

Electrochemical Detection of Specific Gene Related to CaMV35S Using Methylene Blue and Ethylenediamine-modified Glassy Carbon Electrode

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Abstract

Ethylenediamine (En) was introduced onto an electrochemical oxidized glassy carbon electrode using water-soluble 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). DNA was then covalently immobilized onto the En modified GCE with surface-bound primary amino group in the presence of EDC. Cyclic voltammetry and differential pulse voltammetry were used to characterize the DNA modified electrodes using methylene blue (MB) as electro-active indicator. The results showed that ssDNA immobilized using ethylenediamine as connector (ssDNA/En/GCE) could hybridize with target ssDNA more effectively than that immobilized directly on the bare GCE (ssDNA/GCE). The ssDNA/En/GCE was successfully employed for the selective detection of CaMV35S gene (presented in almost all the genetically modified plants) in a fragment of 20-base oligodeoxynucleotides sample. The electro-reduction signal of MB was related to the CaMV35S gene concentration over the range of $5.0 \times 10^{-9} \sim 1.2 \times 10^{-7}$ mol/L.

Key words: DNA immobilization, ethylenediamine, methylene blue, CaMV35S gene.

1. Introduction

Recently, sequence-specific DNA recognition has been used extensively for analytical purposes, including identification of pathogens, monitoring of gene expression, diagnosis of a variety of infectious diseases, environmental control, forensic or pharmaceutical applications, as well as analysis of genetically modified organisms. Nowadays, more and more genetically modified crops (GMC) are planted all over the world. The security of GMC and its effect on the environment are not well known. It is necessary to distinguish GMC from the ordinary ones. GMC can be identified by detecting given DNA segment, such as CaMV35S promoter, NOS terminator et al.

Increased attention has been paid to the electrochemical DNA biosensors for recognition of DNA hybridization because of their advantages, such as low cost, simple design, small dimensions, and low power requirements.¹⁻⁴ The detection of DNA by hybridization is often performed at the surface of a solid support where probes have been immobilized and electro-active indicators are applied to measure the hybridization events between the DNA probes and their complementary DNA fragments. Thus it is a vital step to immobilize probes onto the surface of the substrate electrode.⁵ It is handy and simple to immobilize ssDNA by adsorption, but the immobilized DNA probes readily

leave the support during the utilization and the probes attached on multiple sites have lower reactivity for hybridization.⁶ The ideal condition of coupling the probes onto the electrode firmly with all the relevant bases freely available for the reaction with targets is that the probes are covalently immobilized onto the electrode at one-site through the 5' or 3' end. Gold electrodes have been employed widely due to the self-assembly of thiols and other sulfur-containing compounds on the electrode, which can be used to covalently immobilize DNA.⁷⁻¹⁰ On the other hand, carbonic electrodes have been applied more widely¹¹⁻¹⁷ for their diverse characteristics. In addition, the hybridization efficiency would be decreased if the immobilized DNA was kept too close to the electrode surface to move freely. So it is necessary to link DNA to the electrode by proper connector to make the mobility of DNA more flexible, and improve the approachability of the complementary DNA.^{18,19} Silanization reaction is the dominating organic reaction used to obtain connectors. Pang¹⁷, Liu²⁰ and Sun²¹ introduced connectors with –OH or –NH₂ groups on carbon electrodes via silanization reaction. Connectors with –NH₂ also can be introduced on the surface of glass via silanization reaction.^{22,23} It is a pity that many anhydrous, noxious and relatively expensive organic reagents must be used in this reaction and the process is much too fussy.

In this work, we advanced a convenient method to immobilize DNA. Ethylenediamine (En) was employed as a connector to introduce amino groups on the oxidized glassy carbon electrode for one-site covalent immobilization of probes in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide. The different properties of the ssDNA/GCE and the ssDNA/En/GCE were studied with the techniques of cyclic voltammetry and differential pulse voltammetry using methylene blue as electro-active indicator.^{7, 11, 24} Probes of CaMV35S gene (35S promoter from cauliflower mosaic virus gene presented in almost all the genetically modified plants) was immobilized onto these two kinds of electrodes to prepare electro-chemical DNA biosensors. The hybridization results showed that the ssDNA/En/GCE was more applicable to the recognition event than the ssDNA/GCE. The technique for DNA probe immobilization is illustrated in Figure 1.

