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The Capture of Low-Energy Electrons by PNA versus DNA

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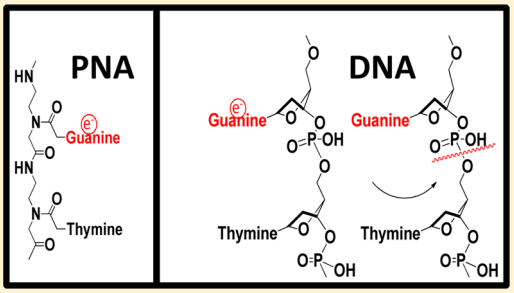
Supporting Information

ABSTRACT: This study provides insight into the mechanism of capturing low energy electrons by peptide nucleic acid (PNA) and the role of the oligonucleotide backbone in the capture of low energy electrons. We studied by photoemission self-assembled monolayers of two types of oligonucleotides, DNA and PNA. PNA is a synthetic analogue of DNA that has a pseudopeptide backbone and which may have important medical and biotechological applications. We found that in both PNA and DNA, the guanine nucleobases capture the electrons more efficiently than thymines. In PNA, once the electrons are captured, their state is at least partially localized on the nucleobases, and the PNA molecule undergoes structural changes that stabilize the electron. This situation is in contrast to DNA, in which the captured electrons are transferred very efficiently to the backbone, and the final state of captured electron is base independent.

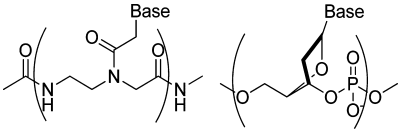


SECTION: Biophysical Chemistry and Biomolecules

The discovery that low energy electrons can damage DNA[1−3] led to intense investigation of the mechanism by which the damage occurs.[4−11] The mechanism that emerged is dissociative electron attachment (DEA). In DEA, the electrons are captured by the DNA nucleobases and are then transferred rapidly to the backbone, where they can cause P−O and C−O bond breaks.[12−15] Many of the studies on electron-induced damage in DNA were performed on DNA deposited on solid substrates because in solution it is difficult to controll the energy of the electron and to analyze the transient products.[16] These studies provided extensive information. For example, studies of DNA with systematically varied sequence led to the conclusion that out of the four nucleobases, guanine (G) captures electrons most efficiently,[17] although the electron affinity of the free guanine is similar to that of the other bases. This property of guanine was attributed to its very low ionization potential that causes it to be slightly positively charged when placed within a DNA strand.[18] The backbone may also affect the charge transfer in DNA, however this issue is not settled yet.[19,20]

Therefore it is important to investigate the role of the DNA backbone in the electron capturing process.[21]In the present work we addressed this subject by the use of synthetic DNA analogues, which has the same nucleobases as DNA but has a a pseudopeptide backbone based on aminoethyl glycine (Aeg) instead of the phosphodiester DNA backbone (Scheme 1).[22−24] Consequently, peptide nucleic acid (PNA) has a C-terminus and an N-terminus in contrast to DNA, which has a 5′-end and a 3′-end, respectively. PNA may have important medical and biotechological applications,[25] and therefore it is of importance to compare its properties to those of DNA. The comparison of the electron capturing properties of DNA and PNA oligonucleotides with the same nucleobases but different backbone provides insight into the role of the backbone in the damage to DNA.

Scheme 1. Chemical Structures of PNA and DNA



Indeed, as will be shown, the backbone has a dramatic effect on the dynamics of the captured electrons. In contrast to DNA, in PNA the backbone is less effective in transferring electrons and the captured electrons are localized, at least partially, on the bases.

For this study, we have synthesized a single strand (ss), thymine-only, PNA 15-mer, and three ssPNA 15-mers containing one, three, or five isolated guanines (Scheme 2). We have also studied the corresponding ssDNA 15-mers with the same sequences of nucleobases as that of the PNAs. The DNA and PNA oligomers were self-assembled on a gold substrate using a thiol group as anchor.[26] The self-assembled monolayers (SAM) were characterized using Fourier transform infrared (FTIR) and X-ray photoelectron spectroscopy (XPS) (Figure S2 and Table S2).

