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Photoelectrochemistry as a novel strategy for DNA hybridization detection

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The special properties of ssDNA and dsDNA molecules in structure and electric behavior, may offer us some new ideas for the fabrication of genosensors and DNA-chips. In this work, the photoelectrochemical method was firstly employed to characterize the photoelectric behavior of a ssDNA probe electrode, which was prepared with the self-assembly technique, and its resulting dsDNA electrode. The obvious decrease in the photocurrent of the dsDNA modified electrode at open potential or a bias voltage indicated that photoelectrochemistry was another useful method for DNA hybridization detection. Using the special design of ssDNA probes, we attempt to discuss further the relationship between the properties of DNA molecules and their photoelectric behaviors. In addition, the electrochemical impedance method was employed to verify the occurrence of some modifications over the electrode interface before and after the hybridization event.

Introduction

The rapid progress in the Human Genome Project and the strong desire for health these days has stimulated development of DNA-chips. Until now, about 3 billion functional gene segments have been found for the human body. With these genes assembled on a few DNA-chips, it is expected that we can quickly check our health condition and prognose diseases in the near future. However, it still needs a continuous effort for the DNA-chip to enter into everyday use. How to assemble as many gene probes as possible on a small chip and how to simultaneously and rapidly recognize these specific binding events are the main problems for the fabrication of DNA-chips. Recently, some techniques have been successfully applied to develop genosensors based upon the combination of a suitable transducer, such as electrochemical,[1–4] chemiluminescent,[5] quartz crystal microbalance,[6–8], fiber optical,[9] evanecent wave,[10] or an acoustic wave devise[11] with an immobilized ssDNA probe for the specific-sequence detection by DNA hybridization. Further understanding of native physical and chemical properties of DNA, will present us with some other new ideas for DNA hybridization detection.

Since Barton *et al.*[12] first reported their exciting discovery that long distance photoinduced electron transfer (> 40 Å) may be mediated by the DNA helix, whether the π-stack DNA system functions as a molecular wire or an insulator, has been the subject of much interest and dispute.[13–15] At least, it has been generally accepted that this π-stack system should have some unique electric properties different from other kinds of biological macromolecules such as proteins or carbohydrates. On the one hand, electrochemical techniques have been shown to directly provide certain information about the electron transfer properties of investigated molecules. On the other hand, electrochemical methods can also be useful tools to qualitatively or quantitatively describe the interaction between two molecules occurring on the electrode interface according to the differences in their interfacial behaviors. As a result, some electrochemical techniques have been employed in this respect. For instance, the voltammetric detection method,[3,4] which is based upon the differences in the redox signal of an indicator, only interacts with the double DNA helix instead of the single-strand DNA. Another method is the ac impedance method,[16] which is based upon the fact that electrode interfacial behaviors are different prior to and after the hybridization event since the charge distribution over a ssDNA molecule differs from that of its resulting dsDNA.

Since Beckquerel first found in 1839 that illuminating certain electrodes in solution generated an electric current, numerous studies of the effect of light on electrodes have been carried out in the proceeding years.[17] Recently it has been used as a powerful method to investigate the properties of semiconductor electrodes, photoinduced long-distance electron transfer and the nature of photosynthesis *etc.* In recent research,[18–20] this technique has also been applied to investigate the photoelectric behavior of supercoiled DNA and the apoptosis process of a cell. On account of the fact that DNA molecules and proteins have some characteristics of semiconductors,[16] photoelectrochemistry may possibly act as a novel approach to investigate some biological processes. In this paper, we aim to study the photoelectric behavior of ssDNA and dsDNA molecules, and thus discuss the possibility of detecting DNA hybridization with this method.

Experimental

Reagents

Two 15-mer thiolated oligonucleotide probes were synthesized by ShangHai Biochemical engineering corporation in China. One sequence only containing G and T was 5′-HS-TTTTTTTTTTGGGTTGGGTTGGGTT-3′ (probe 1), with a complementary oligomer of sequence 3′-CCCAACCCAACC-CAA-5′. The only other oligomer only containing A and C was 5′-HS-CCCCCCCCCCAAACCAAACCAAACC-3′ (probe 2), with a complementary part 3′-TTTGGTTTGGTTTGG-5′. The non-complementary DNA oligomer used was: 3′-TCTACGT-CACAACTA. All the solutions used in the experiment were prepared with sterile distilled water. Electrochemical measurements were conducted with PBS buffer solution (NaCl 136.7 mmol L^{-1}, KCl 2.7 mmol L^{-1}, $Na_2HPO_4 \cdot 12H_2O$ 9.7 mmol L^{-1} and KH_2PO_4 7.3 mmol L^{-1}). Hybridization reactions were performed in 2 × SSC (15 mM Na citrate–ISO mM NaCl) buffer.

