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Electric potential control of DNA immobilization on gold electrode

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Abstract

The assembly of synthetic, controllable molecules is one of the goals in nanotechnology. The primary objective of this contribution is to selectively immobilize DNA on gold via electric potential control. The self-assembly monolayer (SAM) was prepared with 2-aminoethanethiol (AET) on the gold electrode. A new approach based on electric potential was firstly used to control DNA immobilization covalently onto the SAM with the activation of 1-ethyl-3(3-dimethyl-aminopropyl)-carbodiimide (EDC) and N-hydroxysulfosuccinimide (NHS) in low ionic strength solution. The influence of electric potential on DNA immobilization was investigated by means of cyclic voltammogram, A.C. impedance, auger electron spectrometer as well as atomic force microscope (AFM) on template-stripped gold surface. The result proves that controlled potential can affect the course of DNA immobilization. More negative potential can restrain the DNA immobilization, while the more positive potential can accelerate the DNA immobilization. It is of great significance for the control of DNA self-assembly and will find wide application in the fields of DNA-based devices.

Keywords: DNA immobilize; Controlled potential; DNA-based device

1. Introduction

DNA is an important and promising molecule with a diameter of 2 nm, which has all the basic properties necessary for the assembly of nanoscale electronic devices. DNA has a special double-helix structure with one-dimensional charge transport (Fink and Schönenberger, 1999; Porath et al., 2000), phosphorus bridges and hydrogen bonds, which may be a good candidate for tunnel junction and capacitance (Ben-Jacob et al., 1999). Also, DNA templates can direct the growth of semi-conductive nanocrystals and metal wires (Braun et al., 1998). The construction of DNA electronic nanodevices is possible and will also be a predominant technique of new molectron with attendant benefits of miniaturization, low power requirements, high efficiency and low heat generation. DNA immobilization has been considered as a fundamental methodology for construction of molecular logic circuits, and the control of DNA immobilization is clearly an essential step for construction of DNA-based nanodevices.

On the basis of self-assembled monolayer (SAM) technique, a number of different methods for immobilizing DNA onto electrode have been recently reported. Controlled electrostatic adsorption was adopted by Wang and Wu to anchor DNA to carbon paste electrodes (Wang et al., 1996) or highly ordered pyrolytic graphite electrode (Wu et al., 2000). Herlne and Tarlov (1997) and Levicky et al. (1998) synthesized mercapto oligonucleotides and directly immobilized on gold. Sun and Zhao (Sun et al., 1998; Zhao et al., 1999) reported that DNA could be covalently immobilized onto modified electrode using 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysulfosuccinimide (NHS) as the activation coupling reagents. In general, physical adsorption of DNA onto the electrode is simple but either carbon paste or highly ordered pyrolytic graphite electrode is not suitable for the DNA-based device. Functional DNA with mercapto is tedious because the procedure for the modification of DNA to the electrode is very complicated, and the yield of synthesis of mercapto-containing DNA is quite low. In principle, covalently immobilization of DNA onto gold

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via SAM is practicable for DNA-based biosensor, if the self-assembly process can be well controlled. Our efforts are focused on selectively immobilization of calf thymus DNA on gold that is pre-modified with a self-assembly monolayer of 2-amino-ethanethiol (AET) via electric potential control in presence of the activation of EDC and NHS. It is of great significance for the control of DNA immobilization, and the wide application of this technique in the fields of DNA-based device would be expected.

2. Experiment

2.1. Materials

Reagents calf thymus DNA, purchased from Sino-American Biotechnical, was purified as literature (Chaires et al., 1982) to reach a high purity ($A260/A280 \geq 1.8$, where A represents the ultraviolet absorbance). The molecular mass of DNA, estimated by gel electrophoresis in 1% agarose gel, was $4–6 \times 10^6$ Da. AET (Sigma), EDC (Sigma) and NHS (Acros) were used without further purification. $[Co(phen)_3](ClO_4)_3$ was prepared according to the literature (Dollimore and Gillard, 1973). The gold foils used for AES measurements were obtained by vacuum evaporation of high-purity gold onto clean single-crystal silicon wafers, which had been precoated with chromium to improve adhesion. The flat gold foils for AFM were prepared with template-stripped technique reported by Wagner (Wagner et al., 1995) with some modification. Other chemicals were of analytical reagent grade. Millipore water was doubly distilled and sterilized in Milli-Q plus. All solutions were deaerated with the purge of nitrogen.

