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Voltammetric behavior of DNA modified with osmium tetroxide 2,2′-bipyridine at mercury electrodes

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Abstract

Osmium tetroxide complexes with nitrogen ligands (L) are probes of DNA structure and electroactive labels of DNA. Here adducts of single-stranded (ss) DNA with osmium tetroxide 2,2′-bipyridine (DNA-Os,bipy) were studied by cyclic voltammetry for the first time. It was found that at neutral pH DNA-Os,bipy produces three redox couples in the potential range between 0 and −1 V (peaks I–III) and a cathodic peak at about −1.3 V (peak IV). The latter peak decreased with increasing scan rate, and peaks arising from the forward and reverse scans exhibited the same direction, suggesting catalytic nature of the electrode process. We concluded that this peak corresponds to the known differential pulse voltammetric (polarographic) peak of DNA-Os,L adducts for which catalytic hydrogen evolution is responsible. In contrast, currents of cathodic peaks II and III increased almost linearly with increasing scan rate, suggesting involvement of adsorption in the electrode processes. Adsorptive stripping square-wave voltammetry was used to analyze the DNA-Os,bipy at low concentrations. It was shown that at neutral pH, peak III can offer sensitivity in the ppb range, which is only little lower than that reached by catalytic peak IV. The latter peak is, however, superior in sensitivity at acid pH values.

Keywords: Chemical modification of DNA; Osmium tetroxide complexes; Cyclic voltammetry; Mercury electrodes

1. Introduction

Osmium tetroxide complexes (Os,L) have been widely used as probes of the DNA structure [1]. Using DME it was shown [2,3] that osmium tetroxide, pyridine reagent (Os,py), covalently binding to pyrimidine bases (preferentially thymine) in single-stranded (ss) DNA, can serve as electroactive marker and gives several polarographic signals. Later Os,py was used as a probe of the DNA structure in combination with gel electrophoresis and DNA sequencing techniques. Other nitrogen ligands were used providing better stability of the Os,L complex, such as 2,2′-bipyridine (bipy) [4] or 1,10-phenanthroline (phen) [5]. Os,py and Os,bipy selectively reacted with ssDNA while Os,phen bound both ss and dsDNA [5]. Os, L's have been used in studies of local DNA structures such as cruciforms, triplexes, left-handed Z-DNA segments, etc., in vitro and in cells [4,6]. Combination of Os,bipy with DNA sequencing methods was very attractive because it offered a single-nucleotide resolution in DNA structure studies [7,8] while electrochemical techniques were neglected for some time. Recently, electrochemical analysis was used to probe the structure of supercoiled DNA with Os,bipy and a new structural transition (depending on the DNA superhelix density) was observed [9]. The interest in Os,L complexes was renewed in connection with the development of the DNA hybridization sensors [10,11]. It was shown that the catalytic signal of the DNA adduct with Os,bipy (DNA-Os,bipy) offers a highly sensitive DNA determination [12,13] and can be used in the DNA hybridization sensors [14], particularly in connection with the solid dental amalgam electrodes (B. Yosypchuk, E. Palecek et al., in preparation). Optimized analysis at hanging mercury drop electrode (HMDE) allowed detection of DNA-Os,bipy at picogram level [13]. Recently, pyrolytic graphite electrodes have been used to determine DNA-Os,bipy in excess of free Os,bipy [15].

Sufficient evidence has been gathered about the analytical usefulness of the catalytic DNA-Os,bipy signal but the signals occurring at less negative potentials have been little

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investigated. In this paper we have studied these signals by means of adsorptive stripping cyclic voltammetry (AdSCV) at HMDE at neutral pH. We have found three redox couples which may become useful in the detection of the DNA hybridization by means of single-surface techniques [10,11].

