Electrochemistry Communications 11 (2009) 359–362

Contents lists available at ScienceDirect

Electrochemistry Communications

journal homepage: www.elsevier.com/locate/elecom

End-labeling of peptide nucleic acid with osmium complex. Voltammetry at carbon and mercury electrodes

Emil Paleček *, Mojmír Trefulka, Miroslav Fojta

Institute of Biophysics, Academy of Sciences of the Czech Republic, Kralovopolska 135, 612 65 Brno, Czech Republic

ARTICLE INFO

Article history:
Received 3 November 2008
Received in revised form 27 November 2008
Accepted 28 November 2008
Available online 9 December 2008

Keywords:
Peptide nucleic acid end-labeling
Osmium tetroxide complexes
Electroactive labels
Adsorptive stripping voltammetry
Mercury and carbon electrodes
Electrochemical DNA sensors

ABSTRACT

Peptide nucleic acid (PNA), the DNA mimic with electrically neutral pseudopeptide backbone, is intensively used in biotechnologies and particularly in single-base mismatch detection in DNA hybridization sensors. We propose a simple method of covalent end-labeling of PNA with osmium tetroxide, 2,2′-bipyridine (Os,bipy). Os,bipy-modified PNA (PNA–Os,bipy) produces voltammetric stripping peaks at carbon and mercury electrodes. Peak potential (E_p) of one of the anodic peaks of PNA–Os,bipy at the pyrolytic graphite electrode (PGE) differs from E_p of the reagent, allowing PNA–Os,bipy analysis directly in the reaction mixture. At the hanging mercury drop electrode (HMDE) the PNA–Os,bipy yields a catalytic peak Cat_p, in addition to the redox couples. Using Cat_p it is possible to detect purified PNA–Os,bipy down to 1 pM concentration at accumulation time 60 s. To our knowledge this is the highest sensitivity of the electrochemical detection of PNA.

1. Introduction

Fast highly parallel analysis of DNA nucleotide sequences holds great promise for rapid clinical diagnosis, tailor-made therapy, etc. forming a part of the development of the new biomedicine of the 21st century. Among techniques used in nucleotide sequencing electrochemical methods have drawn great attention [1]. Increased interest in these methods arises from low cost, high sensitivity, their compatibility with microfabrication technologies, etc. Peptide nucleic acid (PNA) can be used in DNA hybridization sensors as a surface-immobilized capture probe or a reporter (signaling) probe to detect specific DNA sequence. PNAs are synthetic DNA mimics, which contain 2-aminoethylglycine linkages instead of the negatively charged phosphodiesteric backbone of oligodeoxynucleotides (ODN's) [2]. PNA probes have been particularly useful in detecting single-base mismatches (point mutations in target DNA) [3–5]. In fact the first electrochemical detection of DNA point mutation was achieved more than 10 years ago using PNA [4]. PNA is electroactive [6,7], producing reduction (adenine and cytosine) and oxidation signals (guanine) at mercury electrodes [7] and oxidation signals (adenine and guanine) at carbon electrodes [6] similar to those of DNA and RNA. At these electrodes PNA is also strongly adsorbed [8].

Many of the molecular biological processes involving PNA and particularly the hybridization procedures require affinity nucleotide sequence for the DNA hybridization and a label at PNA terminal. A number of methods of introduction of fluorescent labels into PNA (e.g. fluorescein and rhodamin) have been developed [9]. In addition, PNA was labeled by radioactive ^{32}P as well as by psoralen [10], biotin, etc. Earlier, numerous electroactive DNA labels but not PNA ones were developed [1]. For example, electroactive markers based on osmium tetroxide complexes with nitrogenous ligands [Os,L] have been very useful probes of the DNA structure [11] and DNA electroactive labels [1,12–15] but no such a label have been introduced in PNA. Very recently, ferrocene labeling of PNA was reported [16,17].

In this paper we propose a facile PNA modification by OsO_4, 2,2′-bipyridine (Os,bipy) based on the preferential binding of this agent to thymine residues in single-stranded nucleic acids (NAs) [11]. We show that PNA directly labeled by Os,bipy produces several voltammetric peaks and can be sensitively detected at carbon and mercury electrodes.

