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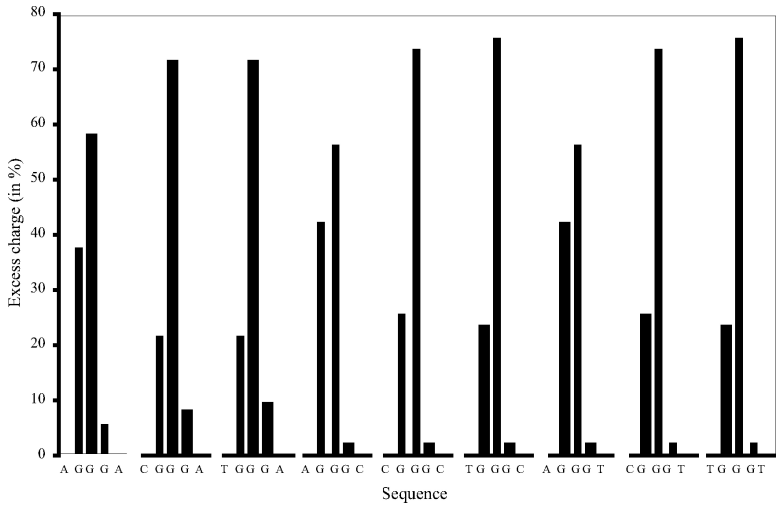

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Mapping the Sites for Selective Oxidation of Guanines in DNA

K. Senthilkumar,[†] F. C. Grozema,[†] C. Fonseca Guerra,[‡] F. M. Bickelhaupt,[‡] and L. D. A. Siebbeles*,[‡]

Radiation Chemistry Department, Interfaculty Reactor Institute, Delft University of Technology, Mekelweg 15, NL-2629 JB Delft, The Netherlands, and Afdeling Theoretische Chemie, Scheikundig Laboratorium der Vrije Universiteit, De Boelelaan 1083, NL-1081 HV Amsterdam, The Netherlands

Received July 2, 2003; E-mail: siebbeles@iri.tudelft.nl

One-electron oxidation of DNA nucleobases has been the focus of considerable investigation not only because of its relevance to DNA-damage causing mutations but also because of the general interest in long-range charge transfer through DNA.[1−3] Positive charges (holes) are predominantly trapped at guanine (G) nucleobases since these have the lowest ionization energy among the four DNA nucleobases. This selective oxidative damage of G bases has been found to depend strongly on the DNA sequence, i.e., not all G moieties are equally susceptible to oxidative damage.[4−8] Experimental studies have shown that sequences with repeated G bases (GG or GGG) show higher reactivity toward oxidation than isolated G bases, and this has been attributed to the formation of delocalized hole traps at -GGG- and -GG- sequences.[9] Ab initio studies have shown that the calculated ionization potentials of stacks containing five bases, -TXGYT- (X, Y = G, A, C, T), agree with the experimental relative reactivity of -XGY- triplets.[9] Voityuk et al.[10] calculated the relative energies of XG^+Y triplets and concluded that the 5′-G in a GG doublet is most reactive for oxidation, as has also been found by Saito et al.[11,12] However, the theoretical studies reported in refs 10−12 do not provide information on the distribution of a positive charge over the individual G's. Such information is essential to understand the cases in which both guanines are significantly oxidized.[6−8,13] Until now no theoretical explanation has been provided for the latter situation. The differences between the reactivity of guanines toward oxidation are most likely due to the influence of neighboring bases on the ionization energy of a certain G.

In the present work, the change in ionization potential of G bases due to the presence of neighboring bases is investigated by density functional theory (DFT) calculations. The ionization potentials and electronic couplings from the DFT calculations are used to calculate the charge distribution within sequences of two or three guanines. The results provide an explanation of experimental data on the selective oxidative damage in DNA containing such sequences.