2.2. Apparatus

Cyclic voltammogram (CV) and AC impedance experiments were performed on CHI 660A workstation (American CH instrument). The electrochemical cell consisted of a three-electrode system with gold or modified gold electrode (0.5 mm in diameter) as the working electrode, a saturated calomel electrode (SCE) and a platinum wire as the reference and the counter electrode, respectively. The experimental temperature was controlled at 25 ± 1 °C. AC impedance experiments were carried out within the frequency range of 10 mHz to 100 kHz. A 10 mV RMS sinusoidal potential signal was applied to the electrode held at open circuit potential. Auger electron spectra (AES) were recorded using an AES-350 electron energy spectrometer (Anelva, Japan). Atomic force microscopic (AFM) images were collected with a Nanoscope IIIa atomic force microscope (American Digital Instruments, Santa Barbara) with the tip of silicon nitride in tapping mode at room temperature.

2.3. Pretreatment of the gold electrode

Gold electrodes were polished firstly with w5 abrasive paper and subsequently with chammy followed by rinsing with water and then sonification in ethanol and water, respectively, for 2 min each. The extent of electrode pretreatment was evaluated by a CV measurement in 1 mM $K_3Fe(CN)_6$ solution containing 0.1 M KCl. The peak-to-peak separation should be less than 70 mV at a scan rate of 100 mV/s.

2.4. Modification of the electrode

Before chemisorptions, the pretreated electrodes or gold foils were treated with 'piranha solution' (concentrated H_2SO_4/30% H_2O_2, 7:3 in v/v) for 5 min at room temperature and subsequently rinsed thoroughly with twice distilled water and finally ultrasonically cleaned with ethanol and twice distilled water for 3 min, respectively. The pretreated electrodes were then immersed in 1 mM AET ethanol solution for 16 h resulting in the AET deposition on the electrodes. After that, the electrodes were thoroughly washed with ethanol and distilled water. The electrode was kept in distilled water till use and denoted as AET/Au.

2.5. Potential control of DNA self-assembly

A gold electrode modified with AET (denoted as AET/Au) was initially immersed in an activation solution containing 5 mM EDC and 8 mM NHS in PB buffer with pH 7.0 for 15 min. The electrode was subsequently used as the working electrode under the controlled potential for 1 h in 1 mg/ml calf thymus DNA solution consisting of 5 mM NaCl and 5 mM Tris–HCl buffer with pH 7.1. The DNA solutions were incubated at 37 °C for 30 min to extend coil state before the immobilization. The DNA modified electrode (denoted as DNA/AET/Au) was then soaked in Tris–HCl buffer for 10 min to remove any non-specifically adsorbed DNA.

2.6. Nonlinear simulation of equivalent circuit

The electrochemical impedance spectroscopes (EIS) were collected on CHI workstation. The data were subsequently converted to text style. The equivalent circuit that we used to model the SAM system was Randles equivalent circuit (Bard and Faulkner, 1980) with the circuit description code (CDS) of R(C[RW]). The capacitances of double-electron layer were simulated by nonlinear least squares (NLLS) using EQUIVCRT.PAS (EQU) (Ver. 4.51) program written

by Boukamp (1993). The errors of capacitance were less than 6%.

3. Results and discussion

3.1. Selections of buffer and controlled time

To preserve DNA physiological pH and to avoid DNA-strand splitting effects (Carter et al., 1989), a pH 7.1 solutions with the constant ionic strength of 50 mM NaCl and 5 mM Tris–HCl buffer was used in electrochemical measurement. The property of the modified electrode was examined by cyclic voltammogram using 0.12 mM $[Co(phen)_3](ClO_4)_3$ as an electro-active species that was added in the Tris–HCl buffer solution. Since EDC and NHS could activate the amide and hydroxide (Desai et al., 1993), the activation solution should be prepared with PB buffer instead of Tris–HCl buffer. A potential controlled time of 1 h was used as the control potential, since at which the CV closely displayed maximum peak currents.