2. Experimental

2.1. Reagents

PNA (Biosynthesis, Lewisville, Texas) contained the purine affinity sequence $(GAA)_4$ and a TTT tail reactive toward Os,bipy (from N-terminus to C-terminus, GAAGAAGAAGAATTT) with three lysines (K-PNA) and without lysines (PNA-1) on its N-terminus; Oligodeoxynucleotide (ODN) 5′-GAAGAAGAAGAATTT-3′ (VBC-GENOMICS,

* Corresponding author. Tel.: +420 541517177; fax: +420 541211293.
E-mail address: palecek@ibp.cz (E. Paleček).

1388-2481/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.elecom.2008.11.049

Austria); Osmium tetroxide (JMC, UK); 2,2′-bipyridine (bipy) min. 99%, Sigma; other chemicals were of analytical grade.

2.2. Instrumentation

Voltammetric measurements were performed in argon atmosphere with an Autolab analyzer (Eco Chemie, Utrecht, The Netherlands) in connection with VA-Stand 663 (Metrohm, Herisau, Switzerland). Three-electrode system was used. Working electrodes: basal-plane pyrolytic graphite electrode (PGE, 14 mm^2) or hanging mercury drop electrode (HMDE, 0.4 mm^2) Ag/AgCl/3 M KCl served as a reference and platinum wire as an auxiliary electrode. PGE was pretreated before each measurement by applying +1.7 V for 60 s and peeled with cellotape. Other details were described in [18–20]. The adsorptive transfer stripping (AdTS, ex situ) technique [21,22] and conventional adsorptive stripping (AdS, in situ) DPV were used.

2.3. Modification of PNA and ODN with osmium tetroxide 2,2′-bipyridine complex [Os,bipy]

End-labeling of PNA was based on the high selectivity of Os,bipy to thymines [11] enabling specific modification of T's in the PNA sequence [11,23]. The reaction mixture containing 100 μM PNA-1, K-PNA or ODN to be modified, 2 mM osmium tetroxide, 3 mM bipy and 50 mM phosphate buffer, pH 7, was incubated at 37 °C for 5 h. The reaction mixture was purified by dialysis on Slide-A-Lyzer Mini Dialysis Units 3500 MWCO (Pierce) for 42 h at 4 °C against 50 mM phosphate, pH 7.0. The final concentration of Os,bipy-modified PNA was determined by absorbance measurement at 312 nm, similarly to DNA [23,24].

3. Results and discussion

3.1. Pyrolytic graphite electrode (PGE)

In AdTS (ex situ) [1,22] purified PNA-1–Os,bipy or K-PNA–Os,bipy were adsorbed on PGE, the PNA-modified PGE was washed and transferred to the blank background electrolyte. Using cyclic voltammetry (CV) these PNAs displayed three anodic faradaic peaks denominated as peaks α_{pa}, β_{pa} and less developed γ_{pa}. Two cathodic peaks α_{pc} and β_{pc} similar to those of DNA-Os,bipy [1,18,20] (Fig. 1A) were observed. AdTS square wave voltammograms (SWV) of PNA-1–Os,bipy and K-PNA–Os,bipy showed two peaks, α_p, at −0.61 V, and β_p, at −0.12 V (Fig. 1B) which were more negative (by 40 or 60 mV, respectively) than those of ODN-Os,bipy (peaks α and β, Fig. 1D) [20]. The electrochemical behavior of both modified PNAs did not practically differ. Importantly, peak α_p was by 170 mV more negative than the corresponding peak I of free Os,bipy (Fig. 1B) allowing discrimination between modified PNA and the free reagent. This enabled us to analyze reaction mixture of PNA and Os,bipy without purification. For analysis of Os,bipy-modified DNAs [12,20,25] or proteins [26] we developed earlier a technique involving removal of unreacted Os,bipy from PGE surface by extraction with organic solvents [12,20,25,26]. Washing of the PNA-modified PGE with 2-propanol after usual washing with water had no effect on the voltammetric responses (Fig. 1B). On the other hand peaks I and II due to free Os,bipy in the absence of PNA were almost eliminated by 2-propanol washing (Fig. 1B). The apparent lack of interference of the unreacted Os,bipy with PNA–Os,bipy determination can be related to very strong competitive adsorption of PNA at the electrode, blocking efficiently the surface and preventing adsorption of free Os,bipy under the given conditions. Fig. 1C shows the dependences of peak α or α_p on the concentrations of