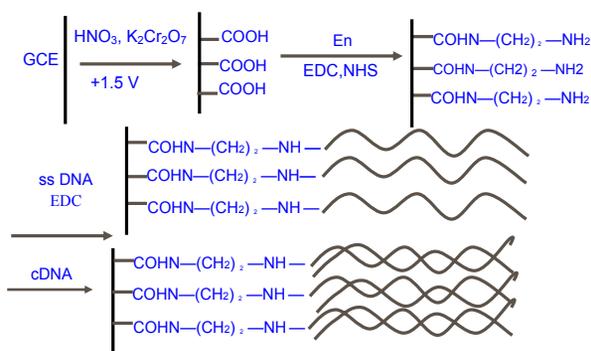


Figure 1. Schematic representation of the immobilization and hybridization of DNA on ethylenediamine modified GCE.

2. Experimental

2.1. Reagents

Three 20-base synthetic oligonucleotides were obtained from SBS Genetech Co., Ltd. (Beijing, China), and they have the following sequences: target A, namely CaMV35S gene sequence (CaMV35S is a heterogenous gene in a transgenic corn): 5'-GTC TCC GTA GAG ATT GCT AC-3', immobilized probe B: 5'-GTA GCA ATC TCT ACG GAG AC-3' and noncomplementary A': 5'-ACG ATA GCC TTT CCT TTA TC-3'. All oligonucleotides stock solutions of 20-base oligomers (1.5×10^{-3} mol/L) were prepared using TE solution (10 mmol/L Tris-HCl+1 mmol/L EDTA, pH 8.0), which was kept frozen. More dilute solutions were obtained via diluting the stock solution with double distilled water prior to use. Double-strand herring fish sperm DNA (dsDNA, Sigma) was used as received. The stock solution of dsDNA was prepared by dissolving appropriate amount of DNA in H₂O and stored at 277 K. The ratio of the absorbance at

260 and 280 nm (A_{260}/A_{280}) was checked to be ~ 1.89 , indicating that the DNA was sufficiently free from protein. Denatured single-stranded DNA (ssDNA) was obtained by heating dsDNA in a water bath at 373 K for 10 min, followed by rapidly cooling in an ice bath. Tris (hydroxymethyl) aminomethane (Tris), ethylenediamine (En), methylene blue (MB) were all purchased from Shanghai Chemical Reagent Company (Shanghai, China). N-hydroxysuccinimide (NHS), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were obtained from Sigma. All other chemicals were of analytical grade. All solutions were made up in double distilled water.

2.2. Preparation of DNA-modified GCE

The GCE was polished successively with 1.0 μm and 0.05 μm alumina suspensions to a mirror finish, sonicated in 1:1 HNO₃, acetone and double distilled water for 2 min, respectively, and then oxidized at +1.5 V for 30 s in an aqueous solution containing 0.085 mol/L K₂Cr₂O₇ and 1.7 mol/L HNO₃. In order to activate the electrode, after being rinsed it was immersed in 0.02 mol/L phosphate buffer solution (pH 6.8) containing 0.02 mol/L EDC and 0.01 mol/L NHS at 353 K for 15 min. The electrode was rinsed again in double distilled water, and then treated with 40 μL of 1.5×10^{-5} mol/L ssDNA (or dsDNA) solution and evaporated to dryness at ambient temperature, rinsed in phosphate buffer solution of pH 7.0 containing 3.5 mmol SDS to remove uncovalently immobilized ssDNA. The modified electrode was denoted as ssDNA (or dsDNA)/GCE throughout.

2.3. Preparation of ssDNA (or dsDNA)-En-modified GCE

The GCE oxidized and activated as above was immersed in 0.3 mol/L En solution at 323 K for 1.5 h to obtain En-modified GCE (En/GCE). Then the En/GCE was treated with 40 μL of 1.5×10^{-5} mol/L ssDNA (or dsDNA) solution containing 0.1 mol/L EDC and evaporated to dryness at ambient temperature. The obtained electrode was rinsed in phosphate buffer solution of pH 7.0 containing 3.5 mmol SDS to remove uncovalently immobilized DNA, and denoted as ssDNA (or dsDNA)/En/GCE throughout.

