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Electronic Structure of DNA - Unique Properties of 8-Oxoguanosine

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Abstract: 8-Oxo-7,8-dihydroguanosine (8-oxoG) is among the most common forms of oxidative DNA damage found in human cells. The question of damage recognition by the repair machinery is a long standing one, and it is intriguing to suggest that the mechanism of efficiently locating damage within the entire genome might be related to modulations in the electronic properties of lesions compared to regular bases. Using laser-based methods combined with organizing various oligomers self-assembled monolayers on gold substrates, we show that indeed 8-oxoG has special electronic properties. By using oligomers containing 8-oxoG and guanine bases which were inserted in an all thymine sequences, we were able to determine the energy of the HOMO and LUMO states and the relative density of electronic states below the vacuum level. Specifically, it was found that when 8-oxoG is placed in the oligomer, the HOMO state is at higher energy than in the other oligomers studied. In contrast, the weakly mutagenic 8-oxo-7,8-dihydroadenosine (8-oxoA) has little or no effect on the electronic properties of DNA.

Introduction

8-Oxo-7,8-dihydroguanosine (8-oxoG) is among the most common forms of oxidative DNA damage found in human cells. One evolutionary outcome is the network of pathways that protect cells from the deleterious effects of this damage, including direct damage removal by 8-oxoG glycosylases, removal of a misinserted adenine opposite the 8-oxoG, and hydrolysis of 8-oxoGTP to prevent its incorporation into the genome during DNA synthesis.[1] Several of the enzymes that bind 8-oxoG have been isolated and crystallized, and the structures of various reaction intermediates have been solved[2] revealing no common feature between the binding of 8-oxoG to these different proteins. 8-OxoG is minimally perturbing to duplex DNA; both 8-oxoG:C and 8-oxoG:A base pairs have only minor effects on double-stranded DNA structure and stability.[3] This makes it particularly challenging for repair enzymes to locate such atomic-level changes.

Past studies have shown a possible correlation between redox reactions and the interactions of proteins with DNA, in general, and between repair enzymes and DNA, in particular.[4] Generally, the question of damage recognition by the repair machinery is a long standing one, and it is intriguing to suggest that the mechanism of efficiently locating damage within the entire genome might be related to modulations in the electronic properties of lesions compared to regular bases. To our knowledge, no detailed microscopic investigation of the electronic properties of modified and regular bases has been performed. Here, using laser-based methods, combined with organizing various oligomers as self-assembled monolayers on gold substrates, we are able to determine the energy of the HOMO and LUMO states and the relative density of electronic states below the vacuum level. Specifically, we find that when 8-oxoG is placed in the oligomer, the HOMO state is at higher energy than in the other oligomers studied. In contrast, when the weakly mutagenic 8-oxo-7,8-dihydroadenosine (8-oxoA)[5] is placed in the oligomer, it has little or no effect on the electronic properties of DNA.

The electronic properties of DNA, and specifically those of the various bases, must play an important role in defining its chemical properties as well as its sensitivity to damage. There are only a handful of techniques that yield detailed information on these properties, most of them were conducted in the gas phase and only on nucleosides and nucleotides, showing that isolated nucleobases undergo dissociative electron attachment (DEA) at subexcitation energies, which leads to the loss of a

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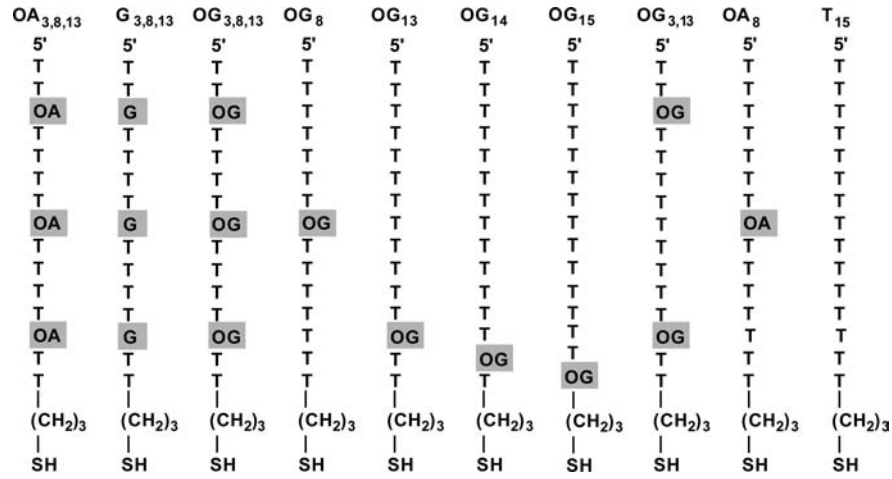