We studied the electron capture by SAMs of DNA or PNA by low energy photoelectron transmission (LEPET) spectroscopy. In LEPET experiments, photons with energy higher than the work function of the system eject electrons from states

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Scheme 2. The Sequences of DNA/PNA Oligonucleotides Used in This Study[a]

PNA/DNA G0	TTT TTT TTT TTT TTT
PNA/DNA G1	TTT TTT **T**G**T** TTT TTT
PNA/DNA G3	TTT **T**G**T** TTT **G**TT TT**G**
PNA/DNA G5	TT**G** TT**G** TT**G** TT**G** TT**G**

[a]All strands are 15 nucleobases long with a varying number of guanines.

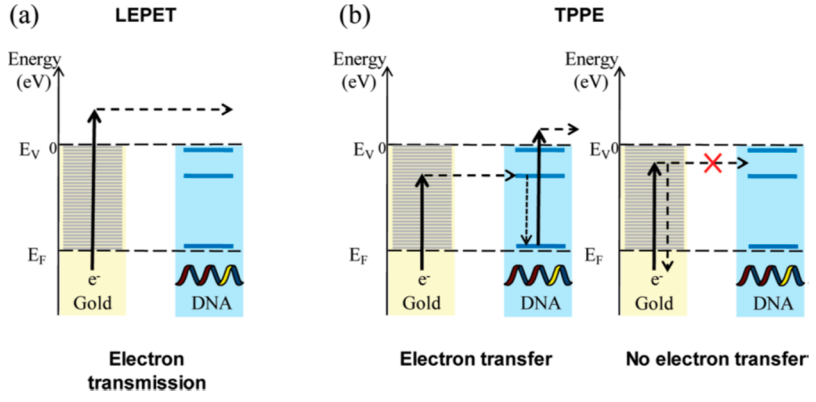
below the Fermi level to above the vacuum level; these electrons are transmitted through the adsorbed film (Scheme 3a). For a given laser intensity, the photoelectron signal depends on the density of states of the system below the Fermi level and on the electron transmission probability through the self-assembled monolayers. Hence, the study of the dependence of the LEPET signal on the sequence of a given nucleic acid provides information on the efficiency of electron capture by different nucleobases in the DNA. The wavelength used in the LEPET experiments was 193 nm corresponding to an energy of 6.42 eV. The laser intensity was 50 pJ/7 ns and 300 pJ/7 ns pulse, for the study of DNA and PNA, respectively, and the overall illumination time of a monolayer sample was only 10 μs to avoid any radiation damage. Four samples of either DNA or PNA were mounted each time on a holder in a UHV chamber, which allowed for the measurement to be conducted by switching back and forth between the mounted samples without varying any parameter of the laser beam. The density of the DNA and PNA monolayers did not vary with their sequence. The density of the PNA monolayers was larger than that of the DNA oligomers, therefore the electron transmission efficiency is lower and higher laser intensity was needed for obtaining a good signal-to-noise ratio.

The LEPET spectra of the SAMs of PNA or DNA molecules showed two peaks (Figure 1). The high-binding energy peak corresponds to the ionization of the guanine bases and as expected, its intensity increases with the number of G nucleobasess in the PNA oligomer. The peak at the low binding energy results from electrons ejected from the Au−S bond, its intensity decreases with the increase in the number of Gs in the sequence of DNA or PNA.[27−29] Since the number of Au−S bonds is the same, irrespective of the number of Gs, the decrease in intensity must be caused by a decrease in the transmission efficency of the SAMs that contain the G's. This result means that G captures electrons more efficiently than T *irrespective of the chemical nature of the backbone of the nucleic acid*. This effect was also independent of the PNA orientation on the Au substrate, i.e., attached to the C-terminus or the N-terminus (see Figure S3, Supporting Information).

