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Bioelectrochemistry 63 (2004) 245–248
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Voltammetry of osmium-modified DNA at a mercury film electrode Application in detecting DNA hybridization

Pavel Kostecka, Ludek Havran, Hana Pivonkova, Miroslav Fojta*

Laboratory of Biophysical Chemistry and Molecular Oncology, Institute of Biophysics, Academy of Sciences of the Czech Republic, Kralovopolska 135, Brno 612 65, Czech Republic

Received 23 June 2003; received in revised form 17 November 2003; accepted 20 November 2003

Abstract

Mercury film electrodes (MFE) have recently been used in nucleic acid electrochemical analysis as alternatives to the classical mercury drop ones. DNA modified with osmium tetroxide, 2,2′ -bipyridine (Os,bipy) can be detected with a high sensitivity at mercury electrodes via measurements of a catalytic osmium signal. In this paper we show that mercury film on a glassy carbon electrode can be used in voltammetric analysis of Os,bipy-modified DNA. Application of the MFE as a detection electrode in double-surface electrochemical DNA hybridization assay involving osmium labeling of target DNA is demonstrated.

Keywords: Osmium; DNA hybridization; Mercury film electrode

1. Introduction

Electrochemical analysis of nucleic acids has employed various electrode materials that proved suitable in different types of measurements (reviewed in Refs. [1–3]). Among them, the mercury electrodes (ME) exhibit unique features when intrinsic DNA redox or tensammetric responses (sensitively reflecting small changes in DNA structure or subtle DNA damage) are measured [1–3]. In addition, modification of DNA with some chemical structural probes, namely osmium tetroxide complexes, can be most sensitively detected at the ME [4,5]. In spite of these facts, the MEs are not very popular among DNA biosensor developers. Aversions against the MEs arise partly from problematic applicability of classical hanging mercury drop electrode (HMDE) ‘‘in the field’’ and difficult incorporation of these devices in miniaturized systems, and partly from rather irrational fears of reputedly poisonous liquid mercury. Development and application of electrodes combining advantages of the mercury and solid ones, including mercury film electrodes (MFE) and solid amalgam electrodes (SAE), are motivated by the need to overcome the difficulties connected with the HMDE. SAEs were recently used [6] for highly sensitive detection of DNA purine bases. Kubicarova et al. [7] showed that mercury film deposited on a glassy carbon electrode (GCE) can be used for measurements of all intrinsic nucleic acid signals previously observed at the HMDE. Both MFE [8] and silver SAE [9] modified with supercoiled DNA can serve as sensors for DNA damage. Hason and Vetterl [10] employed the MFEs in studies of adsorption of nucleic acid constituents and of DNA–drug interactions [11].

Osmium tetroxide complexes (such as osmium tetroxide,2,2′ -bipyridine, Os,bipy) have been originally introduced as excellent single-strand selective DNA probes in studies of local DNA structures [12]. Os,bipy forms a stable covalent adduct with thymine and (in a lesser extent) cytosine residues in single-stranded DNA [12]. Due to their distinct electrochemical properties, the adducts can be used as electroactive DNA labels, yielding a series of signals at mercury [4,5,13] and carbon [14] electrodes. Catalytic osmium signal produced by the adducts at HMDE provides highly sensitive DNA detection (at sub-nanogram per milliliter level) [4]. Recently, we used Os,bipy for electroactive labeling of target DNA [14,15] in electrochemical DNA hybridization assays.

In this paper we used GCE plated with mercury film in measurements of Os,bipy-modified synthetic oligonucleo-

* Corresponding author. Tel.: +42-5-4151-7197; fax: +42-5-4121-1293.
E-mail address: fojta@ibp.cz (M. Fojta).

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doi:10.1016/j.bioelechem.2003.11.005

tides (ODN) and plasmid DNAs. Applicability of the MFE in a DNA hybridization assay is demonstrated.