Figure 1. The 10 oligomers studied. T indicates thymine, G is guanine, and OG and OA are 8-oxoguanine and 8-oxoadenine, respectively.

neutral hydrogen.[6−15] These studies are necessarily limited since they do not probe the properties of the complete DNA oligomer structure in its natural solvated environment. A few other studies investigated the interaction of low energy electrons with plasmid DNA deposited on a substrate.[16] Other experiments were performed on building blocks of DNA in the gas[12,13] and condensed phase.[14,15]

Recently we introduced the possibility of investigating the interaction of electrons possessing well-defined energies with monolayers of single strand (ss) and double strand (ds) DNA oligomers adsorbed on a gold surface as a self-assembled organized monolayer.[17] In our experiments, we use Low Energy Photoelectrons Transmission (LEPET)[18] and two photon photoemission (TPPE)[19−22] spectroscopy on samples of self-assembled thiolated DNA monolayers adsorbed on a conductive substrate. This system provides good control of the composition, structure, and conformation of the DNA. Using this methodology we have previously confirmed the common observation that guanine has the lowest redox potential among all DNA bases and, therefore, can be readily oxidized. In addition, our unique experimental approach revealed that guanine bases also serve as excellent electron capturers, facilitating transfer of an electron to a phosphate on the DNA backbone.[23]

To obtain deeper insight on the effect of oxidative damage of bases on their electronic properties, several DNA oligomers containing either 8-oxoG or 8-oxoA were adsorbed as a self-assembled monolayer on gold substrates, and their electronic properties were studied using LEPET and TPPE and compared to DNA oligomers with no oxidative damage.

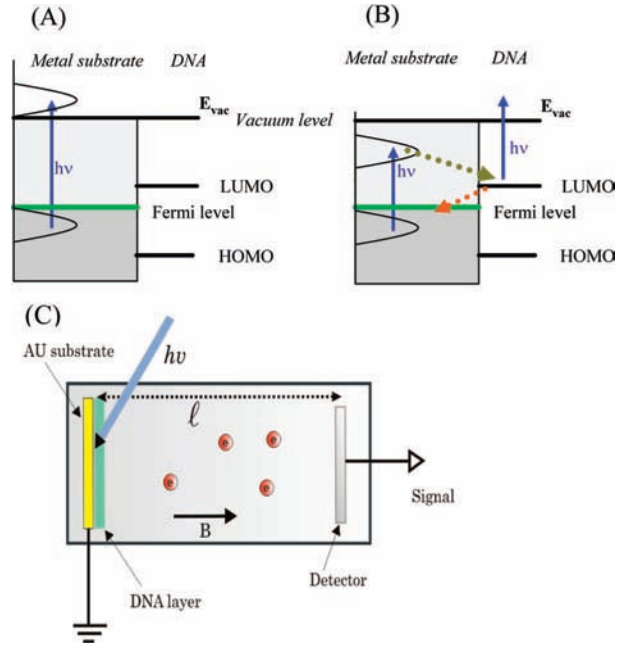

Figure 2. Energy scheme of the methods used. LEPET and TPPE (A and B, respectively). (C) Scheme of the experimental setup.

Materials and Methods

Oligodeoxynucleotide Synthesis and Purification. Oligodeoxynucleotides were initially synthesized at the DNA/Peptide Core

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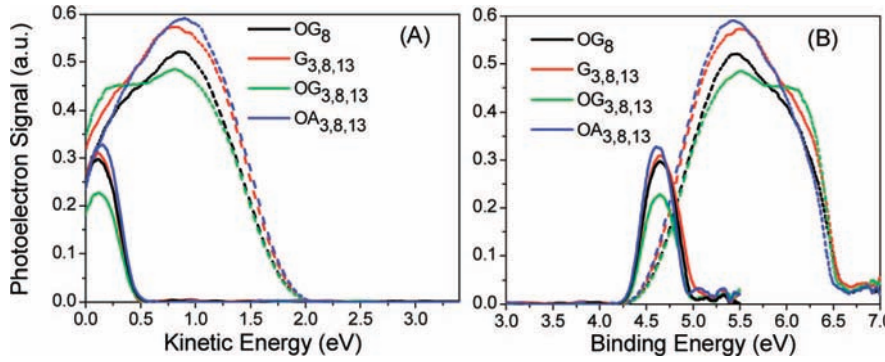

Figure 3. LEPET spectra taken with 6.42 eV photons (dashed lines) and 4.86 eV photons (solid lines) and presented according to either the kinetic energy of the photoelectrons (A) or their binding energy (B).

