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Short communication

Determination of nanogram quantities of osmium-labeled single stranded DNA by differential pulse stripping voltammetry

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Abstract

Earlier, we showed that using differential pulse cathodic stripping voltammetry with hanging mercury drop electrode (HMDE), single-stranded (ss) DNA modified with osmium tetroxide, pyridine reagent (Os,py) can be determined at concentrations down to about 10–5 ng/ml. Here, we show that by exchanging Os,py for osmium tetroxide, 2,2′ -bipyridine (Os,bipy) and decreasing the pH of the background electrolyte from neutrality to about pH 4, ssDNA can be determined at concentrations lower by one order of magnitude. Determination of DNA at such low concentrations may find use in various areas of molecular biology and in biotechnologies, including the development of DNA sensors. © 2002 Published by Elsevier Science B.V.

Keywords: Differential pulse stripping voltammetry; Microdetermination of DNA; Chemical modification of DNA; Osmium tetroxide complexes; HMDE

1. Introduction

Almost 20 years ago, we found [1–3] that osmium tetroxide, pyridine complex (Os,py) covalently bound to pyrimidine bases in (denatured) single-stranded (ss) DNA can serve as an electroactive marker. We showed that Os,py bound to ssDNA can be detected polarographically (with DME) at low concentrations using the catalytic signal at about −1.2 V (against SCE). Os,py was bound not only to ssDNA but also to distorted or damaged regions in the (native) double-stranded (ds) DNA but not to intact B-DNA. On the ground of this finding we developed Os,py as a chemical probe of local DNA structures such a as cruciforms, triplexes, left-handed Z-DNA segments, etc. in recombinant DNAs [4–6]. The osmium binding site in a polynucleotide chain can be detected by gel electrophoresis using single-strand selective nucleases such as S1 or by piperidine cleavage. The latter approach provided information about the osmium binding sites at single-nucleotide resolution (at sequencing gels). Os,py, thus, became one of the first chemical probes of the DNA structure. Later, other ligands such as 2,2′ -bipyridine (bipy) [5] (Fig. 1a) or 1,10-phenanthroline [7] were used instead of pyridine providing better stability of the complex and extending the abilities of the probe for its application in cells [5]. Os,L complexes became versatile probes of the DNA structure analysis *in vitro* and *in vivo*. Because of the importance of the Os,L probes in the DNA structure research, the development of the electrochemical methods of DNA-Os,L adducts was neglected in our laboratory for many years in spite of the fact that the electrochemical behavior of Os,L-modified DNA is interesting and deserves further research. In our earlier polarographic studies of the DNA-Os,py adducts, we showed that these adducts produced in addition to the catalytic signal also other smaller signals at less negative potentials. Using the catalytic signal and stripping voltammetry at HMDE, Os,py-modified ssDNA was detectable at concentrations as low as 10–5 ng/ml at neutral pH [8]. In this paper, we show that with an acid electrolyte and sophisticated base line correction even lower concentrations of the DNA-Os,bipy adduct can be determined.

2. Experimental

2.1. Materials and methods

Calf thymus DNA was isolated as described [1]. Osmium tetroxide was purchased from JMC (England). Other chem-

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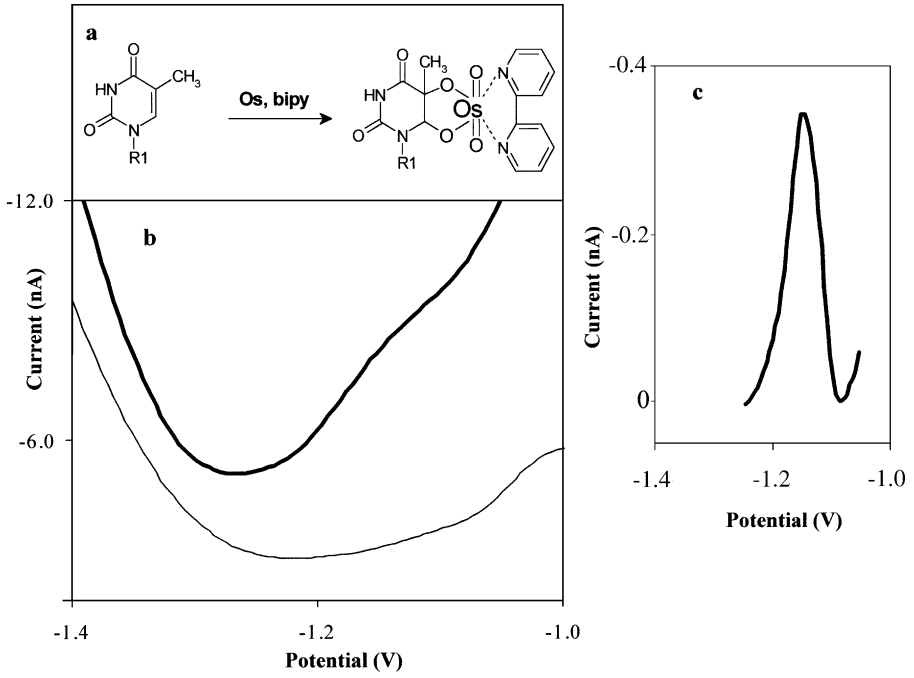