2. Experimental

2.1. DNA samples

Plasmid DNAs pSP64 and pSP64-polyA were isolated, purified, linearized and denatured as described [14,16]. Oligonucleotides, a 71-mer 5′ TTGG$(TTTTTTCTC)_4$TT-TTTG$(A)_{25}$3′ and a 36-mer 5′ $(TTC)_{12}$3′, were purchased from VBC-GENOMICS (Austria). The DNAs (all 50 μg ml^{-1}) were incubated with 2 mM Os,bipy in 0.1 M Tris–HCl (pH 7.4) at 37 °C for 3 h. Unreacted Os,bipy was removed by dialysis.

2.2. DNA hybridization at magnetic beads

Procedure proposed in our previous papers [14,15] was used. Briefly, 10-μl aliquots of magnetic Dynabeads Oligo$(dT)_{25}$ (Dynal, Oslo, Norway) (DBT) were shaken with 20 μl of DNA (10 μg ml^{-1} plasmids or 5 μg ml^{-1} ODNs) samples in 0.3 M NaCl, 10 mM Tris–HCl, pH 7.4 (buffer H) for 30 min at 20 °C. After hybridization, the beads were washed twice in 50 μl of buffer H, and target DNAs were released into 10 μl of TE buffer by heating (85 °C, 2 min). Prior to adsorption at the electrode, NaCl was added to a final concentration 0.2 M.

2.3. Preparation of the MFE

A procedure derived from protocol proposed by Kubicarova et al. [7] was used. GCE (CH Instruments, Austin, TX, USA) was polished by 0.05 μm alumina powder (CH Instruments) on wet cotton. Then it was incubated in hot 20% H_2SO_4 for 5 min and sonicated for 60 s in triplicate distilled water. The mercury film was deposited in 0.1 M $Hg(NO_3)_2$ for 90 s at −1.2 V (vs. Ag/AgCl/3M KCl), rinsed and used for the adsorptive transfer stripping (AdTS) voltammetric measurement. After each measurement, the mercury layer was wiped off by cellulose swab and the procedure was repeated, starting from the ultrasonication step. The whole procedure (starting with GCE polishing) was repeated after each 10th measurement.

2.4. Voltammetric measurements

AdTS differential pulse voltammetry with an Autolab analyzer (Eco Chemie, The Netherlands) was used. Aliquots (5 μl) of the DNA-Os,bipy samples were deposited onto the MFE for 60 s to adsorb the DNA at the electrode. The electrode was washed and transferred into deaerated background electrolyte (Britton–Robinson buffer, pH 4.0). Three-electrode system involving Ag/AgCl/3 M KCl reference and platinum wire auxiliary electrodes was used. DPV settings: initial potential −0.6 V, final potential −1.5 V, pulse amplitude 50 mV, potential step 5 mV, time step 0.5 s.

3. Results and discussion

3.1. Detection of osmium-modified DNA at the MFE

It has been shown that DNA-Os,bipy produce a catalytic signal (peak Os; in Ref. [13], a corresponding signal is denoted as peak IV) around −1.2 V at the HMDE [4,13]. We examined voltammetric behavior of Os,bipy-modified 71-mer ODN and plasmid pSP64 DNA (both at a concentration of 5 μg ml^{-1}, accumulation time 60 s) in the relevant potential region at the MFE (Fig. 1A). Both samples yielded large, broad signals around −1.2 V. Peak Os of the about 3 kilobase long plasmid DNA (Fig. 1A, curve 2) was approximately four times smaller than the peak of the 71-mer ODN (curve 1), in agreement with a lower diffusion rate of the former DNA. Unmodified 71-mer ODN (curve 3) did not produce any signal in the same potential range.

