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Short communication

Elimination voltammetry of nucleic acids on silver electrodes

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Abstract

A newly developed electrochemical method—Elimination Voltammetry with Linear Scan (EVLS)—has been applied to the electrochemical study of nucleic acids (NAs) on a silver electrode. Using the linear combination of the currents measured at different scan rates, the EVLS is capable of eliminating one or two selected particular currents. It was shown that the elimination function conserving the reversible diffusion current and eliminating the charging and kinetic currents provides the significant increase of voltammetric signals of DNA. Due to the high sensitivity and resolution power, the EVLS can contribute to study behaviour of nucleic acids on the charged interface and can be applied to nucleic acid analyses and the development of DNA sensors.

Keywords: Silver electrode; DNA; Elimination voltammetry

1. Introduction

In the research of the properties and structure of the genetic material, significant progress has been done using common voltammetric methods in conjunction with mercury and carbon electrodes. Nucleic acids (NAs) produce redox and tensammetric signals at mercury electrodes and oxidation signals at carbon electrodes (reviewed in Ref. [1]). These signals obtained with mercury electrodes are highly sensitive to the structure, conformation changes, hybridization and damage of NA [2,3]. On the mercury electrode, the electroactivity of polynucleotides is caused by the processes of adenine and cytosine (the joint reduction signal) and guanine (the oxidation signal). Since reduction and oxidation processes proceed in adsorbed state, the detection of polynucleotides can be carried out by mode of adsorptive stripping [1,4].

The application of a silver electrode for the determination of DNA by cyclic and differential pulse voltammetry has been reported recently [5]. The elimination voltammetry with linear scan [6–8] significantly improves cyclic voltammetric results and therefore it can be applied in research of nucleic acids on this charged interface.

In Elimination Voltammetry with Linear Scan (EVLS), the elimination of selected particular currents from the linear scan voltammetry results can be achieved by an elimination function formed by a linear combination of total currents measured at different scan rates [6,7]. For the elimination procedure, it is necessary to fulfill the condition that the eliminated current is expressed as a product of two functions—the scan rate function, independent of electrode potential, $W_j(v)$ and the potential function, independent of scan rate $Y_j(E)$:

$$I_j = W_j(v)Y_j(E). \quad (1)$$

Assuming the linear diffusion of electroactive substance, the currents—diffusion current, I_d, the charging current, I_c and the kinetic current, I_k—can be expressed as:

$$I_d = v^{1/2}Y_d(E) \qquad I_c = v^1 Y_c(E) \qquad I_k = v^0 Y_k(E). \quad (2)$$

The EVLS just employs the differences in the current-scan rate dependence to eliminate the chosen currents. Assuming that the total current is formed by a sum of particular currents ($I = I_d + I_c + I_k + \ldots$), a suitable linear combination of total currents measured at different scan rates, called elimination function, cancels eliminated currents by mutual subtraction of the corresponding terms. The above particular currents, which are neither eliminated nor conserved by selected elimination function, are displayed by this function as multiplied by certain constant. The value of this constant depends on the selected elimination function.

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For above-mentioned particular currents (I_d, I_k and I_c), elimination function with simultaneous I_k and I_c elimination and I_d conservation can be calculated:

$$f(I) = 17.485I - 11.657I_{1/2} - 5.8284I_2. \quad (3)$$

In this equation, I is the reference current, i.e., the total current measured at reference scan rate and $I_{1/2}$ and I_2 are the total currents measured at scan rates corresponding to the half and double values of reference scan rate.

In comparison to ordinary voltammetry, the EVLS provides some significant advances: (a) the expansion of available electrode potential range, (b) the increase of current sensitivity and (c) the improvement of the peak resolution [7]. The last two benefits, (b) and (c), result from the fact that the elimination of kinetic and charging current decreases the irreversible current width and increases the peak height. This effect is particularly pronounced in the case of adsorbed substance—DNA [8]. Moreover, the evidence of adsorption is provided by the unique peak–counterpeak curve form.