Fig. 1. Differential pulse stripping voltammograms of thermally denatured calf thymus DNA modified by Os,bipy at 0.3 M ammonium formate with 0.05 M sodium phosphate, pH = 6.9. (a) Reaction of osmium tetroxide, 2,2′ -bipyridine with thymine. (b) Voltammograms of background electrolyte and ssDNA-Os,bipy at a concentration of 5 ng/ml (raw data). (c) Signal ssDNA-Os,bipy at a concentration of 5 ng/ml after moving average baseline correction. Differential pulse stripping voltammetry, pulse amplitude 50 mV, scan rate 10 mV/s, time of accumulation 120 s, initial potential − 0.6 V, HMDE, stirring.

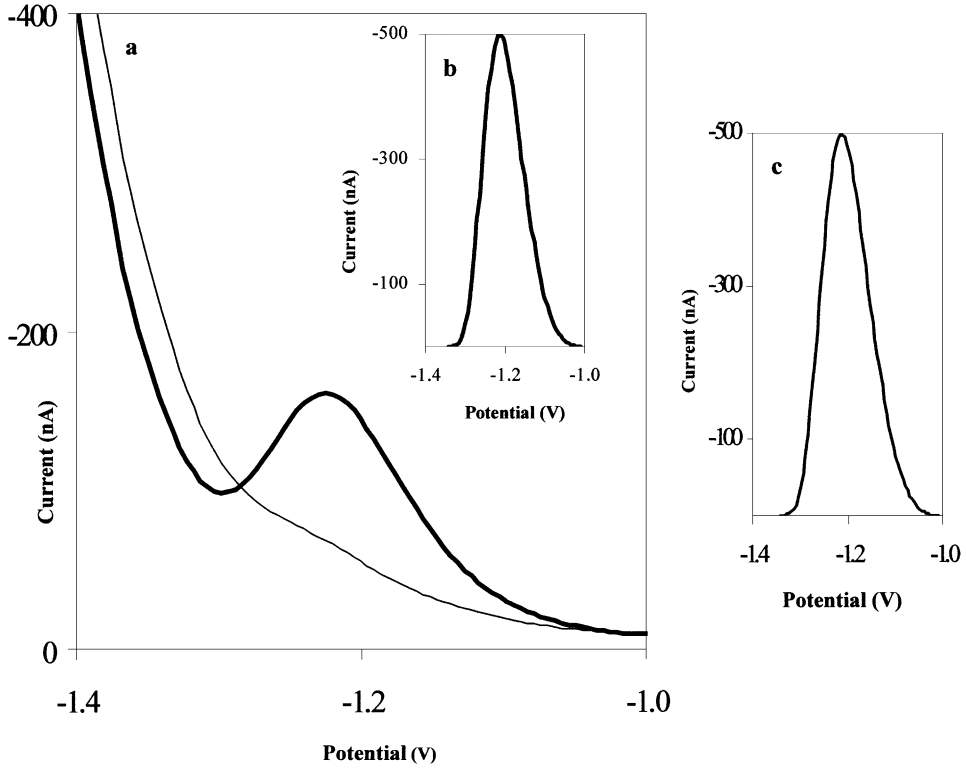

Fig. 2. Differential pulse stripping voltammograms of thermally denatured calf thymus DNA modified by Os,bipy in 0.1 M Britton–Robinson buffer, pH = 4.0. (a) Voltammograms of background electrolyte and ssDNA-Os,bipy at a concentration of 1 ng/ml (raw data). Data after moving average baseline correction (b) at a concentration of 1 ng/ml and (c) 5 ng/ml. Other conditions as in Fig. 1.

icals were of analytical grade. DNA solution was mixed with the 2 mM OsO_4 and bipy in Tris(hydroxymethyl)aminomethane and 0.1 M HCl (pH = 7.0). The reaction was carried out at 26 °C for 24 h. Simple reaction scheme is in Fig. 1a. Further details of modification were published [6].

2.2. Apparatus and procedures

Electrochemical measurements were performed with an AUTOLAB analyzer (EcoChemie, The Netherlands) in connection with a VA-Stand 663 (Metrohm, Zurich, Switzerland). The standard cell with three electrodes was used. The working electrode was the hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2. The reference electrode was the Ag/AgCl/3M KCl electrode and platinum electrode was used as the auxiliary electrode. All experiments were carried out at room temperature.