All calculations were performed using the Amsterdam Density Functional (ADF) theory program,[14] which offers the unique possibility to express the molecular orbitals of stacked triplets (e.g. duplex -GGG-) in terms of the molecular orbitals, φ_i, on the individual nucleobases: the so-called fragment orbitals. The effective ionization potential or site energy, $\epsilon_i = \langle\varphi_i|h_{KS}|\varphi_i\rangle$, of a guanine in an -XGY- triplet is then directly obtained as the diagonal matrix element of the Kohn−Sham (KS) Hamiltonian in terms of the highest occupied fragment orbitals. The off-diagonal elements of the KS Hamiltonian matrix involving the highest occupied fragment orbitals on nucleobases i and j correspond to the charge-transfer integrals $J_{ij} = \langle\varphi_i|h_{KS}|\varphi_j\rangle$.[15]

In the present study the newly developed asymptotically corrected exchange correlation potential, SAOP (statistical average of orbital potentials),[16] was used with a triple-ζ quality basis set consisting of Slater functions. Two sets of polarization functions were included for each atom (TZ2P).[17] Although the use of Koopmans' theorem to obtain ionization potentials from DFT calculations has been considered to give rather poor results, it was shown recently that the SAOP potential yields reliable results.[16] The relative ionization energies (with respect to that for guanine) for isolated A, C, and T were calculated to be 0.34, 0.64, and 1.04 eV, respectively. The calculated values are comparable to the experimental values of 0.20, 0.70, and 0.90 eV,[18] respectively. This indicates that the results obtained with the SAOP potential are sufficiently accurate to evaluate the site energies of G bases in sequences of multiple bases. The charge-transfer integral, J, between the highest occupied molecular orbitals (HOMOs) on two neighboring guanine bases in B-DNA was calculated to be 0.165 eV, and the spatial overlap between the HOMOs was found to be 0.015. The geometries of the stacked triplets (XGY) were generated using the SCHNARP program with standard global helical parameters of B-form DNA.[19]

Table 1. Effective Energy of a Positive Charge (in eV) Localized at the Middle Guanine in 5′-XGY-3′ (X, Y = G, A, C, and T) Sequences

Y	G	A	C	T
GGY	7.890	8.040	8.310	8.290
AGY	7.900	8.060	8.341	8.320
CGY	7.957	8.115	8.383	8.360
TGY	7.965	8.124	8.407	8.381

The calculated effective energies of a positive charge localized on a guanine (the site-energies) flanked by two other nucleobases in different sequences are presented in Table 1. The site energy in a -GGG- triplet was found to be 7.89 eV, which is much lower than that of an isolated G (9.73 eV). This illustrates the strong influence of the neighboring bases on the site energy. Among sequences containing two G's the site energy of the central G increases in the following order -AGG- < -CGG- < -TGG- < -GGA- ≪ -GGT- < -GGC-. The data in Table 1 show that the site energy is strongly influenced by the type of the nucleobase at the 3′ position. When C or T is present at the 3′ position, the site energy at the guanine can be up to 0.44 eV higher than for A or G at this position. The influence of the base at the 5′ position is much smaller; the variation in site energy is less than 0.1 eV. These results demonstrate that the efficiency of a guanine to act as a hole trap in DNA strongly depends on the nature of the flanking nucleobases. The data in Table 1 shows that the site energies of a single guanine flanked by C or T at the 3′ position are significantly higher than for cases when A is present at this position. This provides an explanation for the experimental finding that single guanines in such sequences are almost unreactive toward oxidation.[6,13,20]

The site energies in Table 1 together with the charge-transfer integral and overlap, S, between the fragment orbitals on neighboring G's can be used to calculate the distribution of charge among

[†] Delft University of Technology.
[‡] Scheikundig Laboratorium der Vrije Universiteit.