2. Experimental

Chemicals were analytical grade reagent purchased from Sigma–Aldrich. All solutions were prepared using deionized water (Millipore, Milli-Q). The pH value was verified in solutions before and after electrochemical measurement. DNA (calf thymus) was isolated and thermally denatured according to Ref. [9]. The calf thymus (CT) DNA was precipitated with ethanol, dissolved in 0.002 M KNO_3 (30 ml volume) and, to remove chlorides, dialysed against 250 ml KNO_3 solution of the same concentration. The dialysis procedure was repeated 10 times, always with a fresh portion of KNO_3 solution, so that the total volume of the dialysed bath was 2500 ml.

Cyclic voltammetry (CV) measurements were performed on the AUTOLAB Electrochemical Instrument (EcoChemie, the Netherlands) connected with the VA-Stand 663 (Metrohm, Zurich, Switzerland). The standard cell consisted of three electrodes that includes the working electrode (silver disk with the purity 99.99%), the reference electrode (Ag/AgCl/3 M KCl) and auxiliary electrode (Pt wire). To obtain reproducible results, the silver polycrystalic electrode was cleaned with soft emery paper (600 A), polished with 0.05 μm alumina, then sonicated (ultrasound bath) for 3 min and rinsed with deionized water before each measurement. All experiments were carried out at room temperature.

The software (GPES 4.8) from EcoChemie was employed. Recorded curves were smoothed and were exported into Microsoft Excel, where the elimination function has been created.

3. Results and discussion

Fig. 1 shows the cyclic voltammogram for the native and thermally denatured calf thymus DNA in 0.05 M KNO_3. The pH (7.2) of blank was adjusted by 5×10^{-4} M KOH. During the polarization ($-+-$) in the potential range between -1.75 and $+0.5$ V (vs. Ag/AgCl/3 M KCl), two signals were detected. The first one occurred in the anodic part of cyclic voltammogram at about 0.4 V and the second one was in the cathodic part at about 0.1 V. To obtain anodic and cathodic peaks (A and C in Fig. 1), it was necessary to go to negative starting potentials (above -1.5 V vs. Ag/AgCl/3 M KCl). Both peaks were observed not only for the denatured CT DNA, but also for the native CT DNA. Moreover, the peak of native DNA was higher than denatured DNA (Fig. 1a and b). The greater peak height of the native DNA seems to indicate that the interaction of nucleic acids with the surface of silver electrode proceeds via the sugar-phosphate backbone rather than via the bases. The height and potential of these peaks were strongly dependent on the starting potential and on components in solution, such as the pH and scan rate.

The signals of DNA on silver electrode published in the paper of Ref. [5] were neither observed in acetate buffer nor in KNO_3 solutions. The potentials of above-published peaks correspond to those of chloride ions (unpublished results [10]). Moreover, at starting potentials of about -0.5 V, the DNA without chloride ions (dialysed DNA) gave no signals.

The symmetrical shape of cathodic peak (C) might indicate a surface reaction with the adsorption of depolarizer. This was confirmed by the elimination function in Eq. (3), conserving diffusion current (I_d) and eliminating the charging and kinetic currents (I_c, I_k), the cathodic part of cyclic voltammogram (Fig. 1c). This elimination, using scan rates 8, 16 and 32 mV/s, gives the signal in the form of a peak–counterpeak which indicates the depolarizer adsorption. It was theoretically calculated and experimentally verified [8]. Application of the EVLS to the anodic peak (A) yielded a significant increase of peak height. Experiments performed with the change of switching potential revealed the fact that the anodic and cathodic signals are coupled. From the above results we can conclude that the EVLS enables us:

1. to determine the content of chloride ions in solutions of calf thymus DNA, because chloride ions provide the signals (0.1 V in cathodic and 0.2 V in anodic part) without the electrode polarization from negative potentials (-1.5 V vs. Ag/AgCl/3 M KCl);
2. to determine native DNA and denatured DNA on silver electrode at the electrode polarization from negative potentials;
3. to observe electrode processes proceeding in adsorbed state or process indicating a surface reaction;