All the prepared electrodes were stored in 0.012 mol/L TE buffer of pH 8.0 at 277 K prior to use.⁶

2.4. Hybridization

ssDNA/GCE or ssDNA/En/GCE was immersed into 50 μL hybridization buffer referred as a 2 \times SSC buffer, containing 0.3 mol/L sodium chloride, 0.03 mol/L sodium citrate (pH 7.0) and complementary ssDNA at 315 K for 1.5 h. The same procedure was conducted for the noncomplementary A'. They were respectively

rinsed with $2\times$ SSC buffer to remove the unhybridized DNA. These electrodes were denoted as hybrid/GCE, hybrid/En/GCE or noncomplementary-hybrid/GCE, noncomplementary-hybrid/En/GCE, respectively. The electrodes were stored in 0.012 mol/L TE buffer of pH 8.0 at 277 K prior to use.

2.5. Cyclic voltammetry and differential pulse voltammetry

The cyclic voltammetry (CV) and the differential pulse voltammetry (DPV) were performed with a CHI832 Electrochemical Analyzer (CH Instruments, Shanghai, China). A three-electrode cell, consisting of a modified glassy carbon electrode (GCE) as working electrode, a saturated calomel electrode as reference electrode and a platinum wire auxiliary electrode, was used in electrochemical experiments. The test solution was MB solution containing 0.02 mol/L NaCl in pH 6.3 B-R buffer solution (prepared from an acidic solution that contained each of 0.04 mol/L H_3PO_4 , HOAc, and H_3BO_3 by adjusting to pH 6.3 using 0.2 mol/L NaOH.). Unless otherwise indicated, the scan rate in CV was 100 mV/s and the pulse amplitude, the pulse width and the pulse period in DPV were 50 mV, 50 ms and 0.2 s, respectively. All experiments were conducted at ambient temperature (298.0 ± 0.5 K) and all voltammograms were background subtracted.

3. Results and Discussion

3.1. En modification of the glassy carbon electrode

Carboxyl groups were introduced to the surface of the GCE after the electrochemical oxidation. Three methods have been used to link the amine of En and the carboxyl group on the surface of the GCE: (a) En was immobilized on the oxidized GCE after the carboxylic acid groups were transformed into acyl chloride by treatment with thionyl chloride²⁵, (b) 40 μ L 0.3 mol/L En solution was dropped on the surface of the oxidized GCE and then the electrode was incubated in an oven at 323 K for 2 h, (c) En was immobilized on the oxidized GCE using EDC and NHS as activators. The results showed that the modified electrode obtained from the third method could immobilize DNA more effectively. Carboxyl groups on the surface of the oxidized GCE were initially activated to the *o*-acylisourea by EDC and then converted to NHS ester, which required a strong nucleophile, such as a primary amine, to displace NHS and formed an amide bond. After one amine group of En binding to the activated glassy carbon surface, the other primary amine group was much more difficult to react with the NHS-activated carboxyl group simultaneously for the considerable steric hindrance, thus fewer bridged diamides were formed under this situation. The En/GCE was activated by EDC to allow

the free primary amine group of En immobilized on the electrode to form phosphoramidate bond with the 5'-terminal phosphate group of DNA.

3.2. Cyclic voltammetry of MB at DNA/En/GCE

The influence of the accumulation time of MB at the electrode on the CV was tested. The CV peaks of 2×10^{-5} mol/L MB at all modified electrodes increased with the accumulation time up to 5 min and then tended to remain constant when the accumulation time exceeded 5 min. 5 min of accumulation time was chosen in all experiments.