Facility (University of Utah) using commercially available phosphoramidites. The sequences of oligomers used in this study are shown in Figure 1; all were 15-mers with 3′-terminal propylthiol groups. The dOG and dOA (Glen Research) containing oligodeoxynucleotides were subsequently cleaved from the column overnight in fresh 30% NH_4OH containing 0.25% 3-mercapto-1-propanol followed by deprotection for 17 h at 55 °C. The oligomers were purified by ion exchange HPLC utilizing a linear gradient from 15% B to 100% B over 30 min at a flow rate of 1 mL/min with monitoring at 260 nm (where A = 10% acetonitrile in water and B = 1.5 M sodium acetate pH 7 in 10% acetonitrile in water). The identity and purity of the oligodeoxynucleotides were determined by negative ion ESI-MS on a Micromass Quattro II mass spectrometer. Note that the deprotection step with excess 3-mercapto-1-propanol converted all 3′-terminal thiol groups to disulfides.

Ten types of self-assembled DNA monolayers were prepared (Figure 1) according to standard procedures by depositing 3′ thiolated 15-mers of DNA on clean gold substrates in 0.4 M, pH = 7.2 potassium phosphate buffer. The clean Au slide was covered uniformly with the oligomer solution (20 μM) and kept overnight in a clean and controlled humid environment. After deposition, the slides were washed thoroughly first in 0.4 M potassium phosphate buffer and subsequently by sterile deionized water (Millipore). The samples were then dried in N_2.

^{32}P labeled DNA oligomers were used to characterize the adsorption quantitatively (see ref 17 for details). For all the different ss DNA oligomers, the monolayer density was found to be $N = (1.4 \pm 0.4) \times 10^{13}$ molecules/cm^2, as analyzed by phosphorimager (FLA-5100, FUJI) (Figure 1 supplement data radioactive results). This density is in agreement with a theoretical calculation of the expected density of a close-packed monolayer based on the size of the molecules. The adsorbed oligomers most likely contain structural water molecules, even when placed in an ultrahigh vacuum (UHV) chamber.[24]

LEPET and TPPE Experimental Setup. The experimental setup (Figure 2) was based on ejection of photoelectrons from the gold substrate on which the molecules are adsorbed. The experimental setup is similar to that described in ref 23. The sample was held in an ultrahigh vacuum chamber ($<10^{-8}$ Torr). The sample was exposed to the laser beam for only 20 μs to avoid UV radiation damage. The electrons that are emitted from the gold substrate are transmitted through the DNA monolayer to the vacuum, where their energy is measured by a time-of-flight spectrometer. Electrons that are not transmitted are captured in the layer and transferred back to the grounded metal substrate. Because of the short lifetime of the captured electrons and the low laser intensity and repetition rate, the monolayer does not get charged by electrons between two laser pulses. This was verified by observing a stable electron energy spectrum which does not vary with time.

In the Low Energy Photoelectron Transmission (LEPET) approach, electrons are ejected by a laser with the energy higher than the work function. The photoelectrons are therefore emitted from states below the Fermi level to above the vacuum level and transmitted through the adsorbed film (see Figure 2A). The transmittance properties provide information about the scattering cross section and its dependence on the film's organization, thickness, constituents, and the electrons' kinetic energy.[18] This method also provides information on the density of states of the system below the Fermi level.

In two-photon photoelectron (TPPE) spectroscopy, photons with the energy lower than the work function of the system are used (Figure 2B). The "pump" photons interact with electrons below the Fermi level in the substrate and excite them to states above the Fermi level. If the lasers used are not very intense and are relatively long, the electrons excited by these first photons can relax either back to states in the substrate below the Fermi level or to originally unoccupied states on the organic film, forming transient ions. The second pulse of photons, the "probe" photons, having also energy below the work function of the substrate, are able to eject the electrons from these transient ion states, on the organic film, to above the vacuum level. The measured kinetic energy of these electrons provides information on the binding energy of the electron, namely on the energy of the intermediate states (the LUMO) relative to the vacuum level.