Both 71-mer ODN and the pSP64 plasmid yielded almost linear calibration plots up to 5 μg ml^{-1} (higher concentrations were not tested, Fig. 1B). The measurements exhibited a good reproducibility, with a relative standard deviation of 9 % for the 71-mer ODN at a concentration of 1.25 μg ml^{-1}. The 71-mer ODN was easily detectable at a concentration of 25 ng ml^{-1} (inset in Fig. 1B). The sensitivity of the plasmid DNA detection was remarkably lower, enabling to detect about 1 μg ml^{-1} of DNA-Os,bipy. This observation is in agreement with previous results of Kubicarova et al [7,8] showing that long but not short DNA molecules produce significantly worse-developed voltammetric signals at the MFE, as compared to HMDE.

3.2. Using the MFE to detect DNA hybridization

Recently, we proposed a new approach in electrochemical DNA hybridization assays, the double-surface technique [6,14–16]. This technique involves DNA hybridization at one surface (magnetic beads) followed by electrochemical analysis with a suitable electrode. One of the detection methods introduced in our work is based on labeling of target DNA [14,15] with Os,bipy and detection of the osmium tag at carbon or mercury electrodes. The osmium labeling is particularly suitable when target DNA sequence recognized by the probe contains only purine residues not reacting with Os,bipy [14]. Here we used the 71-mer ODN, involving a $(dA)_{25}$ stretch, and the pSP64-polyA plasmid with a $(dA)_{30}$ stretch. Pyrimidine residues of these target DNAs were modified with Os,bipy and the DNAs were hybridized with $(dT)_{25}$ capture probes at the DBT. After washing, the target DNAs were detached from the DBT and analyzed by AdTS DPV at the MFE. In

addition to the two target DNAs, we used two osmium-labeled nonspecific DNAs, not containing the oligo(dA) stretches (the 36-mer ODN and the pSP64 plasmid). All of the samples yielded peak Os prior to hybridization at the beads (Fig. 2A). After the hybridization procedure (Fig. 2B), only the target DNAs involving the oligo(dA) sequence yielded signals (curves 1 and 3). The nonspecific DNAs did not yield any response (curves 2 and 4) because they could not be captured at the beads.

3.3. Conclusions

Several attempts have been recently made in nucleic acid electroanalysis to replace the HMDE by electrodes retaining electrochemical properties of the mercury surface but possessing advantages of solid electrodes [6–11]. The MFE represents one of the ways to reach the goal [7,8,10,11]. Present results suggest that the MFE can be even better suited for certain types of analysis (e.g. detecting DNA fragmentation, discrimination between short and long DNA molecules) than the HMDE [8]. In this paper we show that measurements of osmium-labeled DNA at the MFE can be utilized for bioanalytical purposes, including detection of DNA hybridization, although the MFE (at glassy carbon substrate) offers a lower sensitivity of detection of the DNA-Os,bipy than the HMDE [4] (by one to two orders of magnitude, resembling the sensitivities attained at carbon electrodes [14]). Nevertheless, our recent results (M. Fojta, R. Fadrna, P. Kostecka, unpublished) obtained with mercury films plated on solid metallic or amalgam substrates suggest that remarkably better sensitivities of DNA-Os,bipy detection (comparable to that at HMDE) can be reached under certain conditions, and that the MFE surfaces can be repeatedly renewed using a solely electrochemical procedure (without mechanical removal of the used film). More results will be published elsewhere.