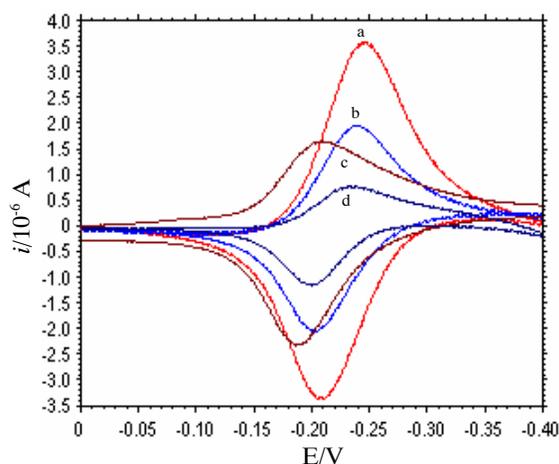


Figure 2. Cyclic voltammograms of 2×10^{-5} mol/L MB in B-R buffer solution containing 0.02 mol/L NaCl (pH 6.3) at (a) ssDNA/En/GCE. (b) dsDNA/En/GCE. (c) bare GCE. (d)

Figure 2 shows the cyclic voltammograms of MB at GCE, En/GCE, ssDNA/En/GCE and ds/En/GCE. At the bare GCE, the anodic peak current (i_{pa}) of MB was much bigger than the cathodic peak current (i_{pc}), $i_{pa}/i_{pc}\approx 1.5$. After immobilizing En on the surface of GCE, the CV signal of MB decreased evidently. Amino groups dominantly existed as $-NH_3^+$ when pH was 6.3, which had an electrostatic repulsion toward MB^+ and made MB^+ difficult to reach to the surface of the electrode, so led to the sharp decrease of i_{pc} . Simultaneously, $-NH_3^+$ made the electrode surface more hydrophilic, which made it difficult for MB to be adsorbed on the electrode surface, thus the i_{pa} of MB was also very small at En/GCE, and that $i_{pa}/i_{pc}\approx 1.4$. After immobilizing ssDNA on the En/GCE obtained from above method (c), the CV signal of MB at the ssDNA/En/GCE rose markedly. By contrast, after immobilizing ssDNA on the En/GCEs obtained from method (a) and (b), the CV signals of MB (not shown) showed little difference. It was suggested that under such situations as above method (a) and (b), En coupled to the GCE surface dominantly through bridged diamides and thus there was fewer free primary amine

groups to bind DNA. MB has a strong affinity to the free guanine bases exposed to the exterior of ssDNA^{11,24} and thus the CV signal of MB at ssDNA/En/GCE was much bigger than that at dsDNA/En/GCE. However, MB could bind dsDNA via an electrostatic interaction, so that peak currents of MB at the dsDNA/En/GCE were higher than those at the En/GCE. It also can be seen that after immobilizing DNA on the surface of the En/GCE, more symmetric cyclic voltammograms were obtained, the ratio of $i_{pa}/i_{pc} \approx 1.0$. The peak current was directly proportional to the scan rate from 0.05~0.40 V/s at both the ssDNA/En/GCE and ds/En/GCE, which indicated that the redox reaction of MB at DNA/En/GCE was the surface process.

A comparison of all the CV data (mean value of three measurements) of MB at different electrodes was listed in Table 1. The peak-potential separation between the E_{pa} and the E_{pc} (ΔE_p) obtained at En/GCE was 9 mV more than that obtained at the bare GCE, showing that the presence of En on the surface of the GCE diminished the velocity of electron transfer. However, after ssDNA was immobilized on the bare GCE or the En/GCE, the value of i_{pc} of MB rose. The value of i_{pc} of MB at the ssDNA/En/GCE was the biggest, which indicated that the immobilization of ssDNA on the En/GCE was more efficient than that on the bare GCE. As a connector, En has a free primary amine group (another amine group had covalently bound with the GCE surface), which can bind with the 5'-terminal phosphate group of ssDNA to form phosphoramidate bond, and En immobilized on the electrode made the DNA molecule be not too close to the surface of the electrode so that it could move. Hence, immobilizing En on the GCE was of benefit for further immobilizing DNA.