3. Results and discussion

Earlier [8], the analysis of DNA-Os,py was performed in the 0.3 M ammonium formate with 0.05 M sodium phosphate, pH = 6.9 (Fig. 1). Recently, we tested conditions of the analysis DNA-Os,bipy and optimized determination of the adduct. Some of the results are reported in this paper. We show that a poorly developed inflexion close to − 1.2 V obtained with DNA-Os,bipy (Fig. 1b) at a concentration of 5 ng/ml can be changed into a well-developed peak by means of moving average baseline correction (Fig. 1c).

The peak height of ssDNA-Os,bipy increases with decreasing pH, in 0.1 M Britton–Robinson buffer [9]. At pH 4.0, DNA-Os,bipy at a concentration of 1 ng/ml produces a peak which without any baseline correction is much better developed (Fig. 2a) than that observed at neutral pH under the same conditions (Fig. 1b). Treating raw data obtained at pH 4 by moving average baseline correction results in a symmetrical, well measurable peak (Fig. 2b and c). Our results show that osmium-labeled ssDNA can be determined by DPV at a concentration as low as 1 ng/ml (Fig 2b). This concentrations does not, however, represent a final detection limit. Substantially lower concentrations of ssDNA-Os,bipy can be determined if the accumulation time is adequately prolonged. To our knowledge, the method introduced in this paper represents the most sensitive electrochemical determination of ss nucleic acid samples.

4. Conclusion

In this paper, we show that chromosomal ssDNA modified with the well-known single-strand selective Os,bipy probe of the DNA structure [5] can be electrochemically determined concentrations as low as 1 ng/ml. Our preliminary results [9] suggest that lower DNA concentrations can be detected under certain conditions. Considering that about 3 μl of DNA-Os,bipy are sufficient for the analysis by the adsorptive stripping transfer voltammetry [10], we can conclude that few picograms of DNA-Os,bipy can be determined. This sensitivity is by about two orders of magnitude higher than that of the most sensitive electrochemical determination of unmodified DNA with mercury or carbon electrodes [11,12]. Such a high sensitivity of the electrochemical determination of DNA may find application in various areas of molecular biology as well as in biotechnologies and particularly in the development of the DNA hybridisation sensors [13]. More detailed studies will be published elsewhere.

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References

[1] E. Paleček, E. Lukášová, F. Jelen, M. Vojtíšková, Electrochemical analysis of polynucleotides, Bioelectrochem. Bioenerg. 8 (1981) 497–506.
[2] E. Lukášová, F. Jelen, E. Paleček, Electrochemistry of osmium nucleic-acid complexes—a probe for single-stranded and distorted double-stranded regions in DNA, Gen. Physiol. Biophys. 1 (1982) 53–70.
[3] E. Lukášová, M. Vojtíšková, F. Jelen, T. Sticzay, E. Paleček, Osmium-induced alteration in DNA-structure, Gen. Physiol. Biophys. 3 (1984) 175–191.
[4] E. Paleček, Local supercoil-stabilized DNA structures, Crit. Rev. Biochem. Mol. Biol. 26 (1991) 151–226.
[5] E. Paleček, Probing of DNA-structure in cells with osmium tetroxide-2,2′ -bipyridine, Methods Enzymol. 212 (1992) 305–318.
[6] E. Paleček, Probing DNA-structure with osmium-tetroxide complexes in vitro, Methods Enzymol. 212 (1992) 139–155.
[7] E. Paleček, D. Vlk, M. Vojtíšková, P. Boublíková, Complex of osmium tetroxide with 1,10-phenanthroline binds covalently to double-stranded DNA, J. Biomol. Struct. Dyn. 13 (1995) 537–546.
[8] E. Paleček, M.A. Hung, Determination of nanogram quantities of osmium-labeled nucleic acids by stripping (inverse) voltammetry, Anal. Biochem. 132 (1983) 239–242.
[9] L. Havran, E. Paleček, in preparation.
[10] E. Paleček, I. Postbieglová, Adsorptive stripping voltammetry of biomacromolecules with transfer of the adsorbed layer, J. Electroanal. Chem. 214 (1986) 359–371.
[11] E. Paleček, M. Fojta, M. Tomschik, J. Wang, Electrochemical biosensors for DNA hybridization and DNA damage, Biosens. Bioelectron. 13 (1998) 621–628.
[12] E. Paleček, From polarography of DNA to microanalysis with nucleic acid-modified electrodes, Electroanalysis 8 (1996) 7–14.
[13] E. Paleček, M. Fojta, Detecting DNA hybridization and damage, Anal. Chem. 73 (2001) 74A–83A.