3.2. Justification of covalently self-assembly of DNA on AET

Covalently self-assembly of DNA on AET was justified by cyclic voltammogram (CV). Fig. 1 shows the comparisons of peak current vs v (a), peak current vs $v^{1/2}$ (b) and CV curve at 50 mV/S (c) for bare Au, AET/Au, DNA/AET/Au electrodes prepared at control potential +0.1 V in pH 7.1 Tris–HCl buffer containing 0.12 mM $[Co(phen)_3](ClO_4)_3$. Comprehensively, the following features were observed: (1) the peak currents obtained for the reduction of $Co(phen)_3^{3+}$ species at DNA/AET/Au were much larger compared to those at AET/Au and bare Au electrodes, and only slightly large peak currents were observed at AET/Au with respect to those obtained at bare Au electrode; (2) the ratio of reduction peak current to oxidation peak current was greater than 1 at DNA/AET/Au electrode but close to 1 at both AET/Au and bare Au electrode; (3) in the cases of AET/Au and bare Au, linear correlations between peak currents and the square root of scan rate were observed, while in the case of DNA/AET/Au, the above correlation was deviated from a straight line and exhibited that the peak currents increased rapidly with increasing scan rate. These data suggest that DNA was efficiently immobilized onto the AET/Au electrode, and that $Co(phen)_3^{3+}$ species can relatively strongly adsorbed onto the DNA/AET/Au electrode. Similar behavior has also been reported for DNA modified electrodes (Pang et al., 1996; Pang and Abruha, 1998).

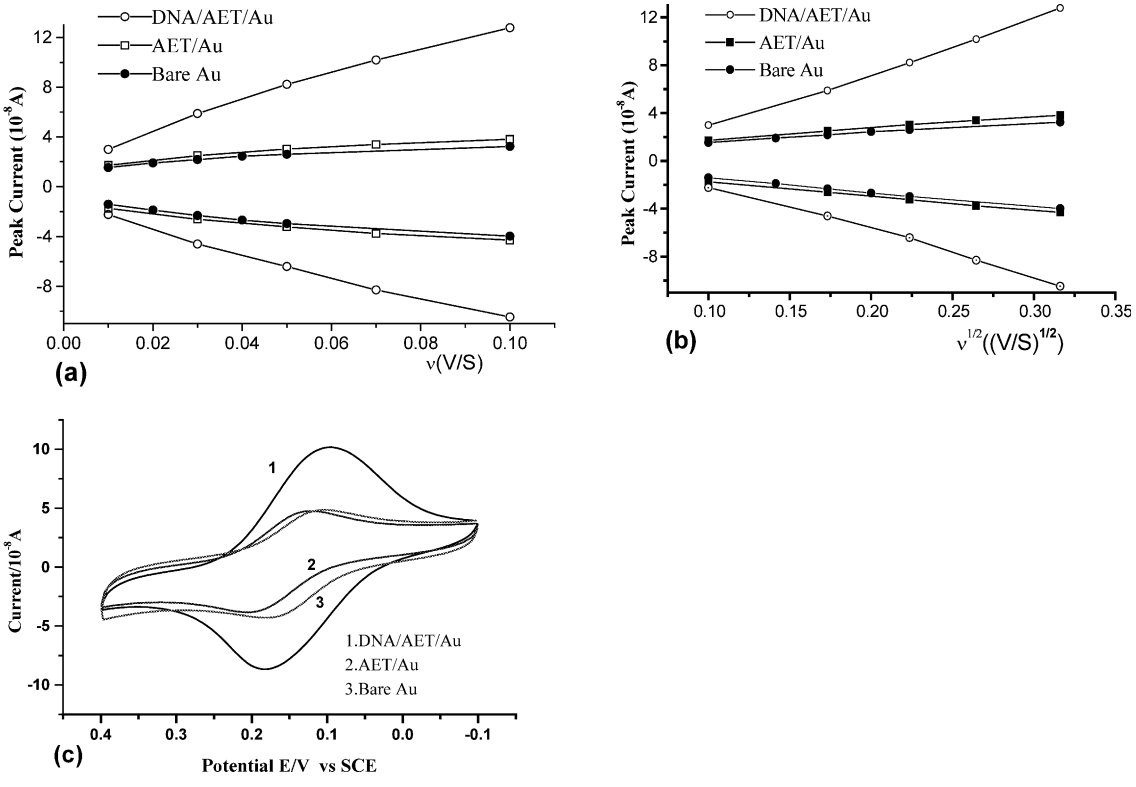

Fig. 1. Cyclic voltammogram (CV) plots for bare Au, AET/Au, DNA/AET/Au electrodes prepared at control potential +0.1 V in pH 7.1 Tris–HCl buffer containing 0.12 mM $[Co(phen)_3](ClO_4)_3$. (a) Peak current vs v, (b) Peak current vs $v^{1/2}$ and (c) CV curve at 50 mV/S.