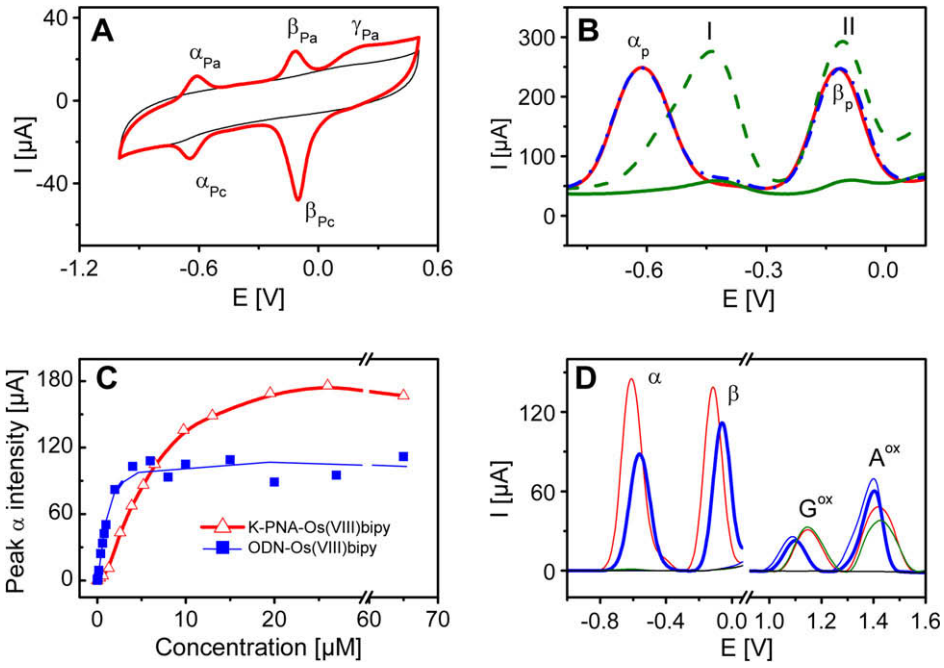

Fig. 1. AdTS (ex situ) voltammetry of Os,bipy-modified PNA or DNA at PGE (A) AdTS CV of 3 μM purified K-PNA–Os,bipy (red) and background electrolyte, 0.2 M acetate, pH 5.0 (black): scan rate 1 V/s, t_A 300 s; (B) AdTS SWV of 100 μM K-PNA adsorbed at PGE from the reaction mixture containing 2 mM Os,bipy (red, blue dashed-dotted line) or 2 mM Os,bipy alone (green). Full lines, after additional 60 s washing with 2-propanol, as described in [20,26], other lines, without this washing; frequency 200 Hz, amplitude 25 mV, t_A 60 s; (C) Dependence of the heights of peak α_p or α on concentration of K-PNA–Os,bipy or ODN-Os,bipy, respectively. Conditions as in (B) but no 2-propanol washing. Concentrations given in the graph were obtained by dilution of the reaction mixture with 0.2 M acetate, pH 5.0; (D) AdTS SWV of unmodified K-PNA (green), purified K-PNA–Os,bipy (red), unmodified ODN (thin blue), purified ODN-Os,bipy (thick blue), and background electrolyte (black). 10 μM NA samples, modified NAs were purified by dialysis, other conditions as in (B).

modified DNA and K-PNA, respectively. Both NAs displayed linear portions and saturation levels of these dependences. The dependence of ODN-Os,bipy was steeper and reached saturation at lower concentrations than that of K-PNA–Os,bipy, which exhibited by about 35% higher signal intensity at full electrode coverage. Such behavior may be related to differences in charges of PNA and DNA affecting their interactions in solution and packing at the electrode surface.

