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Voltammetric Determination of Genomic DNA using Gold-Thiol Self-Assembled Monolayer Electrode

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Abstract

Electrochemical methods have sensitivity, miniaturization capability, low cost and make them suitable for development of portable detection devices. The voltammetric determination of *M. tuberculosis ds*DNA was carried out in this study using gold electrode modified by SAM of thiols. The DPV responses of guanine of dsDNA was found at 0.2 V. The voltammogram showed a linear correlation between the current peak of guanine and the concentration of *ds*DNA over the concentration range 1–25 ppm ($Y = 1.108X - 0.851$, $R^2 = 0{,}993$). This electrochemical method gives slightly different results with biophotometri but provide excellent sensitivity.

Keywords: M.tuberculosis, gold electrode, guanine oxidation, self-assembled monolayer, voltammetry

1. introduction

Nowadays electroanalytical methods are widely used for monitoring different important parameter in environment, food industry, medicine, etc. because of their easy applicability and low cost. Electroanalytical techniques are concerned with the interplay between electricity and chemistry, namely the measurements of electric quantities, such as current, potential, or charge, and their relationship to chemical parameters[6] . An important improvement in DNA determination and application, in term of analysis time and cost, would be made by development of a direct analysis approach using isolate of DNA. Since the thiols leads binding strongly with gold, SAM-gold has been used as excellent electrode for voltammetric analysis of DNA. The SAM is a good strategy for linking biomolecules because, this technique allows an easy formations of monolayer, reasonable stability for extended period, allowing several reliable measurements [8]. DNA immobilization step plays the most important role in determining the performance of an electrochemical genosensor. Control of the DNA binding surface in terms of surface orientation and coverage is essential for the sensitive monitoring of DNA–DNA and compound-DNA interactions by electrochemistry. Our research in this study is focused on gold-based SAM electrodes modified thiols for the determination of genomic DNA through guanine oxidase on the electrode surface. The posibilty of analyzing genomic DNA is very attractive, but so far, only few works operating directly with genomic DNA have been reported[1,2,3,5].

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2. Materials and methods

2.1. Apparatus

Different Pulse Voltammetry (DPV) was carried out using a Metrohm® µAutolab type III potensiostat connected to PC. All measurement were made using a conventional three-electrode system and peformed at room temperature (25 ± 0,5 ^{o}C). three-electrode system consist of a Gold wire (0,5 mm diameter from PT ANTAM, Indonesia) was used as working electrode , an Ag/AgCl (reference electrode), and a platina wire (auxillary electrode). The potential scan dilakukan antara -1 sampai +1 V pada kecepata 50 mV/detik.

2.2. Reagens

double stranded DNA from calf thymus, Glutaraldehide (25%), cysteamine (95%) were obtained from sigma-Aldrich Company (USA), dsDNA stock solutions (100 mg/L) were prepared with phosphate buffer solution (PB; 0,1 mol/L pH7). M.Tuberculosis dsDNA was obtained Biofit company. Others chemicals were of analytical reagen grade.

2.3. the gold electrode pretreatment

The gold electrode was mechanically polished with alumina slurry (0.5 µm diameter, length 2 cm) followed by rinsing with distilled water and sonication (Ultrasonic Cleaner Elmasonic SH050EL, USA) in pure ethanol and water, for 5 min. After mechanical cleaning, the gold electrode suffered a chemical treatment by immersion in a 'piranha solution' (H_2SO_4/H_2O_2, 1 : 3 v/v) for 10 min at room temperature. Afterwards, the gold electrode was immersed in PB and 10 cycles were carried out between + 0.2 and + 1.5 V at 50 mV s–1. Finally, the electrode was rinsed thoroughly with distilled water for 10 min and after exposed to UV radiation for 15 min [8].