In the present setup we used two photons from the same laser pulse to induce the two-photon-photoemission process. Because of the relatively long laser pulse (laser pulse length of 10 nsec) and the low intensities, there is no contribution in the signal from a two-photon coherent process. Due to energy conservation, the kinetic energy of electrons resulting from such a process would be $E = 2h\nu - e\Phi$. Consistently, we obtain no signal at this energy, which means that, in the TPPE process, once the electrons are excited by the first photon, they relax to some long-lived state from where it is excited by the second photon. In order for these intermediate states to be long-lived, they must be localized on the monolayer; namely they are the LUMO in the monolayer. Hence, the kinetic energy of the electrons reflects their binding energy in the monolayer so that $E_b = |E - h\nu|$.

In all of these studies, the sample was biased by -1 V versus the detector.

Results

Current understanding of damage recognition and removal by the DNA glycosylases mainly focuses on characterization of the protein moieties involved in binding to the modified base and characterization of the structural fit between the protein and the base. To our knowledge, no information exists on the contribution of the electronic differences between the regular

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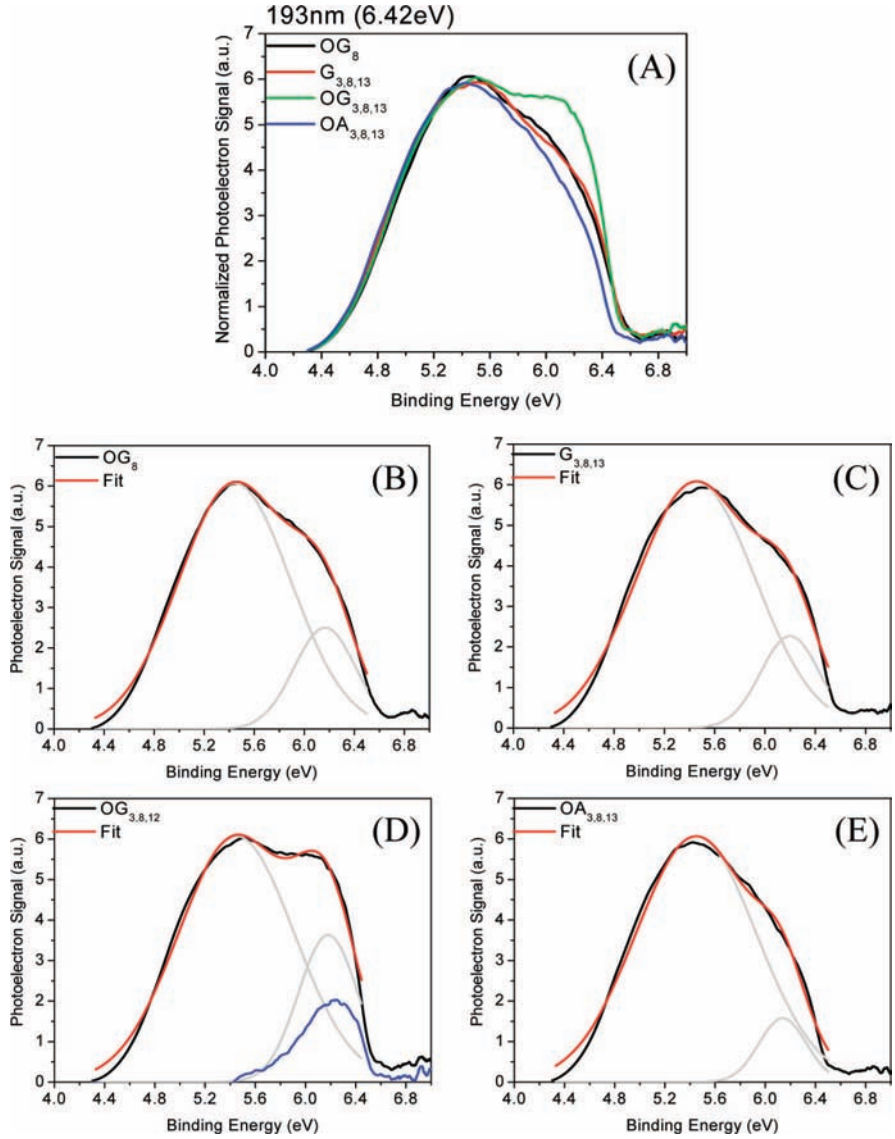

Figure 4. (A) Normalized LEPET spectra. These spectra could be fitted assuming two peaks (gray lines) (B−E) for the different oligomers. In 4D also shown (blue line) is the peak at 6.2 eV after subtracting from the spectrum of $OG_{3,8,13}$ the spectrum of $OA_{3,8,13}$.

bases and their oxidized forms to the process of damage recognition. We therefore sought to characterize the electronic spectrum of DNA oligomers containing oxidized bases in comparison to their nonoxidized counterparts.