2. Experimental

Calf thymus DNA was isolated and denatured as described [2]. Osmium tetroxide of analytical grade was purchased from JMC (UK), other chemicals were of analytical grade. The DNA samples were treated with 2 mM Os,bipy in 0.1 M Tris–HCl (pH 7.5). The reaction was carried out at 26 °C for 24 h. The nonreacted Os,bipy was removed by dialysis against 0.1 M Tris–HCl (pH 7.5) at 5 °C for 12 h. Electrochemical measurements were performed with an AUTOLAB analyzer (EcoChemie, The Netherlands) in connection with a VA-Stand 663 (Metrohm, Herisau, Switzerland). A standard cell with three electrodes was used. The working electrode was hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2. The reference electrode was Ag/AgCl/3M KCl electrode, and platinum wire was used as the auxiliary electrode. AdSCV measurements were performed with the following settings: concentration of DNA-Os,bipy 10 μg/ml, accumulation time (t_a) 1 min, accumulation potential (Ea) 0 V, initial and final potential 0 V, switching potential −1.85 V. A 0.3 M ammonium formate with 0.05 M phosphate buffer (pH 6.98) was used as background electrolyte. Adsorptive stripping differential pulse voltammetry measurements were performed with the following settings: t_a = 1 min, pulse amplitude 0.05 V, scan rate 0.01 V/s, Ea −0.6 V. Britton–Robinson buffer pH 4.0 was used as background electrolyte. Adsorptive stripping square wave voltammetry (AdS SWV) measurements were performed with the following settings: frequency 10 Hz, amplitude 0.025 V, scan rate 0.05 V/s, accumulation potential 0 V, equal background electrolyte as in ASCV measurements was used. The analyte solution was deaerated before each experiment by bubbling argon. All electrochemical measurements were carried out at room temperature.

3. Results and discussion

First electrochemical experiments with Os,L were performed with Os,py [2,3,16]. To obtain fully modified DNA it was necessary to use strong modification conditions and a large excess of py. Exchange of py for bipy made it possible to obtain more stable DNA adducts and to work in absence of the ligand excess. In this paper we modified DNA with Os,py using equimolar concentration of OsO_4 with py (under otherwise the equal conditions as with Os,bipy). Fig. 1C shows that DNA-Os,bipy at a concentration of 800 ng/ml yields at pH 4 a well-developed DPV peak IVc at a relatively short t_a (t_a = 60 s). Under the same conditions DNA treated with Os,py reagent (containing py concentra-