Apparatus

AC impedance experiments were carried out with a CHI660A electrochemical workstation (CH Instruments, Cordova, TN, USA). The photoelectric measurements were performed with a Xe light souce (Muller, light intensity 121.4 mW cm^{-2}), the

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CHI660A workstation was used to impose a bias voltage and record photocurrent simultaneously.

Electrode preparation

A gold electrode with area 0.3 mm^2 was first polished with ultrafine alumina, then put in a warm piranha solution (70% concentrated sulfuric acid and 30% peroxide solution) for 15 min, and then rinsed with water thoroughly. The ssDNA modified electrode was prepared by immersing a pretreated gold electrode in 20 μM solution of an oligonucleotide probe in PBS overnight, then rinsing with water carefully before use. Hybridization was performed at 37 °C for 1 h in a 2 × SSC buffer, the concentration of complementary and noncomplementary target was 20 μM. Upon removal from the hybridization reaction solution, the electrode was rinsed with water and PBS solution thoroughly. This was the so-called dsDNA modified electrode.

Procedures

AC impedance experiments were performed in a single-compartment cell of 10 mL volume. The working electrodes were prepared as described above. A saturated calomel electrode (SCE) and platinum wire served as reference and counter electrode respectively. PBS solution was used as supporting electrolyte. Photoelectrochemical measurements were carried out in a quartz crystal rectangular cell of 5 mL volume, other experimental conditions were the same as above.

Results and discussion

Fig. 1 shows the photoelectric response behavior of two probe modified electrodes and their complementary dsDNA modified electrodes in PBS buffer respectively, when they are illuminated with a Xe light power at 110 mW cm^{-2} at 200 mV bias voltage. It reveals that the photocurrent of the bare electrode in buffer is much lower than that of the ssDNA modified electrode. For the ssDNA modified electrode, the photocurrent increased with ion strength of supporting electrolyte, implying that the change of the ion strength of the buffer may modify original double-layer structures of the electrode interface. The obtained negative photocurrent indicated that the electrons transferred from the DNA molecule to the substrate electrode, and increased linearly with bias voltage up to 500 mV. As higher voltage would damage the DNA probe by oxidation of the Au–S bond, all the photoelectric experiments were consequently performed with the applied voltage less than this critical value. From Fig. 1, it can be observed that the photocurrent of the dsDNA modified electrode decreased after hybridization. The higher the bias voltage, the greater the decrease in photocurrent. However, if the above experiments were conducted using both probe electrodes with their noncomplementary strand (3′-TCTACGTCACAACTA), little change was observed in their response photocurrent, suggesting that photoelectrochemistry was an available method to recognize DNA hybridization. Fig. 2 shows the action spectrum of ssDNA and dsDNA modified electrodes respectively. Both electrodes attained their maximum photocurrents at about 400 nm, which was almost in agreement with the result obtained from plasma DNA.[19] Therefore, it can be concluded that the observed photoelectric behaviors reflect the properties of immobilized DNA molecules.

With these two specially designed DNA probes, we can make a detailed discussion on the mechanism of their photoelectric response. The DNA molecule can be considered as a semiconductor. It has been shown that electron transfer along the DNA helix has a close relationship with the properties of G.[12,13,21–23] As the oxidation potential of G is lower than A and the other two bases can not be oxidized near the oxidation potential range of G and A, the DNA molecule with a sequence of probe 1 ought to be a good mediator for electron transfer. Owing to the lower oxidation potential of G, probe 1 might also possibly act as a photoactive molecule in which G serves as a donor. Under this assumption, when the probe 1 modified electrode was illuminated, the observed photoelectric behavior should result from the electrons hoping from G to the substrate electrode mediated by the DNA helix. When hybridized with its complementary part, the photocurrent of the dsDNA modified electrode decreased. This might be ascribed to the fact that the activity of G decreased when specifically binding with C. This assumption seemed in good agreement with the observed results. In order to further confirm the accuracy of this assumption, another probe was specially devised to have only A and T in its structure and employed to repeat the above experiment. To our surprise, we also observed that when the probe 2 modified electrode was hybridized with its complementary part, the photoelectric current of the dsDNA electrode decreased as well. This seemed unreasonable based on the above hypothesis, since there were more G in the complementary part, the addition of G in dsDNA helix would be expected to result in the increase in the photocurrent of dsDNA electrode as G was considered the only source of donor in this system. Therefore, the experimental results indicated that the observed photoelectric phenomenon did not merely come from