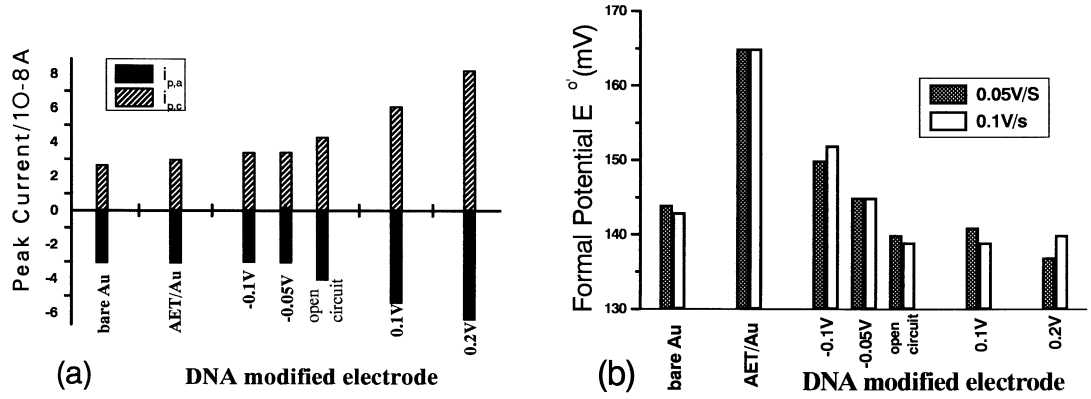

Fig. 2. Comparison of peak currents (a) ($u = 50$ mV/S) and formal potential $E^{0\prime}$ (b) for bare Au, AET/Au, DNA/AET/Au electrodes prepared at different control potentials.

3.3. Analysis of peak current i and formal potential $E^{0\prime}$ at different electrodes

The CVs for the reduction of $Co(phen)_3^{3+}$ at DNA/AET/Au electrodes obtained under different control potential values were measured at a scan rate of 50 mV/s and 100 mV/s. Fig. 2 shows comparisons of the peak current i (a) (at 50 mV/s) and formal potential $E^{0\prime}$ (b) at different electrodes. It is clear that the peak current at DNA/AET/Au electrode steadily increases as the controlled potential becomes more positive, while those with controlled potentials of -0.05 and -0.10 V versus SCE are nearly equal to those obtained from a bare Au and AET/Au electrode. The shift of formal potential $E^{0\prime}$ could characterize interaction between $[Co(phen)_3]^{3+}$ and DNA, which could quantify DNA immobilized on AET/Au. The $E^{0\prime}$ values for the electrodes prepared by controlling potential at -0.05 and -0.1 V versus SCE shift positively compared with that for bare gold, but they shift negatively relative to that for AET/Au. The more positive of the controlled potential applied for DNA immobilization, the more negative shift of the formal potentials. It seems that the immobilization of DNA onto the AET/Au was insignificant at a controlled potential value of -0.1 V versus SCE. This can be readily explained, since DNA is a poly-anion with negative charges and negative potentials could keep DNA from approaching the electrode, while positive potential could accelerate the DNA immobilization. This finding suggests that DNA immobilization could be selectively controlled with electric potential, which is significant for manufacture of DNA-based devices.

3.4. AC impedance analysis for different electrodes

AC impedance data for bare Au, AET/Au, DNA/AET/Au electrodes prepared at different control potentials were recorded in the electrolyte solution of 1 mM $K_3Fe(CN)_6$ and 1 mM $K_4Fe(CN)_6$ containing 0.1 M KCl. The Nyquist curves for DNA/AET/Au electrodes prepared at the controlled potential values of $+0.1$ and -0.1 V versus SCE were presented in Fig. 3. Similar behavior between the bare Au and the AET/Au electrode is evident. In both cases, small charge transfer resistances are obtained. However, after modification of DNA onto the AET/Au electrode, particularly when a control potential value of $+0.1$ V versus SCE was used, the charge transfer resistances increase significantly. The capacitance of the electrode was also varied largely with the presence of DNA. It was found that after immobilization of DNA onto the AET/Au electrode, the capacitance changed from 6.19×10^{-8} F for AET/Au to a much small value of 2.85×10^{-8} F for DNA/AET/Au electrode prepared at a control potential value of $+0.1$ V versus SCE, while that of electrode prepared at a control potential value of -0.1 V was 4.293×10^{-8} F. The reason for difference between AET/Au electrode and DNA/AET/Au electrode prepared at a control potential value of -0.1 V may be the activation before potential control of DNA immobilization, suggesting

