backbone was modified as described above. The geometry of the stacked base pairs in the Z form was generated from the crystallographic coordinates of 5′-d(m^5CGGGm^5CG)-3′/5′-d(m^5CGCCm^5CG)-3′ (PDB 145D). The dinucleotide parts were extracted from the hexamer, and the sugar phosphate backbone was modified as described above.

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JA9609821

(20) (a) Cozzarelli, N. R., Wang, J. C., Eds. *DNA Topology and Its Biological Effects*; Cold Spring Harbor Laboratory Press: New York, 1990. (b) Sinden, R. R., Ed. *DNA Structure and Function*; Academic Press: New York, 1994.

(21) Rich, A.; Nordheim, A.; Wang, A. H.-J. *Annu. Rev. Biochem.* **1984**, *53*, 791.

(22) Schroth, G. P.; Kagawa, T. F.; Ho, P. S. *Biochemistry* **1993**, *32*, 13381.

(23) Sugiyama, H.; Kawai, K.; Matsunaga, A.; Fujimoto, K.; Saito, I.; Robinson, H.; Wang, A. H.-J. *Nucleic Acids Res.* **1996**, *24*, 1272.