2.4. Self assembled monolayer and dsDNA immobilization

The pretreated electrodes were immersed into 25 mmol L^{-1} ethanolic solution of Cys for 2 h at room temperature (25°C) to allow an intimate contact between the gold and the thiol groups and subsequent formation of Au–Cys SAMs on the electrode surface. After that, the electrode was washed with distilled water. The immobilization of dsDNA was accomplished after incubating the Au–Cys electrode in a Glu solution (2.5% of Glu in 0.1 mol L^{-1} PB pH 7.0 at 4°C for 50 min). The electrode was then exhaustively washed with ultra pure water and incubated with dsDNA from calf thymus prepared in 0.1 mol L^{-1} PB pH 7.0 at 100 µL. The incubation time was maintained for 1 h at room temperature (25°C). Then, the electrode was washed for 2 min twice with PB buffer (pH 7.0) to remove the non-binding probe dsDNA.

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2.5. Immobilization of the dsDNA M.Tubercuosis

The gold electrode modified SAM on electrode surface. After that, the electrode was washed with a ultra pure water and incubated with M. Tuberculosis dsDNA in 0.1 mol L^{-1} PB pH 7.0 at 100 µL. The incubation time was maintained for 1 h at room temperature (25°C). Then, the electrode was washed for 2 min twice with PB buffer (pH 7.0).

2.6. Electrochemical measuremant

The immobilization of dsDNA on the Au–Cys–Glu, containing PB buffer solution, was scanned by different pulse voltammetry and attached to Autolab potentiostat, equipped with ANOVA software. Differential pulse voltammetry was applied to detect electrochemical signals, whereby a potential sweep was applied between 0.5 and 1.2 V, scan rate of 50mV.

2.7. Biophotometer measurement

Determination *M. tuberculosis ds*DNA sample was carried out using Biophotometer Plus eppendorf (USA). A solution of 50 µL DNA sample isolated from a pure bacteria culture was diluted in 0.1 mol L^{-1} PB (1:50). The TB blank solution using a ultra-pure water. All measurement was performanced at 25 ± 2 ^{o}C .

3. Result and discussion

3.1. pretreatment of gold electrode

Pretreatment of gold electrode was investigated by cyclic voltammetry (CV) to indicate the condition of the electrode surface clean of dsDNA in this study.. For pretrearment, Gold electrodes applied potential -1 to +1 V for 12 minutes using a cyclic voltammetry with scan rate of 50 mV. After that the gold electrode was rinsed thoroughly with distilled water for 10 min and after exposed to UV radiation for 15 min then A gold electrode immobilized in to 10 ppm dsDNA solution for 1 hour and B gold electrode without dsDNA immobilization then responds was measured using cyclic voltammetry with applied potential -0.4 to 0.6 V.

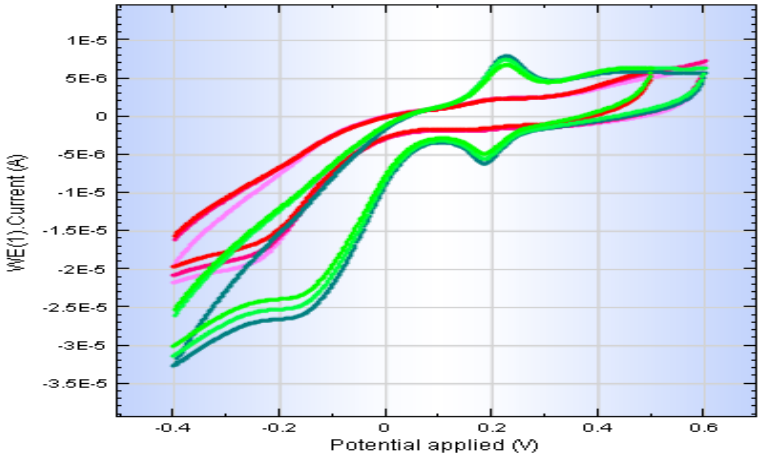

Fig.3A. cyclic voltammogram (CV) of gold electrode pretreatment . a (green line) and b (red line) representing a gold-dsDNA and bare electrode.

. The voltammograms show a peak current of double strain DNA (green line) and no peak current (red line). Figure 3.1.1. shows the behavior of the gold electrode after immobilization dsDNA and pretreatment electrodes (bare electrodes) . important to note that there are different, when dsDNA adsorbed on a gold electrode

and a clear surface gold electrode (bare electrodes) . The cleaning process was polished with alumina slurry until there is no peak at 0.2 volts. Therefore, the cleaning process is very important to obtain an accurate result.