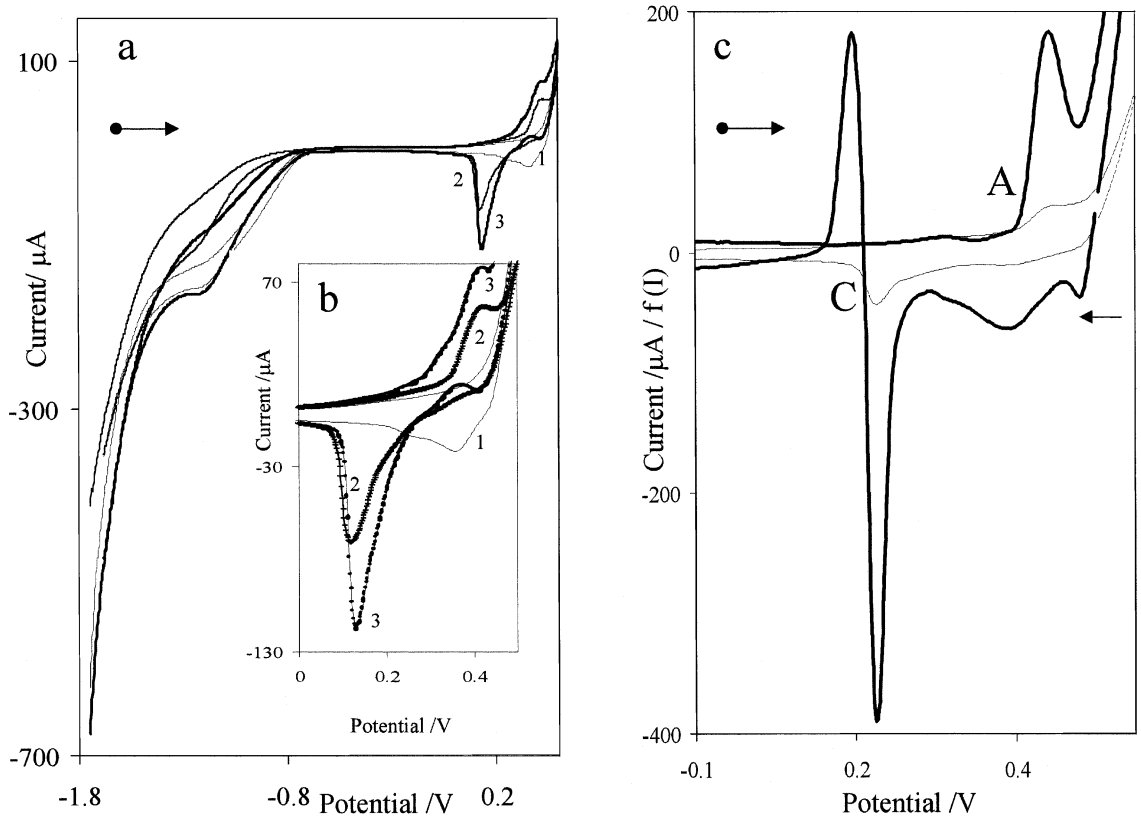

Fig. 1. (a) Cyclic voltammograms of native DNA and thermally denatured DNA (calf thymus) on silver electrode. The concentration of both DNAs were 623 μg/ml. Supporting electrolyte 0.05 M KNO_3 (pH 7.2), starting potential −1.75 V, switching potential 0.5 V, reference scan rates 16 mV/s, 1—supporting electrolyte, 2—denatured DNA, 3—native DNA. The arrow shows the direction of electrode polarization (−+−). (b) The zoom of peaks 1, 2 and 3. (c) Elimination and cyclic voltammograms of thermally denatured DNA (calf thymus) on AgE. — CV, reference scan rate 16 mV/s, — EVLS, A—anodic process, C—cathodic process. Other conditions as in (a).

4. to give a good and readable EVLS peak–counterpeak which can be successfully used in the analysis of DNA.

4. Conclusion

We focused our attention on voltammetric study of nucleic acids (native and denatured calf thymus DNA) on a silver electrode and selected the type of elimination with respect to the sensitivity and the resolution. The best results were obtained with the elimination function with I_k and I_c elimination and I_d conservation. The elimination voltammetry with linear scan might provide new possibilities for further electrochemical research and determination of nucleic acids. It can be considered as one of the most useful techniques in developing new original biosensors for nucleic acids.

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