We have also studied the SAMs of DNA or PNA by two-photon photoelectron (TPPE) spectroscopy. In the TPPE experiments, pulses of "pump" and "probe" photons with energy lower than that of the work function of the system are used (Scheme 3b). Unlike in many other studies, such as cited in refs 30−32, here the two photons are within a single nanosecond pulse. 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 are not very intense and are relatively long (500 nJ/pulse, 10 Hz, 7 ns pulse length), the electrons excited by the"pump" photons can relax either back to states in the substrate situated below the Fermi level, or to unoccupied states in the SAM, i.e., the lowest unoccupied molecular orbital (LUMO), and form transient ions. In the TPPE experiments, the overall illumination time of a monolayer sample was only 10 μs to avoid any radiation damage.

The "probe" photons eject electrons from these transient ion states to above the vacuum level. As was demonstrated previously in ref 33, an intense TPPE signal means either that the layer captures excited electrons very efficiently, or that the lifetime of the electrons in the LUMO is very long, allowing for a high transient population to form. The measured kinetic energy of the ejected electrons provides information on the binding energy of the electrons, namely, the energy of the LUMO. Figure 2 clearly indicates that the intensity of the TPPE signal increases with the number of G nucleobases in the PNA strand, and thus that the G nucleobases play an important role in the capture of electrons by PNA. This result is

Scheme 3. Schematic Representation of the Principle Behind LEPET (a) and TPPE (b) Spectroscopies Using DNA as an Example[a]



[a](a) Electrons ejected with a single photon from below the Fermi of the Au substrate to the vacuum pass through the monolayer. Some of them are captured by a SAM of DNA. (b) Electrons are excited to states above the Fermi level. These electrons are transferred to the monolayer only when their energy matches that of a negative ion state of the DNA/PNA molecules in the SAM. A second photon ejects the electrons from the molecules to the vacuum.

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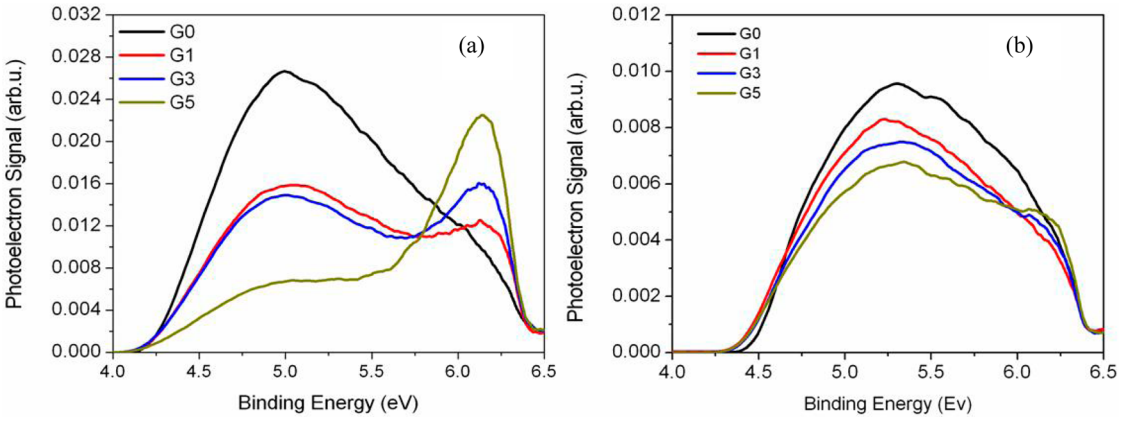

Figure 1. LEPET spectra for SAMs of (a) ssPNA (b) ssDNA with different G content. The high binding energy peak results from direct ionization of the guanine bases. This peak is cut off and less noticeable in panel b because of the higher work function of the gold covered with DNA SAMs as compared to gold with the PNA SAMs. The low energy peak corresponds to electrons excited from the Au−S bond.