3.3. Detection of the target gene

Probe B was immobilized on the oxidized GCE and the En/GCE, respectively, to prepare CaMV35S gene electrochemical biosensors. After being tested in MB solution and rinsed in double distilled water, the ssDNA (probe B)/GCE and the ssDNA (probe B)/En/GCE were, respectively, immersed into target A solution for

hybridizing. The CV curves of MB were respectively almost the same as those obtained at the electrodes before hybridization, which suggested that MB bound to ssDNA-modified electrodes could not be rinsed off with water and made the hybridization of ssDNA impossible. If the above two electrodes were soaked in 1 mol/L KNO₃ solution at 315 K for 15 min, rinsed in double distilled water, and then hybridized with target A, the CV peaks of MB under the same conditions above decreased greatly, with respect to those obtained at the electrodes without hybridization. It was suggested that MB adsorbed on the electrodes could be removed off the electrodes in the solution of high ionic strength because the binding reaction between ssDNA and MB was electrostatic in nature.¹¹ The reduction of the CV peak of MB at the hybrid/En/GCE was much larger than that at the hybrid/GCE. This result indicated that the connector En could improve the approach ability of target A to probe B^{19,20}, resulting in the increase of the efficiency of the hybridization and the sensitivity of the gene sensor. So the ssDNA (probe B)/En/GCE was chosen to detect target A.

The hybridization of target A with probe B was also dependent on the ionic strength, temperature and the hybridization time. 2×SSC buffer was chosen as the hybridization solution and 315K as the hybridization temperature.^{9,20,26} Figure 3 showed the dependence of the DPV peak current (i_p) of 2×10⁻⁵ mol/L MB on the hybridization time. The i_p of MB decreased with the hybridization time up to 75 min and then tend to remain constant. A hybridization time of 90 min was selected to insure all the immobilized probes involving in hybridization. The ssDNA (probe B)/En/GCE was regenerated as previous work²⁷ and stored in 0.012 mol/L TE buffer of pH 8.0 at 277 K prior to use. To prevent ssDNA from folding and hybridizing with themselves, the period should not be longer than three days. Otherwise the ssDNA/En/GCE should be regenerated again before hybridization.

The quantitative analysis should use the same electrode for each step, because the DPV signal of MB had a relative standard deviation of 4.9% at different electrodes.

Table 1. CV data of 2×10⁻⁵ mol/L methylene blue in the B-R buffer of pH 6.3, containing 0.02 mol/L NaCl at different electrodes (mean value of three measurements)

Electrode	E_{pc} (mV)	E_{pa} (mV)	ΔE_p (mV)	i_{pc} (μ A)	i_{pa} (μ A)
Bare GCE	-210	-186	24	1.504	-2.278
En/GCE	-235	-202	33	0.849	-1.163
ssDNA/GCE	-230	-202	28	2.560	-2.705
dsDNA/GCE	-231	-202	29	2.119	-2.257
ssDNA/En/GCE	-248	-209	38	3.705	-3.507
dsDNA/En/GCE	-242	-205	37	2.028	-2.115

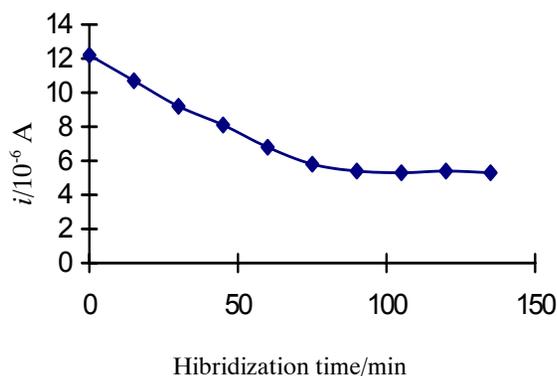


Figure 3. The dependence of the DPV peak current of 2×10^{-5} mol/L MB in B-R buffer of pH 6.3 containing 0.02 mol/L NaCl at the hybrid/En/GCE on the hybridization time. The concentration of target A was 1.5×10^{-5} mol/L.