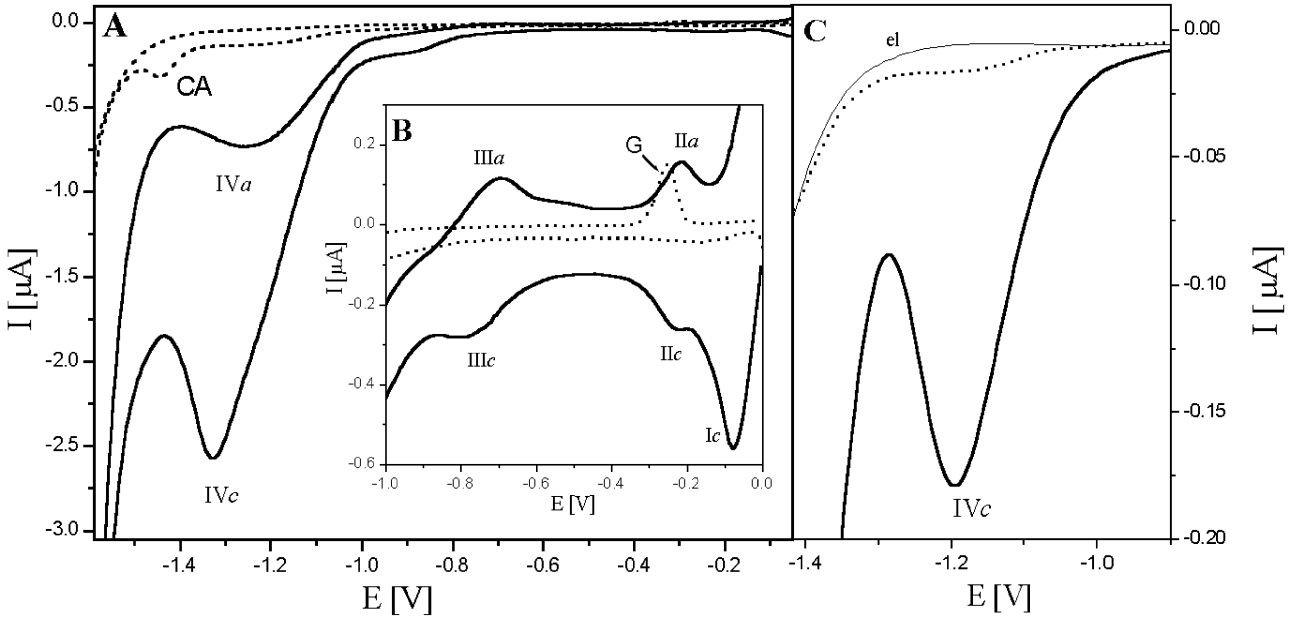

Fig. 1. (A) Adsorptive stripping cyclic voltammograms (AdSCV) of unmodified single-strand (ss) DNA (dashed line) and ss DNA modified with 2 mM Os,bipy (DNA-Os,bipy) (full line) at a concentration of 10 μg/ml. Background electrolyte: 0.3 M ammonium formate and 0.05 M sodium phosphate (pH 7.0), t_a 1 min, scan rate 0.1 V/s, initial potential 0 V, switching potential −1.85 V. (B) Section of the DNA and DNA-Os,bipy cyclic voltammograms measured at scan rate 1 V/s, other conditions as in (A). (C) Adsorptive stripping differential pulse voltammograms of ss DNA modified with 2 mM Os,bipy (full line) and with 2 mM Os,py (dashed line) at a concentration of 800 ng/ml. Background electrolyte (thin full line): Britton–Robinson buffer pH 4.0, t_a 1 min, pulse amplitude 0.05 V, scan rate 0.01 V/s, Ea −0.6 V.

tion equimolar to osmium tetroxide) produced only negligible signal, showing clearly the advantage of Os,bipy in modification of ssDNA.

In our further work we studied only DNA-Os,bipy. Fig. 1A,B compares cyclic voltammograms of unmodified calf thymus ssDNA and the same DNA modified by Os,bipy. As expected, the unmodified ssDNA produced two signals, one cathodic peak due to irreversible reduction of adenine and cytosine (CA peak) at − 1.4 V (Fig. 1A) [17] and an anodic peak due to guanine (peak G) (Fig. 1B). G was reduced to 7,8-dihydroguanine at potentials near to hydrogen discharge and 7,8-dihydroguanine was oxidized back to G at about − 0.3 V [18–21].

DNA-Os,bipy produced three couples of cathodic and anodic peaks (see Fig. 1B). First of them occurred close to the potential of the dissolution of mercury; this signal will be a subject of separate paper. The other two couples occurred at − 0.22 V, II*cathodic*(*c*); − 0.22 V, II*anodic*(*a*); − 0,76, V III*c*; − 0.70 V, III*a*. Another signal appeared at a potential about − 1.3 V. This peak was remarkably higher than the former signals and exhibited opposite direction in cathodic reverse branch of the cyclic voltammogram (Fig. 1A).

Earlier, some evidence was presented that the signal produced by DNA-Os,py and DNA-Os,bipy was due to catalytic hydrogen evolution [22]. No characteristics of the more positive signals have been, however, published. To obtain more information on the nature of individual signals of DNA-Os,bipy, in this paper we measured (a) the dependence of peak currents on scan rate (Fig. 2) and (b) the dependence of peak III*c* on the accumulation time at relatively low concentration of DNA-Os,bipy. From the theory of cyclic voltammetry [23,24], it is known that in case of diffusion controlled electrode process peak current increases linearly with square root of scan rate. On the other hand, if an electrode process involves catalytic reactions, the peak current decreases with scan rate, and signals arising from the forward and reverse scans exhibit the same direction [23,24]. Fig. 2 shows dependencies of the heights of peaks II*c*, III*c* and IV*c* on square root of scan rate (v). Currents of peak II*c* and peak III*c* increased with v, albeit the dependency exhibited significant deviations from linearity. This non-linearity can be explained by influence of adsorption on the electrode process in agreement with the fact that the reacting substance was in adsorbed state. Almost linear dependence of peak III*c* height on the scan rate (Fig. 2, inset) supports the adsorption involvement. In contrast to the less negative peaks II*c* and III*c*, peak IV*c* decreased with increasing scan rate, in agreement with its catalytic nature supported also by the cathodic direction of IVa (Fig. 1A). Fig. 3A shows the dependence of peak III*c* (measured by AdS SWV) on t_a at a DNA-Os,bipy concentration of 250 ng/ml and pH close to neutral. Under these conditions the SWV peak III*c* increased almost linearly with t_a up to about 120 s and at about 300 s it leveled off. Baseline corrected curves obtained at t_a 60 and 300 s are shown in Fig. 3B and C. There is no doubt that peak IV*c* at acid pH's is superior in sensitivity of the determination DNA-Os,bipy [12,13]. If however the measurements have to be performed close to neutral pH (e.g. in single-surface DNA hybridization techniques [10]), peak III*c* can provide sensitivity only little lower than that of peak IV*c*. Peak III and peak II measured at the HMDE corresponded to peak α