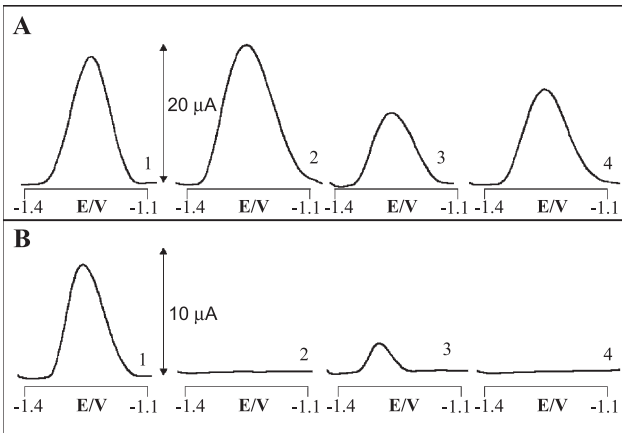

Fig. 2. Detection of DNA hybridization at the DBT using the MFE. (A) Details of baseline-corrected peak Os yielded by (1) 71-mer target ODN; (2) 36-mer nonspecific ODN; (3) plasmid pSP64-polyA target DNA; (4) plasmid pSP64 nonspecific DNA, prior to hybridization at the beads. Concentration of the ODNs was 5 μg ml^{-1}, concentration of the plasmids 10 μg ml^{-1}. (B) Hybridization responses of the same DNA samples. The DNA solutions (20 μl) were 30 min incubated with the DBT, followed by removal of unbound DNA solution, release of captured target DNAs by heating and detection of the latter at the MFE. Numbering of the curves as in (A); for other details, see Experimental and Fig. 1.

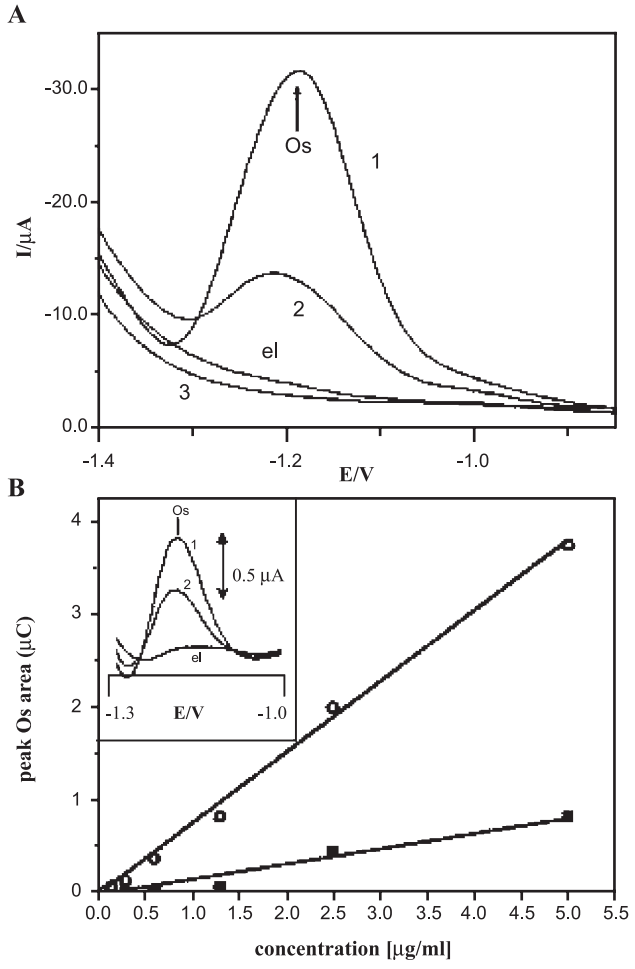

Fig. 1. (A) Differential pulse voltammograms of: curve 1, Os,bipy-modified 71-mer ODN; curve 2, Os,bipy-modified plasmid pSP64; curve 3, unmodified 71-mer ODN; el, background electrolyte. DNA samples (all 5 μg ml^{-1}) were adsorbed at the MFE surface from 5 μl of 0.2 M NaCl, 10 mM TE, for 60 s. Then the DNA-modified MFE was washed, immersed into voltammetric cell containing Britton–Robinson buffer, pH 4, and the voltammograms were recorded. (B) Dependence of the peak Os area on DNA-Os,bipy concentration. (○) 71-mer ODN; (■) plasmid pSP64 DNA. Inset: Detail of baseline-corrected peak Os yielded by the modified 71-mer ODN at concentrations of (1) 50 ng ml^{-1} or (2) 25 ng ml^{-1}; el, background electrolyte; other details as in (A).