Figure 3 shows the LEPET spectra for 4 oligomers (OG_8, $G_{3,8,13}$, $OG_{3,8,13}$, and $OA_{3,8,13}$) of the 10 oligomers studied (Figure 1) all containing thiols at their 3′ end. Thiol binding to the gold surface results in formation of DNA self-assembled monolayers such that we can control the amount of 8-oxoG and 8-oxoA on the surface, while keeping the same DNA density. In addition, we could also vary the average distance of the oxidized base from the gold surface for each of the oligomers. The spectra in Figure 3 were produced by using photons with an energy of either 6.42 or 4.86 eV. Figure 3A presents a spectrum of electrons reaching the detector according to their kinetic energy. The high energy cutoff in the spectra results from electrons near the Fermi level that are the most easily ejected to above the vacuum level. Their kinetic energy is therefore simply $E = h\nu - e\Phi$, where $e\Phi$ is the substrate work function which is 4.35 ± 0.1 eV for the DNA-coated gold. The spectra obtained applying the 6.42 eV photons can be qualitatively described as having two characteristic peaks, whose maximal kinetic energies are 1 and 0.3 eV, respectively. The interesting feature stemming from these two peaks is that the shape of the spectrum for all 4 oligomers is similar but their relative intensities change from the 1 eV peak to the 0.3 eV peak. That is, the $OG_{3,8,13}$ oligomer has the highest intensity of the 4 oligomers in the 0.3 eV peak but the lowest intensity at the 1 eV peak (Figure 3A).

To elucidate the origin of the difference between the oligomers containing 8-oxoG and all others, we must realize that the LEPET spectrum, $S(E)$, is a result of two parameters, the electron energy distribution that was excited by the photon, $P(E)$, and the probability of the excited electrons to be transmitted through the self-assembled monolayer, $T(E)$. Hence, as discussed in ref 18.

$$S(E) = P(E_b)\, T(E) \tag{1}$$

where E is the final kinetic energy of the electrons as measured in the system, and E_b is the binding energy of the electrons, namely the initial state from which they were excited to above the vacuum level (see Figure 2A).

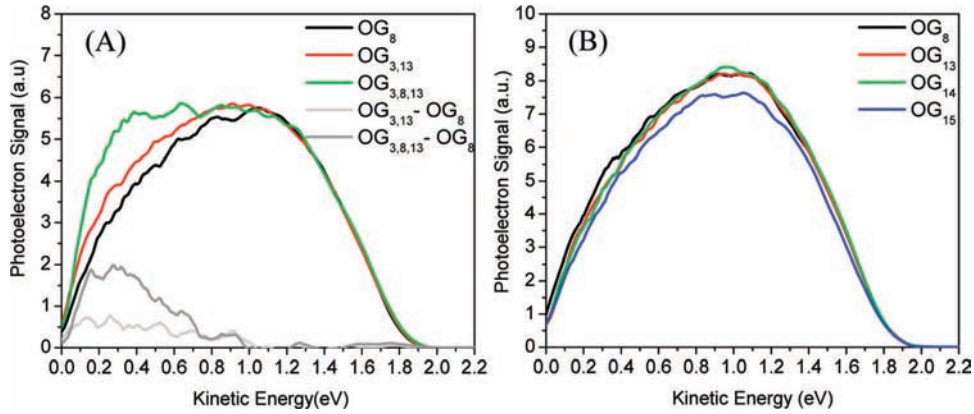

Figure 5. LEPET spectra obtained with monolayers made from (A) three different oligomers containing one, two, and three 8-oxoG. A clear difference in the specta is seen as the number of 8-oxoG is increased. This difference is quantified by subtracting the spectrum obtained with one 8-oxoG from the other two spectra. The ratio between the two subtractions is ~2, indicating an additive effect of the 8-oxoG. (B) Four different oligomers each containing a single 8-oxoG base at a different distance from the thiol bound to the surface. No distance effect could be observed by changing the position of the 8-oxoG from the 8th position to the 15th position.