3.2. Voltammetry measurement

The electrochemical determination of *ds*DNA was performed in this study by using DPV with a disposable gold . this investigation used two gold electrode are treated differently which the *ds*DNA was immobilized on to both gold modified by SAM and unmodified , the oxidation signal of guanine was then measured then from both trials resulting voltammogram peak currents which the electrode modified by SAM have higher peak currents than the unmodified. the strong binding of thiols to gold can be used to attach nucleic acid strands to electrode surface and increase the peak current.

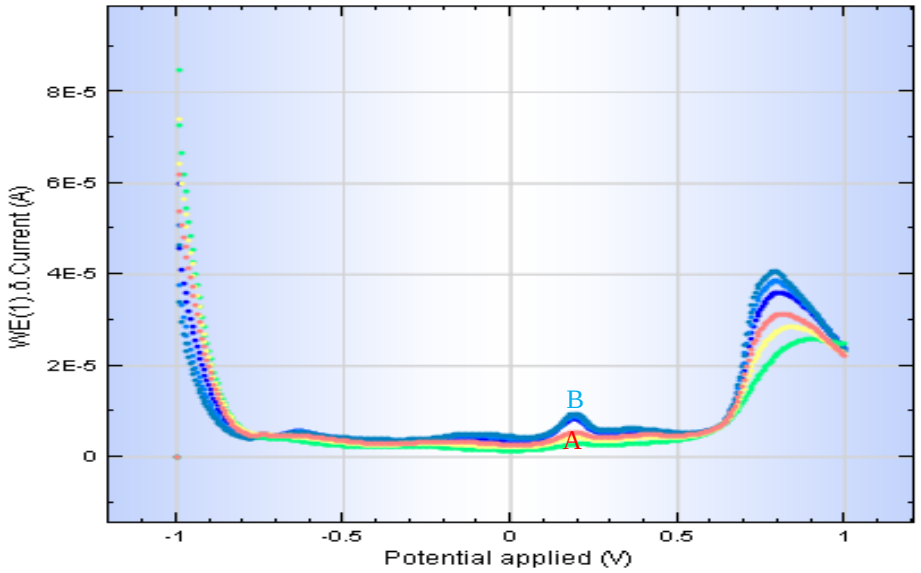

Fig.3B . (A). Differential pulse voltammogram of Gold-*ds*DNA immobilization at 20 ppm without SAM and (B) Gold-SAM-*ds*DNA immobilization at 5 ppm .

DNA is both hydrophobic and hydrophilic. The backbone of DNA is hydrophilic while bases of DNA are hydrophobic. Au (111) has a hydrophobic interaction, they can give to dsDNA adsorption interactions so as to produce a peak current of calfthymus found at 0.2 V, but the SAM-modified gold is more stable and has a higher peak currents from the gold electrode without modification. Therefore for the next step we chose to use the SAM modified gold for determining the concentration of dsDNA.

After that dsDNA in different concentrations as 1, 3, 5, 7, 9, 20, 25 ppm was immobilized onto the Au-SAM followed by dip-coating procedure during 1 h, there was an increase observed at guanine oxidation signal gradually in linear to increasing concentrations of dsDNA at 0.2 V. The signal increases gradually with dsDNA concentrations up to 1 to 9 ppm (Fig. 1A).

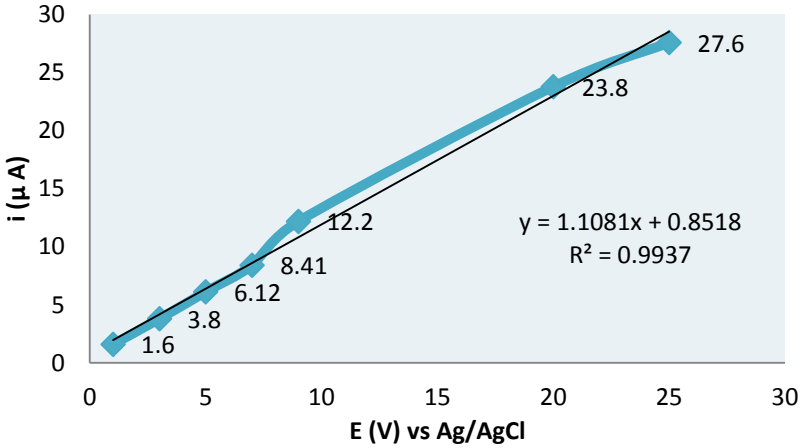

Fig. 3C. Graph of representing guanine oxidation signal obtained in different consentrations of dsDNA at 1 to 25 ppm