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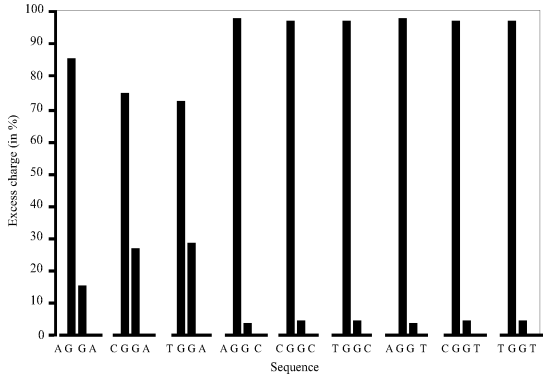

Figure 1. Distribution of excess charge (in %) on guanines in 5′-XGGY-3′ (X,Y = A, C, T) duplexes.

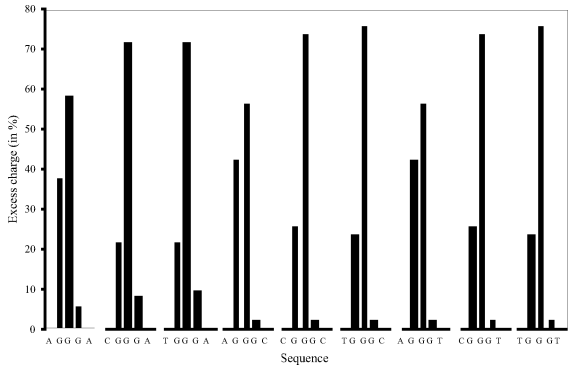

Figure 2. Distribution of excess charge (in %) on guanines in 5′-XGGGY-3′ (X,Y = A, C, T) duplexes.

the guanines in 5′-XGGY-3′ and 5′-XGGGY-3′, due to removal of an electron from the HOMO. The amount of charge on a certain G can be calculated from the coefficients of the guanine fragment orbitals, and the overlap, according to

$$q_i = c_i^2 + \sum_{j(\neq i)} c_i c_j S_{ij}$$

This approach is reliable if overlaps are small, which is the case in our systems. The fragment orbital coefficients were obtained by solving the eigenvalue equation, $HC = SCE$, where H is the Hamiltonian matrix with the site energies and charge-transfer integrals involving the guanines in the sequence considered. This method can be used for any DNA sequence with the assumption that non-nearest neighbor interactions can be neglected.

In Figures 1 and 2 the calculated charge distribution on G's in different sequences is shown. If it is assumed that the oxidative damage on guanines in -GG- and -GGG- sequences is proportional to the amount of charge present on the different G's, the experimental damage yields can be compared directly to Figures 1 and 2 for sequences containing two or three G's.

For the sequences in Figure 1, it is clear that when the base at the 3′ position is C or T the guanine damage predominately occurs at the 5′-G. If the 3′ neighbor is A, both guanines in the -XGGY-sequence are oxidized to a considerable extent, irrespective of the base at the 5′ position. These results agree with those from the experiments reported in refs 4−8, 13, and 20−21. Similar results were obtained for sequences containing three guanines. Figure 2 shows that the first and second G's from the 5′ side are most susceptible to oxidation. The relative amount of oxidation on the first G (5′) is significantly enhanced if the 5′ neighbor is an adenine. The extent of oxidation of the third guanine at the 3′ side increases by the presence of an adenine at the 3′ side. If Y = C, T in the 5′-XGGGY-3′ sequence, the oxidation of the third G in the sequence is negligible. The results calculated for the relative oxidation of the guanines in a sequence consisting of three G's agree with the experimental results in refs 4−6 and 22.

It is concluded that the neighboring base at the 3′ position determines to a large extent the charge distribution and therefore the oxidative damage on a sequence of guanine bases. If the 3′ neighbor is C or T the damage on the 3′ G will be negligible. The above calculations show that the experimentally observed selective oxidation of guanine nucleobases within a sequence of adjacent guanines is a consequence of the differences between the site energies. The effective energy of a positive charge localized on a specific guanine strongly depends on the type of the flanking nucleobases.

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Supporting Information Available: Computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

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