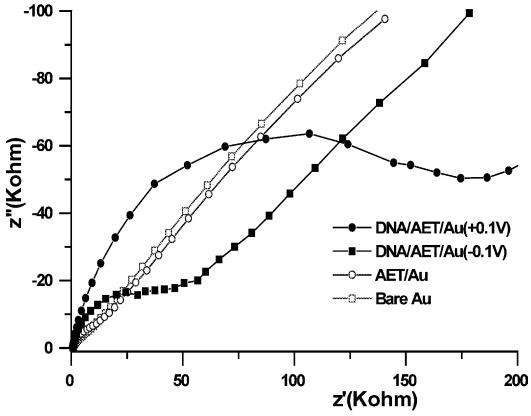

Fig. 3. Nyquist curve of bare Au, AET/Au and DNA-modified electrode at -0.1, $+0.1$ V control for 1 h. Supporting electrolyte solution is 1 mM $K_3Fe(CN)_6$ and 1 mM $K_4Fe(CN)_6$ containing 0.1 M KCl.

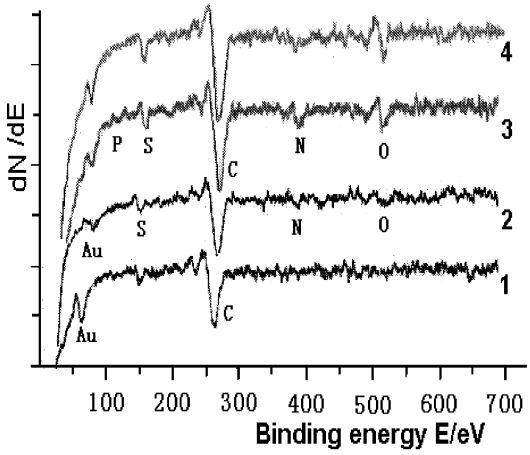

Fig. 4. Auger electron energy spectra of bare gold foil (1), AET modified gold foil (2), DNA self assembly gold foil (controlled potential at +0.1 V) (3) and DNA self-assembly gold foil (controlled potential at −0.1 V) (4).

that DNA could not be immobilized onto gold foil modified SAM of AET under control potential −0.1 V.

3.5. Auger electron spectra (AES)

Auger electron energy spectra of bare gold foil (1), AET modified gold foil (2) and DNA self-assembly gold foils (at a control potential of +0.1 (3) or −0.1 V (4) vs. SCE) are presented in Fig. 4. The main peaks observed on bare gold foil were C and Au, indicating that these elements existed on bare gold foil. The presence of C on Au is probably because of the adsorption of CO_2 from atmosphere. The main peaks observed on AET modified gold foil were N and O besides C, indicating the presence of AET on gold foil. The P peak was present on the DNA self-assembly gold foil (at the controlled potential +0.1 V) accompanying relatively strong N, O peaks, confirming the strong immobilization of DNA onto the surface of AET/Au, while the P peak cannot be observed on the DNA self-assembly gold foil prepared at the controlled potential of −0.1 V. Consistent with the previous data obtained from the CV and AC impedance, results based on the AES experiments suggest DNA can only be well adsorbed on the surface of AET/Au electrode at positive potentials relative to SCE.