In this work, DPV, which was more sensitive than CV, was chosen to detect target A. Figure 4 showed the differential pulse voltammograms for detecting target A. Curve a was the differential pulse voltammogram of MB at probe B/En/GCE, which had a well-defined differential pulse voltammetric peak at about -0.18 V. Curve b was that at noncomplementary-hybrid/En/GCE, the peak of which was almost the same as curve a demonstrating noncomplementary A' could not hybridize with immobilized probe B. Curve c, d, e, f and g were differential pulse voltammograms at hybrid/En/GCE hybridizing with different contents of target A, respectively. The decrease of the peak height was directly proportional to the content of target A over the range from 5.0×10^{-9} mol/L to 1.2×10^{-7} mol/L, with the regression equation $y = -0.5234x + 11.707$ ($r = 0.9982$), where y was the peak height in μA and x the content of target A in 10^{-8} mol/L.

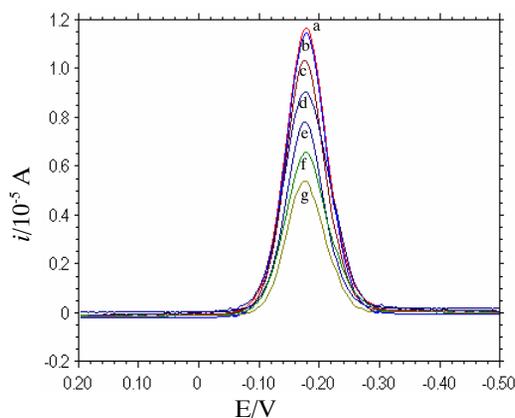


Figure 4. Differential pulse voltammograms of 2×10^{-5} mol/L MB in B-R buffer of pH 6.3 containing 0.02 mol/L NaCl at (a) probe B/En/GCE, (b) noncomplementary-hybrid/En/GCE, (c), (d), (e), (f) and (g) hybrid/En/GCE, the content of target A was 2.4×10^{-8} , 4.8×10^{-8} , 7.2×10^{-8} , 9.6×10^{-8} and 1.2×10^{-7} mol/L, respectively

4. Conclusion

In this research, we have demonstrated a novel method to introduce amine groups on the GCE and obtained En/GCE. Using coupling reagent, both ssDNA and dsDNA could be covalently immobilized on the surface of the GCE or En/GCE. The properties of both ssDNA/GCE and ssDNA/En/GCE were measured with the techniques of CV and DPV using MB as electro-active indicator. The efficiency of ssDNA immobilization and the hybridization efficiency of the immobilized ssDNA using En as connector were both higher than immobilizing ssDNA directly on the GCE surface. The inexpensive, sensitive, and reliable biosensor was successfully applied to the detection of the CaMV35S gene qualitatively and quantitatively in a fragment of 20-base oligodeoxynucleotides sample. The main advantage of this DNA biosensor is its cost-effectiveness because it is handy, regenerative, and needless of expensive or noxious solvent and further modification of nucleic acid. The developed method also has a sufficient low detection limit for real-world analysis.

5. Acknowledgements

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6. References

1. J. Wang, *Anal. Chem.* **1999**, *71*, 328–332.
2. M. I. Pividori, A. Merkoci, S. Alegret, *Biosensors and Bioelectronics* **2000**, *15*, 291–303.
3. C. Xu, H. Cai, P. G. He, Y. Z. Fang, *Analyst* **2001**, *126*, 62–65.
4. F. Yan, A. Erdem, B. Meric, K. Kerman, M. Ozsoz, O. A. Sadik, *Electrochem. Commun.* **2001**, *3*, 224–228.
5. V. Dugas, G. Depret, Y. Chevalier, X. Nesme, E. *Sens. Actuators, B, Chem.* **2004**, *101*, 112–121.
6. K. Hashimoto, K. Ito, Y. Ishimori, *Anal. Chem.* **1994**, *66*, 3830–3833.
7. A. Tani, A. J. Thomson, J. N. Butt, *Analyst* **2001**, *126*, 1756–1759.
8. D. W. Pang, H. D. Abruna, *Anal. Chem.* **1998**, *70*, 3162–3169.
9. X. Y. Sun, P.G. He, S. H. Liu, J. N. Ye, Y. Z. Fang, *Talanta* **1998**, *47*, 487–495.
10. S. Takenaka, K. Yamashita, M. Takagi, Y. Uto, H. Kondo, *Anal. Chem.* **2000**, *72*, 1334–1341.
11. B. Meric, K. Kerman, D. Ozkan, P. Kara, S. Erensoy, U.S. Akarca, M. Mascini, M. Ozsoz, *Talanta* **2002**, *56*, 837–846.