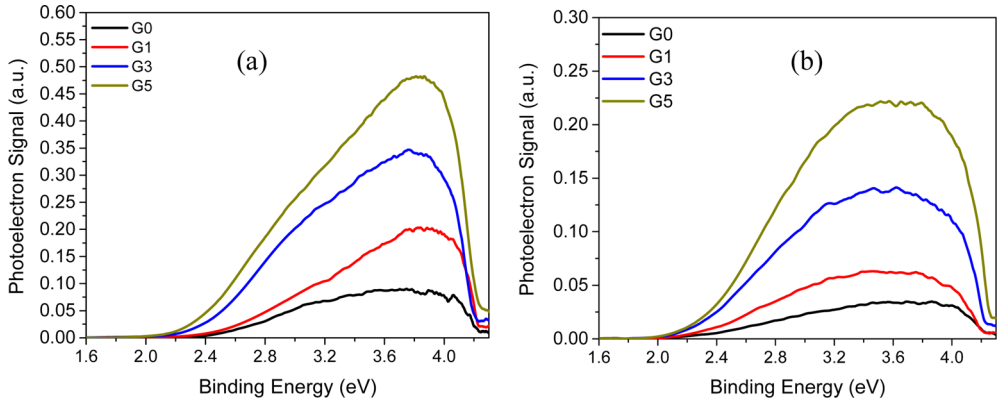

Figure 2. The TPPE spectra for PNA (a) and DNA (b) obtained for monolayers made from the different oligomers. The spectra are composed from two bands: one centered at about 3.8 eV and the other at 3.0 eV as explained in the Supporting Information (Figure S5). As the number of G bases in the oligomer increases, the intensity of the low energy peak increases more than the intensity of the high energy one. The spectra were obtained by illuminating the samples with radiation of 295 nm wavelength.

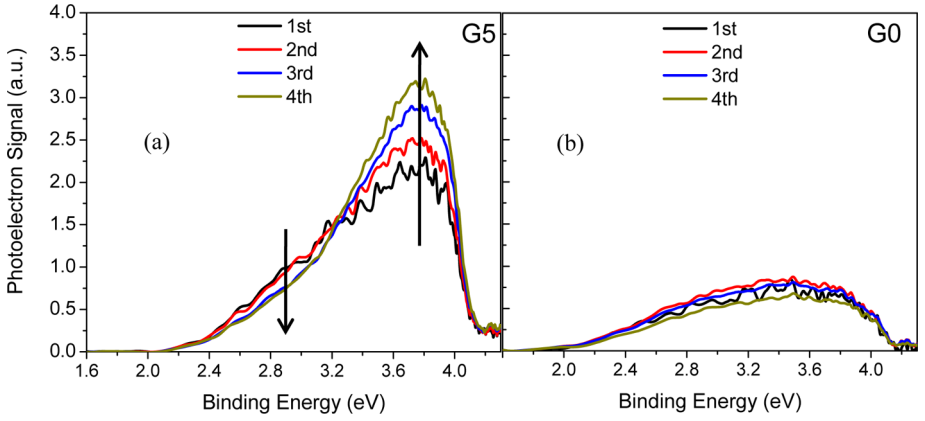

Figure 3. TPPE spectra for SAMs of ss PNA G5 (A) and G0 (B). The spectra were taken every 100 laser shots. In the case of G5, the spectrum evolved over time. Specifically, the intensity of the low binding energy peak (3.0 eV) decreased with time, while the high binding energy peak (3.8 eV) increased. In the case of G0, the spectrum was independent of time within the signal-to noise ratio.

consistent with the LEPET results (see above), which indicated that the capturing probability increased as the number of Guanines increased, as well as with the results obtained for DNA.

Previous studies showed that the binding energy for electrons residing on DNAs does not depend on the sequence of the DNA, and led to the conclusion that after the electrons are captured by the nucleobases, they are transferred rapidly to the LUMO of the DNA, which is localized primarily on the DNA backbone.[14] By contrast, the results obtained for the PNA monlayers show two TPPE peaks and their relative intensity vary as a function of the number of G bases (Figure 2). Hence, unlike the LUMO of DNA, the LUMO of PNA *is sensitive to the*

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sequence of nuclebases, which means that it is at least partially localized on the nucleobases.