Figure 3C was obtained from different concentrations of dsDNA between 1 and 25 ppm and result a linearity y =1.108x-0.851 with correlation regression (R^2=0.993) When the same procedure was performed by using ssDNA, the similar results were obtained based on magnitude of guanine, but in response to a concentration 2-fold have a better linearity (R^2=0.993) than the 5-fold obtained in the presence of dsDNA move to the surface of the gold-SAM. Dramatic decrease in signal observed by immobilizing dsDNA guanine about 25ppm compared to that observed with dsDNA at a concentration level of 20 ppm. This decrease is attributed to the limited surface area of gold on the surface oxidation process. As revealed by Chiti et al, One initial major handicap of the use of thiol-subtituted DNA by its self was the tendency of DNA to bind lying flat to the gold. According the results obtained with different dsDNA concentrations, more 25 ppm was chosen as the optimum concentration for full coverage surface of gold by SAM technique from thiols.

Seven subsequent experiments performed for electrochemical detection of DNA at concentration level of dsDNA as 15 ppm immobilized onto Gold-SAM during 1 h gave an RSD value of 17,6 % (n = 3). The detection limit (DL) and LOQ estimated from S/N = 0.22 ppm and 25 ppm in the 100 μL samples. The overall performance of electrochemical DNA analysis method is based on repeated use of electrodes to determine the concentration of DNA. For this purpose, the effect of DNA immobilization time onto Gold-SAM was studied followed by dip-coating procedure. However, the DPV did show a increase after 25 ppm. The decrease in the DPV of guanine signal indicated that over disposal double helix DNA had cause decrease conductivity on gold electrode suface, which at 50 ppm dsDNA immobilization present peak current decreased sharply about ± 10.1 uA.

Table 3.2.2. Result of measurement isolated dsDNA of *M. Tuberculosis* with voltammetry and Biophotometry methods.

Measurement	Voltammetry		Biophotometer
	(μA)	ppm	
I	17.5	15.26	16.7
II	18.5	15.93	16.8
III	18.9	16.28	16.8
Average	15.92 ppm		16.77 ppm

Table 3.1. Shows the measurement of the two methods for samples of M. tuberculosis isolates dsDNA. In the isolation of M. tuberculosis results carried out a guanine oxidation signal at +0.3 V to the gold-SAM surface. Guanine signal shift is probably caused by impurities. DNA has a very simple structure : it is a linear long chain polimer constituted by only four different monomeric unit, the deoxyribonucleotides, containing the nitrogenous bases adenine, cytosine, guanine and thymine. The genetic information of an organism is due to the sequence of bases the DNA. In thypical superior organism, DNA contains about 3×10^9 nucleotides and it is important to note that in the extraction and purification of DNA undergo fission chain therefore the length of each dsDNA varied. The length of the dsDNA sequence is not uniformly cause interference measurement, where one of the major defects on the use of DNA SAM is a tendency to lay flat with the surface of the gold.

The measurement results obtained using the method biophotometer 16.77 ppm, but for voltammetry method is 15.92 ppm. This difference may be due to the measurements biophotometer wear measurement system based on a wave UV (260-280 nm) is absorbed into the DNA molecule that is based on four different monomers whereas voltammetry method is based on measuring the guanine oxidation signal.

4.Conclusions

Electrochemical method using a gold-SAM has a little bit of difference to the outcome biophotometer (15.92 and 16.77 ppm). Although different electrochemical measurement results based on the gold-SAM has a fairly good linearity (R2 = 0993). As for the limit of detection (LOD) and limit the quantity is 0.22 and 25 ppm. the use of electrochemical method is very beneficial because it is very sensitive and low cost of analysis.

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