In addition to the above-mentioned peaks, typical for thymine-Os,bipy adducts in PNA or DNA, we observed oxidation peaks of guanine (peak G^{ox}) and adenine (peak A^{ox}) (Fig. 1D). In agreement with previous data obtained with modified ODNs [13], these peaks were barely affected by the modification, showing almost identical peak G^{ox} in the modified and unmodified NAs. Peak G^{ox} is thus useful for normalizing the adduct-specific signals in determination of the NA modification extent [27].

3.2. Hanging mercury drop electrode (HMDE)

We studied PNA–Os,bipy by AdTS CV (ex situ) at different pH's and scan rates. PNA–Os,bipy displayed three cathodic faradaic peaks between 0 and −0.9 V (peaks I_{pc}, II_{pc} and III_{pc}) and their anodic counterparts (peaks I_{pa}, II_{pa} and III_{pa}) (Fig. 2C) and a catalytic peak Cat_p at about −1.2 V (Fig. 2A). Optimum conditions for faradaic peaks were around pH 7 and at higher scan rates, such as 1–3 V/s. On the other hand, peak Cat_p was well-developed at acid pH's (e.g. pH 4.8) and at slow scan rates (such as 0.1 V/s). This peak is due to catalytic hydrogen evolution [28] and is remarkably higher than the faradaic peaks under optimal conditions [1,29,30]. 500 nM K-PNA–Os,bipy produced Cat_p and its cathodically directed counter-peak Cat_{pa} (Fig. 2A) characteristic for electrocatalytic processes.

We measured Cat_p by AdTS DPV and, at lower concentrations, also by conventional AdS DPV. In the range from 0.5 to about 25 nM PNA-1–Os,bipy the height of AdTS Cat_p was linearly dependent on the concentration of PNA-1–Os,bipy while between 200 and 1000 nM the peak height changed only slightly, suggesting that a full electrode coverage was reached (Fig. 2D). E_p shifted slightly with increasing concentration to less negative values. 1 pM PNA-1–Os,bipy produced at t_A = 60 s a well-developed peak (Fig. 2B), while free Os,bipy was not detectable below 50 pM under the same conditions.

4. Conclusions

In this article, we propose a simple method of covalent end-labeling of PNA with Os,bipy. This method can be performed in any biological or chemical laboratory lacking solid-state chemistry equipment and/or highly qualified personnel. PNA–Os,bipy produces well-developed peaks at carbon and mercury electrodes, which can be utilized in DNA sensors [1]. Earlier it was shown that DNA-Os,bipy produced well resolved signals also at gold electrodes [18,31]; it can be thus expected that PNA–Os,bipy will be useful also in the Au-electrode based sensors. Hydrogen evolution catalyzed by only 1 pM PNA–Os,bipy resulted in a well-developed peak Cat_p at HMDE at t_A = 60 s (Fig. 2B) suggesting that at longer t_A even subpicomolar concentrations could be detectable. This electrode does not represent the best transducer for a DNA sensor. It has been shown, however, that HMDE can be replaced by solid amalgam electrodes without loosing the high sensitivity of the DNA-Os,bipy catalytic peak [29]. The great sensitivity of peak Cat_p can be further increased using multiple (Os,bipy) labels, as in the DNA labeling [1,13], and by further optimization of the conditions. On the other hand, to our knowledge, none of the known PNA electroactive labels [16,17] offers such a high sensitivity as the Cat_p peak. For example, for PNA monomer labeled with ferrocenyl-azobenzene, detection limits 1 μM (DPV) and 100 nM (UV–vis) were reported [17]. Peak α_p at PGE (Fig. 1B) required higher PNA–Os,bipy concentration (Fig. 1C) than Cat_p (Fig. 2B and D) at HMDE but with PGE it was possible to apply PNA at the electrode in the reaction