12. J. Wang, G. Rivas, C. Parrado, X. Cai, M. N. Flair, *Talanta* **1997**, *44*, 2003–2010.
13. K. M. Millan, A. Saraullo, S. R. Mikkelsen, *Anal. Chem.* **1994**, *66*, 2943–2948.
14. K. M. Millan, S. R. Mikkelsen, *Anal. Chem.* **1993**, *65*, 2317–2323.
15. H. Cai, X. N. Cao, Y. Jiang, P. G. He, *Anal. Bioanal. Chem.* **2003**, *375*, 287–293.
16. P. Kara, D. Ozkan, K. Kerman, B. Meric, A. Erdem, M. Ozsoz, *Anal. Bioanal. Chem.* **2002**, *373*, 710–716.
17. D. W. Pang, M. Zhang, Z. L. Wang, Y. P. Qi, J. K. Cheng, Z. Y. Liu, *J. Electroanal. Chem.* **1996**, *403*, 183–188.
18. M. H. Bune, *Nucleic Acids Res.* **1982**, *10*, 7181–7196.
19. M. H. Bune, P. Westhoff, R. G. Herrmann, *Nucleic Acids Res.* **1982**, *10*, 7163–7180.
20. S. H. Liu, J. N. Ye, P. G. He, Y. Z. Fang, *Anal. Chim. Acta* **1996**, *335*, 239–243.
21. Y. Sun, C. Xu, S. H. Liu, P. G. He, Y. Z. Fang, *Chem. J. Chinese Universities* **2002**, *19*, 1393–1396.
22. M. K. Walsh, X. Wang, B. C. Weimer, *J. Biochem. Biophys. Methods* **2001**, *47*, 221–231.
23. M. Yang, R. Y. C. Kong, N. Kazmi, A. K. C. Leung, *Chem. Lett.* **1998**, *27*, 257–258.
24. Y. Z. Fang, S. H. Liu, P. G. He, *Chem. J. Chinese Universities* **1996**, *7*, 1222–1224.
25. K. Jiao, G. Y. Xu, X. Z. Zhang, C. H. Mi, *Chem. J. Chinese Universities* **2005**, *26*, 841–843.
26. K. Kerman, D. Ozkan, P. Kara, B. Meric, J. J. Gooding, M. Ozsoz, *Anal. Chim. Acta* **2002**, *462*, 39–47.
27. Y. Liu, D. C. Wu, W. D. Zhang, J. Xuan, C. B. He, T. S. Chung, S. H. Goh, K. W. Leong, *Angew. Chem.* **2005**, *117*, 4860–4863.

Povzetek

Na elektrokemijsko oksidirano elektrodo iz steklastega grafita (GCE) smo uvedli etilendiamin (En) z uporabo vodotopnega 1-etil-3-(3-dimetil-aminopropil) karbodiimida hidroklorida (EDC) in N-hidroksisukcinimida (NHS). Nato smo DNA kovalentno imobilizirali na elektrodo modificirano z En. Za karakterizacijo tako modificirane elektrode smo uporabili ciklično voltometrijo in diferencialno pulzno voltometrijo z uporabo metilen modrega kot elektroaktivnega indikatorja. Rezultati kažejo, da se imobilizirana ssDNA povezana z En na GCE (ssDNA/En/GCE) hibridizira s ciljno ssDNA bolj učinkovito kot tista, ki je povezana neposredno na GCE (ssDNA/GCE). Tako smo uspešno uporabili ssDNA/En/GCE za selektivno detekcijo CaMV35S gena, ki je prisoten v skoraj vseh genetsko modificiranih rastlinah. Signal, ki predstavlja redukcijo metilen modrega, je bil povezan s koncentracijo gena CaMV35S v intervalu $5,0 \times 10^{-9}$ – $1,2 \times 10^{-7}$ mol/L.