The relative contribution of $P(E)$ and $T(E)$ can be evaluated by comparing the spectra obtained with two different photon energies. In our case, we used photons of 6.42 and 4.86 eV. The photon with the lower energy ejects only a subpopulation of the electrons ejected by the high energy photon from below the Fermi level. It is clear that the relative intensities of the different oligomers changed dramatically (compare dashed line and solid line spectra in Figure 3A), resulting in $OG_{3,8,13}$ having the highest intensity for the 0.3 eV kinetic energy peak in the spectra taken with the 6.42 eV photons, while having the lowest intensity for the same peak in the spectra when taken with the 4.86 eV photons. If the origin of the difference between the oligomers was due to electron transfer, $[T(E)]$, no difference in relative intensities for the different oligomers was expected to be observed in Figure 3A upon changing the photon energy. The fact that the intensities do vary indicates that the different behavior of the $OG_{3,8,13}$ oligomer must stem from differences in $P(E_b)$. These differences are better demonstrated in Figure 3B, where the LEPET spectrum is presented as a function of E_b, the initial binding energy of the electrons. Indeed we see that $P(E_b)$ is the function controlling the spectra. Namely, the relative intensities of the spectra of the 4.86 eV are similar to the relative intensities of the spectra of the initial density of states from which the electrons were excited.

Another way of emphasizing the different behavior of $OG_{3,8,13}$ is by resolving the $S(E)$ spectrum into the two peaks of which it is comprised. The peak corresponding to kinetic energy of 0.3 eV when presented as binding energy appears at 6.1 eV and becomes very dominant in the case of $OG_{3,8,13}$ (Figure 4). The special contribution of 8-oxoG to the LEPET spectrum is emphasized by subtracting from the spectrum of $OG_{3,8,13}$, the spectrum obtained with other oligomers (like $OA_{3,8,13}$ as an example). Figure 4D shows a clear peak at ~6.2 eV that appears, as a result of this subtraction, and relates to the specific contribution to the signal due to the existence of the 8-oxoG in the oligomer.

The simplest explanation of our results is that the two peaks at kinetic energies of 1 and 0.3 eV in the LEPET spectra, which are not present in bare gold, refer to two states with binding energies of 5.4 and 6.1 eV, namely located at 1.1 and 1.8 eV, respectively, below the Fermi level. Previous observations relating the state at 1.1 eV below the Fermi level to thiols adsorbed on gold[25,26] and benzenethiol on copper[27] support it. In addition, calculations have shown that chemisorption of thiols can result in the formation of a metal/molecule band near this energy.[28−30] We therefore suggest that the band seen in the present spectra, which is common to all four oligomers, stems from their 3′ thiol binding to the gold surface. The novel feature in these spectra is the state that corresponds to the higher binding energy (1.8 eV below the Fermi level) which depends on the nature of the DNA oligomer. The density of states in this band is significantly higher for the monolayer made from $OG_{3,8,13}$.

To investigate the density of states in this band, strands containing one, two, or three 8-oxoG's (Figure 1) were used. Interestingly, the photoemission signal at this band (at 1.8 eV below the Fermi level) was found to be additive (see Figure 5A). Since these oligomers differ from each other not only by the number of 8-oxoG's but also by the proximity of the 8-oxoG to the thiol, we probed the distance effect by measuring the LEPET spectrum of oligomers with a single 8-oxoG at various distances from the thiol (Figures 1 and 5B). The intensity of the 1.8 eV band in $OG_{3,8,13}$ (Figure 4D) was always higher than that for oligomers containing only a single 8-oxoG base, even if this base is located at the 15th position (Figure 5B), in high proximity to the substrate. That is, no distance effect could be observed by changing the position of the 8-oxoG from the 8th position to the 15th position and all oligomers showed almost identical spectra (Figure 5B). Hence, the new band found at 1.8 eV below the Fermi level most likely originates from the ionization of the strands containing 8-oxoG. It corresponds to an ionization energy of 6.1 eV. This finding is in line with former calculations indicating that the ionization potential of 8-oxoG can be as low as 6.3 eV and is strongly affected by its