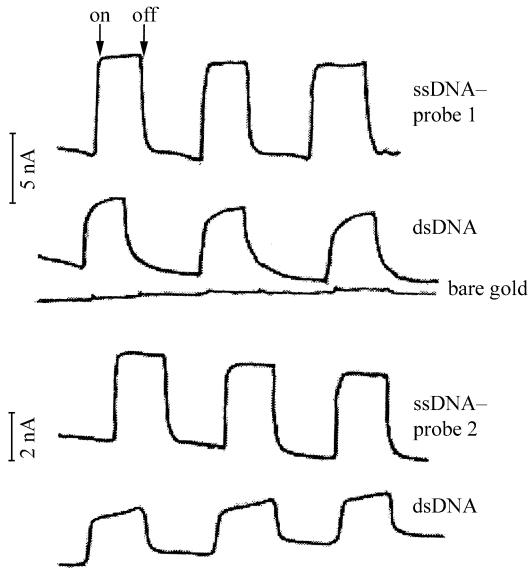

Fig. 1 Photoelectric behavior of bare gold electrode, ssDNA and dsDNA modified electrodes illuminated with a Xe light power at 110 mW cm^{-2} at 200 mV bias voltage.

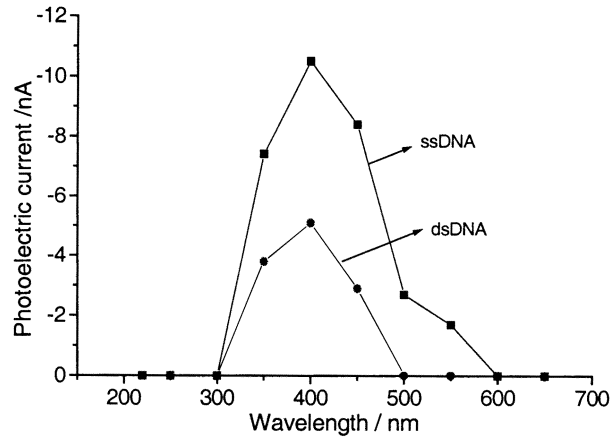

Fig. 2 The effect of light wavelength on the photocurrent of ssDNA and dsDNA modified electrodes.

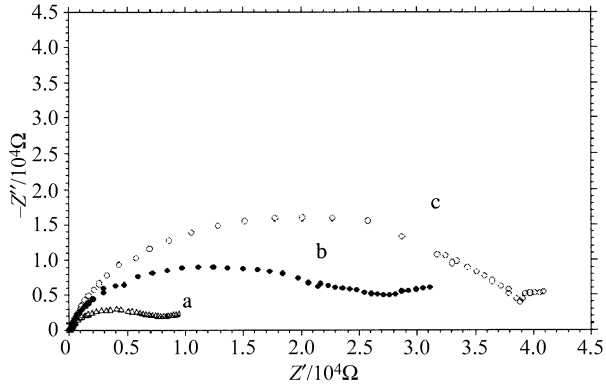

Fig. 3 Nyquist plots for faradaic impedance measurements of a gold electrode in the presence of 2 mM $[Fe(CN)_6]^{3-/4-}$: (a) bare electrode, (b) Au electrode with self-assembly of a monolayer of ssDNA (probe 2), (c) dsDNA–Au electrode after hybridization with its complementary part.

one base in the DNA helix but reflected the total interfacial properties of the modified electrodes. The DNA molecule is a kind of polyanion. The self-assembly of negatively-charged ssDNA and specifically formed double-stranded DNA molecule would alter the double-layer potential at the electrode surface, and further the Fermi energy difference at the electrode/solution interface, and eventually lead to the different photoelectric behaviors of each kind of electrode. The changes in the interfacial state of the electrode before and after the hybridization event can be clearly verified with the ac impedance measurement results shown in Fig. 3. Fig. 3 shows the electrochemical impedance spectra of gold electrodes before and after modification with ssDNA and dsDNA molecules. It can be observed that the formation of self-assembling monolayers of a ssDNA probe on gold, and latter the formation of dsDNA through the hybridization with its complementary part, resulted in an obvious increase in the curvature of the plots. Hybridization of both probes with their noncomplementary parts almost retained the original impedence behaviours of their ssDNA′ parts respectively.

In conclusion, owing to the special properties of the π-stack DNA system, and large differences in the photoelectric behaviors of the ssDNA modified electrode and resulting dsDNA electrode, photoelectrochemistry can also be used as a powerful tool to rapidly detect DNA hybridization. In addition, based upon the special electronic properties of the DNA helix before and after the hydridization event, its detection sensitivity was expected to be increased by introducing a donor or acceptor which had a special interaction with ssDNA or dsDNA.

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