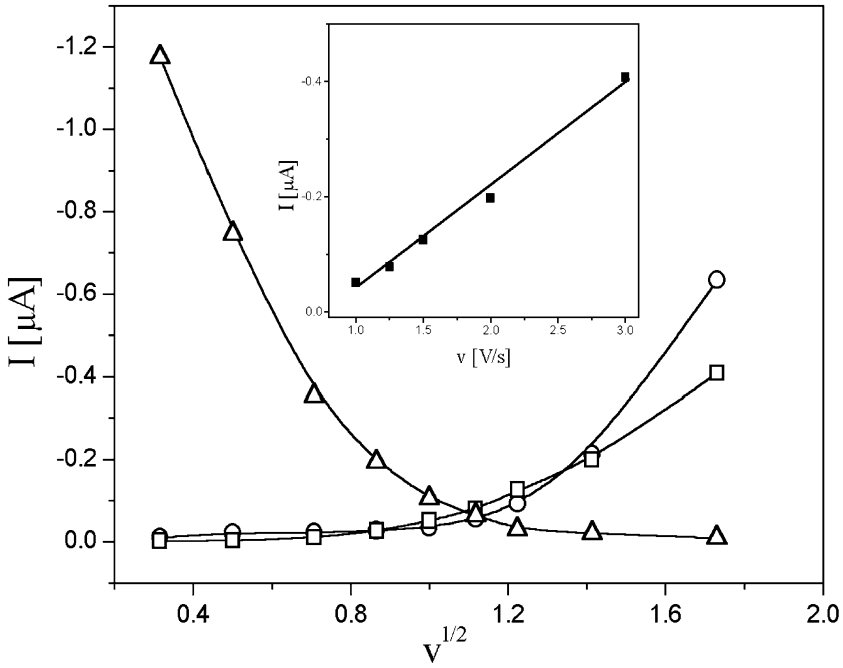

Fig. 2. Dependence of the heights of AdSCV cathodic signals of DNA-Os,bipy at a concentration of 10 μg/ml on square root of scan rate. ○ II*c*, □ III*c*, △ IV*c*. Inset: dependence of the height of AdSCV peak III*c* on scan rate. For other details see Fig. 1.