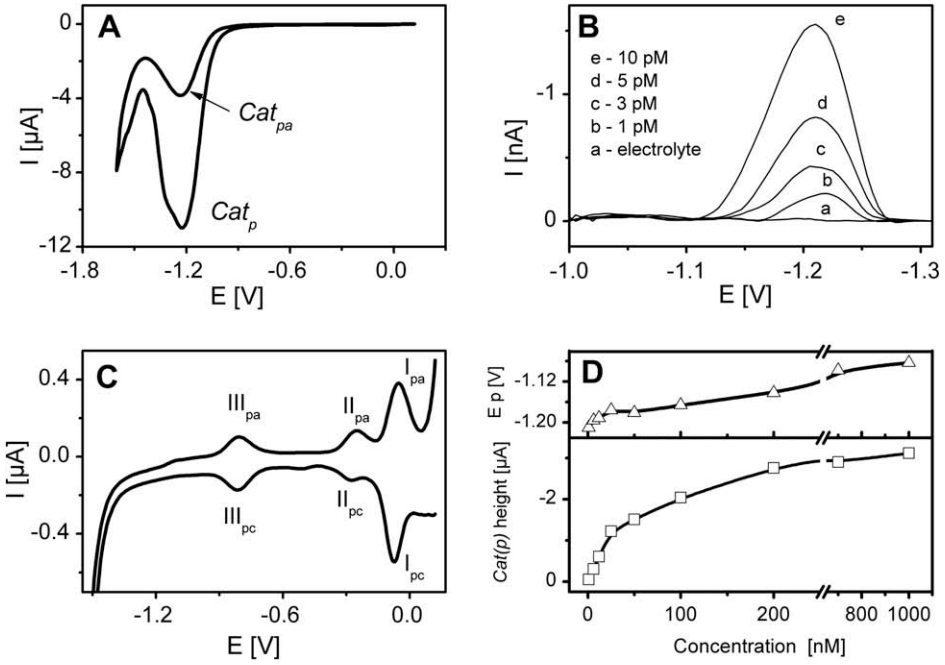

Fig. 2. Voltammetry of PNA–Os,bipy at HMDE (A) AdTS CV of 500 nM purified K-PNA–Os,bipy, scan rate 0.1 V/s; (B) Catalytic peak Cat_p at different concentrations of purified PNA-1–Os,bipy, conventional AdS (in situ) DPV. 0.1 M Britton–Robinson buffer, pH 4.8, t_A 60 s, accumulation potential −0.6 V, with stirring (1500 rev/min), modulation amplitude 50 mV, moving average baseline correction; (C) AdTS CV of 3 μM K-PNA–Os,bipy, pH 7.0, scan rate 1 V/s; (D) Dependence of Cat_p peak potential (E_p) and height on PNA-1–Os,bipy concentration, AdTS DPV; (A, C and D) Adsorption at open current circuit for t_A 60 s, purified PNA–Os,bipy-modified electrode was washed and transferred in electrolytic cell with blank 0.1 M Britton–Robinson buffer, pH 4.7.

mixture without any purification. This procedure showed its usefulness both in DNA [20] and protein analysis (in proteins accessible tryptophans are modified [26,32,33]). Moreover, DNA-Os,L adducts containing different nitrogenous ligands were prepared which displayed signals at different potentials at carbon electrodes, enabling "multicolor labeling" of DNA [15]. Our preliminary results suggest that similar labeling will be possible also with PNA.

Acknowledgements

The authors are indebted to T. Doneux for critical reading of the manuscript.

This work was supported by the Academy of Sciences of the Czech Republic (KAN400310651), by the Ministry of Education, Youth and Sports of the CR (LC06035) and by institutional grants AV0Z50040507 and AV0Z50040702.