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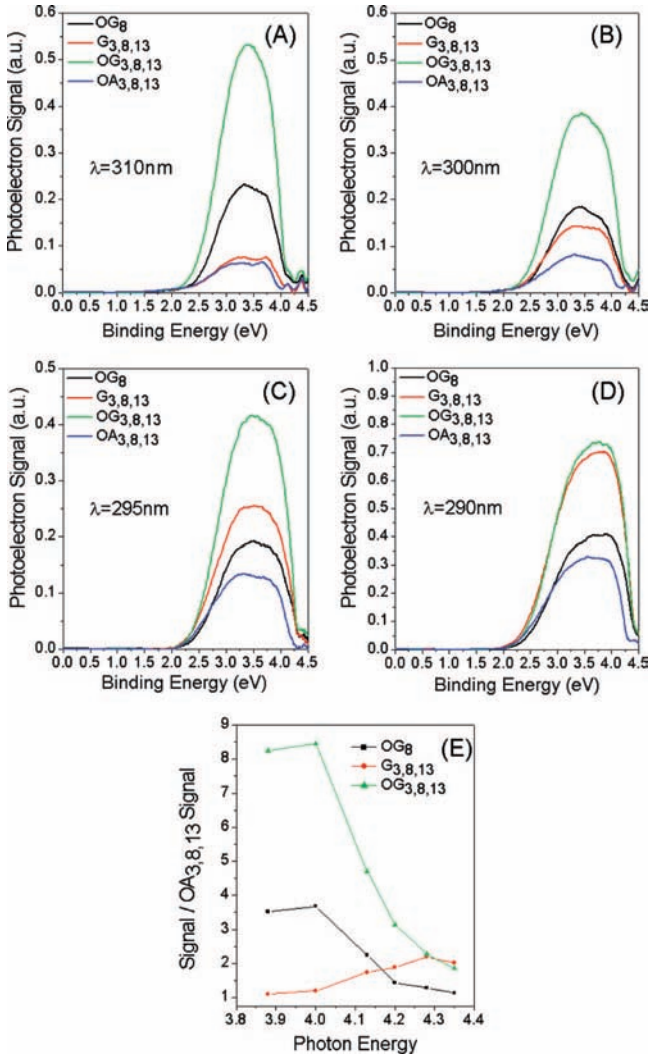

Figure 6. TPPE spectra obtained for four oligomers when excited with different photon energies (A−D). In (E) the relative intensity of the TPPE spectrum for each oligomer is shown as a function of the photon energy. The intensities are normalized to the intensity obtained for the monolayer made from the $OA_{3,8,13}$ oligomer.

environment.[31] This ionization potential is lower than those reported for all other nucleic acid bases (∼8.4 eV).[32]

TPPE. Figure 6A−D presents the TPPE spectra obtained for the 4 oligomers whose LEPET was shown in Figure 3, at various photon energies, all below the work function. The shape of the spectra looks very similar for all energies used and for all 4 oligomers. This indicates that the LUMO, from which electrons are ejected by the second photons, are the same for all oligomers. Interestingly, the relative intensities of the spectra for the different oligomers vary quite significantly with wavelength, as summarized in Figure 6E. The TPPE results show variation in the relative intensity for the different oligomers as a function of photon energy (summarized in Figure 6E). The most pronounced effect is the strong signal from $OG_{3,8,13}$ compared to the signal from $OA_{3,8,13}$ at relatively low photon energies and its reduction as the photon's energy increases.

The intensity of the TPPE spectrum depends both on the probability to inject electrons into the intermediate state, the LUMO of the monolayer, and on the state's lifetime (see Figure 2B). All of the spectra have peaks at around the same energy (∼3.3 and ∼4 eV). This means that the binding energy of the electrons in the monolayer is oligomer independent. Namely, since the LUMO is the same for all oligomers and for all wavelengths excited, its lifetime cannot explain variations among the different samples or changes in TPPE intensity as a function of photon energy. Therefore, the variation in relative intensities of the spectra as a function of wavelength must depend on the probability of injecting the electrons into this LUMO state.