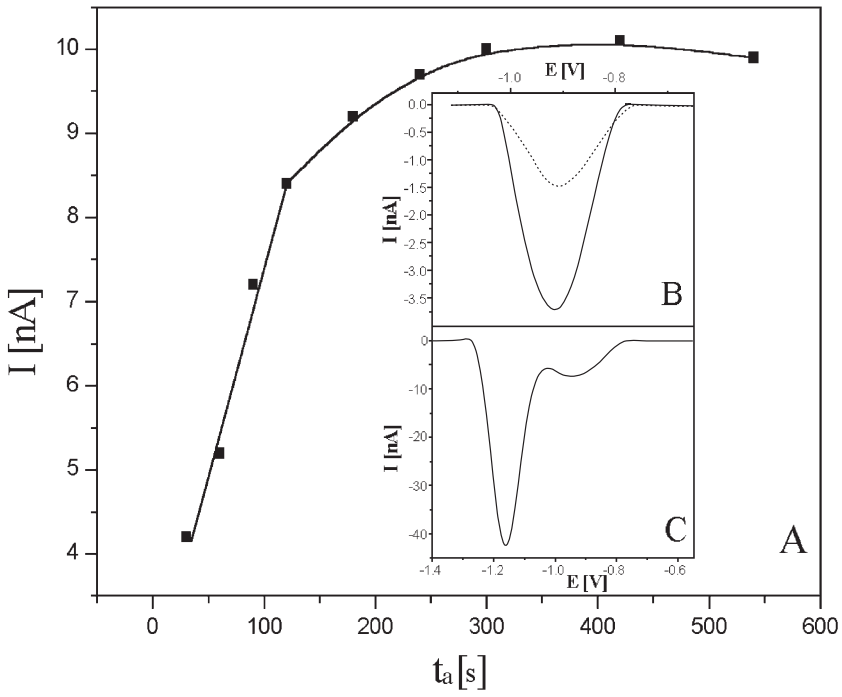

Fig. 3. (A) Dependence of the height of peak III*c* of DNA-Os,bipy on accumulation time. DNA-Os,bipy at a concentration of 250 ng/ml, adsorptive stripping square wave voltammetry (AdS SWV), frequency 10 Hz, amplitude 0.025 V, scan rate 0.05 V/s, accumulation potential 0 V, initial potential 0 V, end potential − 1.45 V, background electrolyte: 0.3 M ammonium formate and 0.05 M sodium phosphate (pH 7.0). (B) Sections of baseline corrected AdS SWV peak III at t_a 60 s (dashed line) and at t_a 300 s (full line). (C) Section of baseline corrected AdS SWV peak III*c* and peak IVc (ta 300 s).

and peak β, respectively, yielded by the DNA-Os,bipy at carbon electrodes [15]. Using carbon paste or pyrolytic graphite (PGE) electrodes, the latter peaks have been recently utilized in DNA hybridization assays [14,25,26]. Differences between cathodic and anodic peak potentials of the respective redox couples measured at HMDE or PGE under otherwise the same conditions did not exceed 20 mV (not shown). No signal corresponding to the catalytic peak IV was detected at the PGE.

It can be expected that the distinct electrochemical features of the DNA-Os,bipy are derived from that of OsO_4. Polarographic behavior of osmium tetroxide at mercury dropping electrode in different electrolytes was studied [27,28]. The authors found four individual polarographic waves of consecutive osmium tetroxide reduction. First two steps were described as two-electron reduction from Os(VIII) to Os(VI) and from Os(VI) to Os(IV). The third one was attributed to one-electron reduction from Os(IV) to Os(III). The most negative signal observed in neutral and weakly acidic media was ascribed to catalytic hydrogen evolution [27]. The mechanism of catalytic hydrogen evolution on mercury electrodes from solutions containing compounds of platinum metals was described [29]. These metals form "islands" (clusters of atoms) of deposited metal at the mercury surface. Since the hydrogen overvoltage at these metals is much lower, compared to mercury, evolution of hydrogen occurs at less negative potentials. Moreover, some platinum metals form low-valence complexes, which catalyse hydrogen evolution too [29].

The osmium-modified DNA undoubtedly represents a more complicated analyte than free osmium tetroxide. Long DNA molecules at the electrode surface involve strongly adsorbed polynucleotide segments and loops of DNA strands stretching to the solution [17]. Osmium moieties in the DNA-Os,bipy may thus differ in their accessibility for the electrode reactions. Moreover, adsorption of the negatively charged DNA is influenced by the electrode potential [17]. Individual signals of DNA-Os,bipy may therefore be differently affected by DNA adsorption. Electrochemical behavior of DNA-Os,bipy may thus somewhat differ from simple species such as OsO_4, free Os,bipy and Os,bipy adducts of thymine or thymidine. More details will be published elsewhere.

4. Conclusions

The results confirmed the previous assumption, made from polarographic measurements [3,16], that the signals occurring at potentials more positive than the catalytic one (peak IV in this paper) are due to diffusion controlled faradaic electrode processes. The catalytic peak has been utilized as a signal allowing highly sensitive voltammetric determination of the DNA-Os,bipy adducts, mainly in weak acidic media [12,13]. Some analytical applications, such as electrochemical single-surface DNA hybridization assay [10,11], require measurements in neutral media. Under such conditions, the peaks I–III of DNA-Os,bipy appear to provide similar sensitivity as the catalytic one (peak IV).

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