3.6. AFM images

To correlate with our characterization of CV and AC impedance spectra, we directly visualized the immobilized DNA, and more importantly to determine the presence of DNA. As shown in Fig. 5, atomic force microscope (AFM) was employed to examine gold foil modified with AET (a), gold foil self-assembled with DNA at a controlled potential +0.1 V (b) and −0.1 V

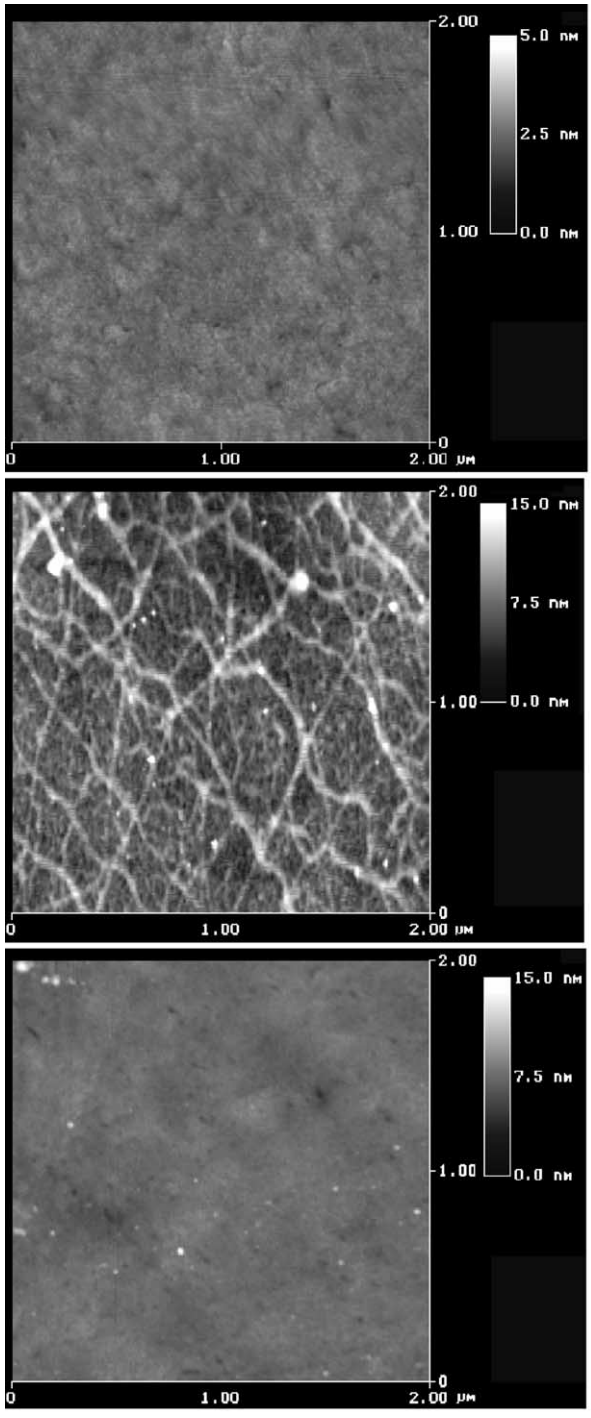

Fig. 5. Tapping mode AFM images of gold foil modified with AET (a), gold foil self-assembled with DNA at a controlled potential of + 0.1 V (b) and −0.1 V (c).

(c). From Fig. 5(a), the gold foil modified with AET with a roughness of 0.205 nm was very flat distinctly. It was nearly as flat as polishing silicon wafer, which was sufficient for the structural basis of DNA immobilization for AFM measurement. Fig. 5(b) shows the image of gold foil self-assembled with DNA at a controlled

potential +0.1 V with the displays of the individual widths of 15–20 nm, which were clearly different from the topology in Fig. 5(a), suggesting that the calf thymus DNA was inter-twisted together with some extent of aggregation.

The wideness of DNA in AFM was 15–20 nm because of the magnification effect (Hansma et al., 1992; Lyubchenko et al., 1993) for the presence of interaction between the tip and sample. The interaction includes Van de Waals attraction, capillary adsorption and electrostatic force. From Fig. 5(c), the appearance of gold foil self-assembled with DNA at a controlled potential of −0.1 V was similar to that modified with AET, despite of some difference for the activation before control potential of DNA self-assembly. The results also indicate that the modification of DNA to AET/Au at a negative potential value is insignificant and sometimes is not detectable, which is consistent with previous findings.

4. Conclusion

This work demonstrated that ds-DNA immobilization could be controlled by potential. Negative potentials can restrain DNA immobilization, while the positive potentials can accelerate DNA immobilization. It is of great significance for the control of DNA immobilization and the technique reported above could be very useful in the fields of DNA-based devices, despite further studies are clearly required before any practical applications. The studies could include the selection of DNA, the supporting electrolyte effect and the aggregation of DNA.