Additionally, for PNAs containing one or more G bases, the TPPE spectrum changed gradually as a function of illumination time (Figure 3). Specifically, the low binding energy peak decreased while the high binding peak increased with time (a few seconds). This effect was reversible, and the original spectra was reacquired after leaving the sample a few tens of seconds in the dark; this indicates that the SAM was not damaged by illumination. The time scale of seconds on which these changes occur is long, suggesting that the molecular events that cause them are not (only) electronic in nature but may involve large-amplitude, structural reorganization of the molecules, such as the reorientation of the amino-acid groups to stabilize the captured electron. Another possibility is that a cooperative change takes place in the structure of the monolayer that also stabilizes the electrons. For probing the existence of cooperativity, we studied PNA monolayers that contained a mixture of G0 and G3 oligomers. SAMs were formed by incubation with solutions containing different ratios of the G0 and G3 oligomers (G3:G0 = 1:0, 1:1, or 1:4). The change of the TPPE spectra as a function of illumination time was observed irrespective of the G3:G0 ratios in the solution from which the SAMs were formed (Figure S4). This result suggests that the effect is a molecular property rather than a cooperative one.

The present work shows that the guanine plays a special role in the capture of electrons by both DNA and PNA. In DNA, the studies showed that the LUMO is localized mainly on the backbone of DNA. It is important to appreciate that the LUMO is the state of the negatively charged DNA/PNA and it is not simply related to the excited state of the neutral DNA. The peptide carbonyl group in the linker that connects the PNA nucleobases to the backbone may play in PNA a role similar to that played by the phosphate groups in DNA. Specifically, these groups would attract some negative charge from the guanine, leaving it partially positively charged. The TPPE results suggest that after the electron is captured by G in PNA, it remains close to the nucleobase.

This study provides first insight in the process of electron capturing by PNA and the comparison between the electron capture by single stranded DNA and PNA strengthens earlier conclusions regarding the mechanism of low energy electron capturing by DNA. We observed that the guanine plays an important role in capturing low energy electrons by DNA, and its interaction with the phosphate in the backbone of DNA has a crucial role in the fast transfer of the electrons captured by the DNA nucleobases to the backbone. This is due to the large electron-affinity of the phosphate. In PNA, where the phosphate is not present and there is no group on the backbone with such high electron affinity, no fast transfer to the backbone occurs; once captured, the low energy electrons are localized, at least partially, on the nucleobases. The present study indicates that the electronic interaction between the backbone and the nucleobases plays an important role in the electron capturing by oligonucleotides and thus in the single-strand breaks caused to DNA in the DEA process.

EXPERIMENTAL METHODS

We have synthesized the PNA oligomers listed in Table S1. The PNA oligomers were purified by high-performance liquid chromatography (HPLC) and characterized by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-ToF). Self-assembled monolayers of 3′-thiolated DNA 15-mers were prepared according to standard procedures[6] by incubating clean gold substrates with a 20 μM solution of the ss DNA in pH 7.2 0.4 M potassium phosphate buffer. The clean Au slide was covered uniformly with the oligomer solution and kept overnight in a clean and controlled humid environment within a desiccator. After deposition, the slides were washed thoroughly first with pH 7.2 0.4 M potassium phosphate buffer and subsequently with deionized water (Millipore). The samples were then dried in N2.

Self-assembled monolayers of PNA were prepared similarly by incubating the clean gold substrates with 25 μM solutions of the thiol-modified PNAs in pH 7.2 10 mM potassium phosphate buffer. The clean Au slide covered uniformly with the oligomer solution was kept overnight in a clean and controlled humid environment. After deposition, the slides were washed thoroughly first with the pH 7.2 10 mM potassium phosphate buffer and subsequently by deionized water (Millipore). The samples were then dried with N2.

DNA and PNA monolayers were characterized by FTIR (Figure S2) and XPS (Table S2). Based on both techniques, we conclude that the PNA monolayers were more densely packed than the DNA monolayers. The XPS results showed no free thiols, which indicates that the monolayers are densely packed and that there is no multilayer stacking. Additionally, extensive rinsing of the PNA samples with 50% acetonitrile−water solution did not show any significant effect on the FTIR and LEPET spectra of the 2ET-modified PNA monolayers.

ASSOCIATED CONTENT

Supporting Information

PNA synthesis and characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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