Discussion

In the present study we investigated in detail the orbital energies of oligomers that contain oxidized bases such as 8-oxoG and 8-oxoA. Figure 7 summarizes schematically the spectral analysis of the oligomers containing the oxidized bases, by showing a graphic depiction of the density of states of these oligomers, as inferred from the combination of LEPET and TPPE measurements. The analysis clearly indicates that the 8-oxoG-gold system has some special properties compared to the other oligomers studied. Specifically, a dramatic difference between the electronic configuration of $OG_{3,8,13}$ and the other oligomers is seen in bands both below (LEPET spectra) and above (TPPE spectra) the Fermi energy. We show that when 8-oxoG is present in the strand, the HOMO of the strand is at ∼6.1 eV below the vacuum level. In addition, for oligomers that contain 8-oxoG there is an increase in the density of states at 0.45 eV beneath the vacuum level. This high density of states may result in an efficient electron capturing probability by oligomers containing 8-oxoG.

Characterization of the unique states of the oligomers containing 8-oxoG has indicated the following: (i) They are independent of the distance of 8-oxoG from the gold (Figure 5B). (ii) Their intensity is additive. That is, comparing oligomers with one, two, or three 8-oxoG nucleotides yielded a linear additive effect (Figure 5A). (iii) Unlike the 8-oxoG oligomers, the 8-oxoA-containing oligomers show no pronounced effect on the density of states below the Fermi level. In the TPPE experiments, the spectrum taken with 8-oxoA varied the least as a function of the photon energy, indicating that there are no resonances (or high density of states) in the region between the Fermi level and the vacuum level. Hence, in contrast to 8-oxoG, 8-oxoA does not modify significantly the electronic structure of the DNA. Interestingly, 8-oxoA and G are isomers; however their solution electrochemistry differs. This may be related to our observation (Figure 7) that the G-containing oligomers have a higher density of states below the vacuum level than 8-oxoA containing oligomers. Taken together, this analysis supports the notion that the new states observed are characteristic of the presence of 8-oxoG nucleotide in the oligomer and not due to the attachment of the oligomers to the gold surface.

Interestingly, we do not find that 8-oxoG interacts differently from the other oligomers studied with electrons possessing energies above the vacuum level. Namely the shape of the LEPET spectra is controlled by the density of states of the system below the Fermi energy and not by the transmission of the electrons, which was found to be independent of the monolayer.

Our findings are consistent with the notion that the electronic properties of 8-oxoG may play a role in its recognition or removal by the repair machinery. It is important to note that the increase in density of states below the vacuum level was also detected, to some extent, in the $G_{3,8,13}$ oligomer. Therefore,

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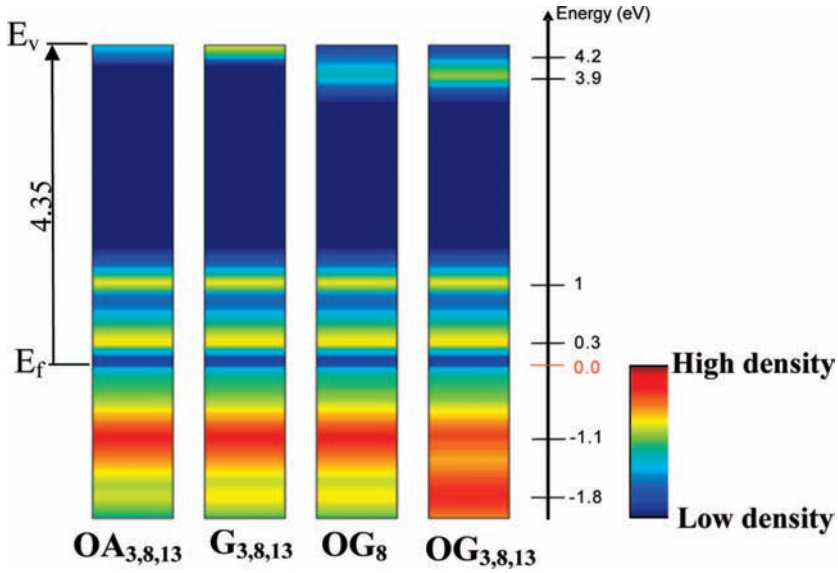

Figure 7. Relative density of states obtained based on the LEPET and TPPE experiments for four types of monolayers. The high density near −1.1 eV below the Fermi energy (E_f) corresponds to the known band caused by the formation of thiol−gold bonds.

we suggest that this property of 8-oxoG may most likely be attributed to electronic properties of G bases that are enhanced by the oxidation of G to 8-oxoG. These states below the vacuum were not observed in the $OA_{3,8,13}$ oligomer (comprised of 8-oxoA and T), further supporting the notion that 8-oxoG may have unique properties among the bases.

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

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