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References

Bard, A.J., Faulkner, L.R., 1980. Electrochemical methods. In: Fundamental and Applications. Chapter 9. Wiley, New York.

Ben-Jacob, E., Hermon, Z., Caspi, S., 1999. DNA transistor and quantum bit element: realization of nano-biomolecular logical devices. Phys. Lett. A 263, 199–202.

Boukamp, B.A., 1993. Equivalent Circuits. Users Manual, 2nd edition. University of Twente, The Netherlands.

Braun, E., Eichen, Y., et al., 1998. DNA-templated assembly and electrode attachment of a conducting silver wire. Nature 391, 775–778.

Carter, M.T., Rodriguez, M., Bard, A.J., 1989. Voltammetric studies of the interaction of metal chelates with DNA. 2. Tris-chelated complexes of cobalt(III) and Iron(II) with 1,10-phenanthroline and 2,2'-bipyridine. J. Am. Chem. Soc. 111, 8901–8911.

Chaires, J.B., Dattagupta, N., Crothers, D.M., 1982. Studies on interaction of anthracycline antibiotics and deoxyribonucleic acid: equilibrium binding studies on interaction of daunomycin with deoxyribonucleic acid. Biochemistry 21, 3933–3937.

Desai, M.C., Linda, M., Stramiello, S., 1993. Polymer bound EDC (P-EDC): a convenient reagent for formation of an amide bond. Tetrahedron Lett. 34 (48), 7685–7688.

Dollimore, L.S., Gillard, R.D., 1973. Optically active co-ordination compounds. PartXXX. Potassium (+)Tris-[L-cysteinesulphinato(2-)-SN] cobaltate (?): a versatile agent for resolution of 3+ species. J. Chem. Soc., Dalton Trans, 933–940.

Fink, H.W., Schönenberger, C., 1999. Electrical conduction through DNA molecules. Nature 398, 407–410.

Hansma, H.G., Sinsheimer, R.L., Li, M.Q., et al., 1992. Atomic force microscopy of single-stranded and double-stranded DNA. Nucleic Acids Res. 20 (14), 3585–3590.

Herlne, T.M., Tarlov, M.J., 1997. Characterization of DNA probes immobilized on gold surfaces. J. Am. Chem. Soc. 119, 8916–8920.

Levicky, R., Herne, T.M., et al., 1998. Using self-assembly to control the structure of DNA monolayers on gold: a neutron reflectivity study. J. Am. Chem. Soc. 120 (38), 9787–9792.

Lyubchenko, Y., Shlyakhtenko, L., Harrington, R., et al., 1993. Atomic force microscopy of long DNA-imaging in air and under water. Proc. Natl. Acad. Sci. USA 90 (6), 2137–2140.

Pang, D.W., Abruha, H.D., 1998. Micromethod for the investigation of the interactions between DNA and redoxactive molecules. Anal. Chem. 70 (15), 3162–3169.

Pang, D.W., Zhang, M., Wang, Z.L., et al., 1996. Modification of glassy carbon and gold electrode with DNA. J. Electroanal. Chem. 403, 183–188.

Porath, D., Bezryadin, A., de Vries, S., Dekker, C., 2000. Direct measurement of electrical transport through DNA molecules. Nature 403, 635–638.

Sun, X., He, P., et al., 1998. Immobilization of single-stranded deoxyribonucleic acid on gold electrode with self-assembled aminoethanethiol monolayer for DNA electrochemical sensor applications. Talanta 47, 487–495.

Wagner, P., Hegner, M., Güntherodt, H.J., et al., 1995. Formation and in situ modification of monolayers chemisorbed on ultraflat template-stripped gold surfaces. Langmuir 11, 3867–3875.

Wang, J., Cai, X., Rivas, G., Shiraishi, H., 1996. Stripping potentiometric transudation of DNA hybridization processes. Anal. Chim. Acta 326, 141–147.

Wu, L., Zhou, J., Luo, J., Lin, Z., 2000. Oxidation and adsorption of deoxyribonucleic acid at highly ordered pyrolytic graphite electrode. Electrochim. Acta 45, 2923–2927.

Zhao, Y., Pang, D., et al., 1999. DNA-modified electrodes: part 4: optimization of covalent immobilization of DNA on self-assembled monolayers. Talanta 49, 751–756.