References

[1] E. Palecek, F. Jelen, in: E. Palecek, F. Scheller, J. Wang (Eds.), Perspectives in Bioanalysis, Electrochemistry of Nucleic Acids and Proteins. Towards Electrochemical Sensors for Genomics and Proteomics, vol. 1, Elsevier, Amsterdam, 2005, pp. 73–174.
[2] P.E. Nielsen, G. Haaima, Chem. Soc. Rev. 26 (1997) 73.
[3] O. Brandt, J.D. Hoheisel, Trends Biotechnol. 22 (2004) 617.
[4] J. Wang, E. Palecek, P.E. Nielsen, G. Rivas, X.H. Cai, H. Shiraishi, N. Dontha, D.B. Luo, P.A.M. Farias, J. Am. Chem. Soc. 118 (1996) 7667.
[5] M. Steichen, Y. Decrem, E. Godfroid, C. Buess-Herman, Biosens. Bioelectron. 22 (2007) 2237.
[6] J. Wang, G. Rivas, X.H. Cai, M. Chicharro, N. Dontha, D.B. Luo, E. Palecek, P.E. Nielsen, Electroanalysis 9 (1997) 120.
[7] M. Tomschik, F. Jelen, L. Havran, L. Trnkova, P.E. Nielsen, E. Palecek, J. Electroanal. Chem. 476 (1999) 71.
[8] M. Fojta, V. Vetterl, M. Tomschik, F. Jelen, P. Nielsen, J. Wang, E. Palecek, Biophys. J. 72 (1997) 2285.
[9] H. Ørum, R. Casale, M. Egholm, in: P.E. Nielsen, M. Egholm (Eds.), Peptide Nucleic Acids Protocols and Applications, Horizon Scientific Press, Wymondham, 1999, pp. 81–86.
[10] G.F. Ross, P.M. Smith, A. McGregor, D.M. Turnbull, R.N. Lightowlers, Bioconjugate Chem. 14 (2003) 962.
[11] E. Palecek, Methods Enzymol. 212 (1992) 139.
[12] M. Fojta, L. Havran, S. Billova, P. Kostecka, M. Masarik, R. Kizek, Electroanalysis 15 (2003) 431.
[13] M. Fojta, L. Havran, R. Kizek, S. Billova, E. Palecek, Biosens. Bioelectron. 20 (2004) 985.
[14] M. Fojta, L. Havran, M. Vojtiskova, E. Palecek, J. Am. Chem. Soc. 126 (2004) 6532.
[15] M. Fojta, P. Kostecka, M. Trefulka, L. Havran, E. Palecek, Anal. Chem. 79 (2007) 1022.
[16] G. Gasser, N. Husken, S.D. Koster, N. Metzler-Nolte, Chem. Commun. (2008) 3675.
[17] J.D. Li, M. Chen, H.B. Zhang, S. Liu, Inorg. Chem. Commun. 11 (2008) 392.
[18] M. Trefulka, N. Ferreyra, V. Ostatna, M. Fojta, G. Rivas, E. Palecek, Electroanalysis 19 (2007) 1334.
[19] M. Trefulka, V. Ostatna, L. Havran, M. Fojta, E. Palecek, Electroanalysis 19 (2007) 1281.
[20] M. Fojta, L. Havran, R. Kizek, S. Billova, Talanta 56 (2002) 867.
[21] E. Palecek, F. Jelen, C. Teijeiro, V. Fucik, T.M. Jovin, Anal. Chim. Acta 273 (1993) 175.
[22] E. Palecek, I. Postbieglova, J. Electroanal. Chem. 214 (1986) 359.
[23] F. Jelen, P. Karlovsky, E. Makaturova, P. Pecinka, E. Palecek, Gen. Physiol. Biophys. 10 (1991) 461.
[24] C.H. Chang, M. Beer, L.G. Marzilli, Biochemistry 16 (1977) 33.
[25] L. Havran, J. Vacek, K. Cahova, M. Fojta, Anal. Bioanal. Chem. 391 (2008) 1751.
[26] M. Fojta, S. Billova, L. Havran, H. Pivonkova, H. Cernocka, P. Horakova, E. Palecek, Anal. Chem. 80 (2008) 4598.
[27] P. Brázdilova, M. Vrabel, R. Pohl, H. Pivonkova, L. Havran, M. Hocek, M. Fojta, Chem.-Eur. J. 13 (2007) 9527.
[28] E. Palecek, M.A. Hung, Anal. Biochem. 132 (1983) 236.
[29] B. Yosypchuk, M. Fojta, L. Havran, M. Heyrovsky, E. Palecek, Electroanalysis 18 (2006) 186.
[30] H. Sopha, W. Falko, G.U. Flechsig, Electrochem. Commun. 10 (2008) 1614.
[31] G.U. Flechsig, T. Reske, Anal. Chem. 79 (2007) 2125.
[32] S. Billova, R. Kizek, E. Palecek, Bioelectrochemistry 56 (2002) 63.
[33] O. Sedo, S. Billova, E.M. Pena-Medrez, E. Palecek, J. Havel, Anal. Chim. Acta 515 (2004) 261.