Acknowledgements

This work was supported by grants from the Grant Agency of the Academy of Sciences of the Czech Republic nos. A4004108 to MF, KJB4004302 to LH, and a grant no. Z 5004920 from the Academy of Sciences of the Czech Republic. The authors are grateful to Professor Emil Palecek for critically reading of the manuscript.

References

[1] M. Fojta, Electrochemical sensors for DNA interactions and damage, Electroanalysis 14 (2002) 1449–1463.

[2] E. Palecek, M. Fojta, Detecting DNA hybridization and damage, Anal. Chem. 73 (2001) 74A–83A.

[3] E. Palecek, Past, presence and future of nucleic acids electrochemistry, Talanta 56 (2002) 809–819.

[4] R. Kizek, L. Havran, M. Fojta, E. Palecek, Determination of nanogram quantities of osmium-labeled single stranded DNA by differential pulse stripping voltammetry, Bioelectrochemistry 55 (2002) 119–121.

[5] E. Palecek, M.A. Hung, Determination of nanogram quantities of osmium-labeled nucleic acids by stripping (inverse) voltammetry, Anal. Biochem. 132 (1983) 236–242.

[6] F. Jelen, B. Yosypchuk, A. Kourilova, L. Novotny, E. Palecek, Label-free determination of picogram quantities of DNA by stripping voltammetry with solid copper amalgam or mercury electrodes in presence of copper, Anal. Chem. 74 (2002) 4788–4793.

[7] T. Kubicarova, M. Fojta, J. Vidic, D. Suznjevic, M. Tomschik, E. Palecek, Voltammetric and chronopotentiometric measurements with nucleic acids-modified mercury film at glassy carbon electrode, Electroanalysis 12 (2000) 1390–1396.

[8] T. Kubicarova, M. Fojta, J. Vidic, L. Havran, E. Palecek, Mercury film electrode as a sensor for the detection of DNA damage, Electroanalysis 12 (2000) 1422–1425.

[9] K. Kucharikova, L. Novotny, B. Yosypchuk, M. Fojta, Detecting DNA damage with a silver solid amalgam electrode, Electroanalysis (2004) (in press).

[10] S. Hason, V. Vetterl, Application of carbon electrodes modified with a mercury layer of a different thickness for studies of the adsorption and kinetics of phase transients of cytidine, J. Electroanal. Chem. 536 (2002) 19–35.

[11] S. Hason, J. Dvorak, F. Jelen, V. Vetterl, Impedance analysis of DNA and DNA–drug interactions on thin mercury film electrodes, Crit. Rev. Anal. Chem. 32 (2002) 167–179.

[12] E. Palecek, Probing of DNA structure with osmium tetroxide complexes in vitro, Methods Enzymol. 212 (1992) 139–155.

[13] L. Havran, M. Fojta, E. Palecek, Voltammetric behavior of DNA modified with osmium tetroxide 2,2′-bipyridine at mercury electrodes, Bioelectrochemistry (2004) (doi: 10.1016/j.bioelechem.2003.11.004).

[14] M. Fojta, L. Havran, S. Billova, P. Kostecka, M. Masarik, R. Kizek, Two-surface strategy in electrochemical DNA hybridization assays: detection of osmium-labeled target DNA at carbon electrodes, Elecroanalysis 15 (2004) 431–440.

[15] E. Palecek, R. Kizek, L. Havran, S. Billova, M. Fojta, Electrochemical enzyme-linked immunoassay in a DNA hybridization sensor, Anal. Chim. Acta 469 (2002) 73–83.

[16] E. Palecek, S. Billova, L. Havran, R. Kizek, A. Miculkova, F. Jelen, DNA hybridization at microbeads with cathodic stripping voltammetric detection, Talanta 56 (2002) 919–930.