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Facile end-labeling of RNA with electroactive Os(VI) complexes

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ABSTRACT

Ribose at the 3′-end of oligonucleotides (oligos) selectively modified by Os(VI)2,2′-bipyridine (bipy) produced two CV redox couples at pyrolytic graphite electrode. Using square wave voltammetry (SWV) 22-mer oligos can be detected down to 250 nM. At mercury electrodes the Os(VI)bipy-oligo adducts produced an electrocatalytic peak at ~−1.2 V allowing their determination down to picomolar concentrations. High specificity of Os(VI)bipy for ribose in nucleic acids and high sensitivity of the determination at mercury and solid amalgam electrodes give promise for new efficient methods of microRNA determination.

1. Introduction

Electrochemical analysis of nucleic acids and particularly the development of the DNA hybridization sensors is at present a booming field as documented by hundreds of papers per year and over 15 review articles published in 2009 and 2010 (e.g., [1–7]). Among the hundreds of papers only a very small fraction deals with RNA [8–16]. It can be expected that the number of electrochemical papers devoted to RNA will greatly increase in the near future because RNA became a hot science topic due to the discovery of microRNAs (miRNA). The initial discovery of miRNA dates back to 1993 [17] but only after 2001 miRNA research attracted attention of a very large number of laboratories. It has been shown that miRNAs are short strands of RNA between 18 and 25 nucleotides in length, performing gene regulation either directly or indirectly via translation repression. There is a growing evidence of links between miRNA expression and the onset of cancer and other diseases. Currently miRNAs are predominantly analyzed using Northern blot, PCR and microarray analysis with optical detection. Only recently several electrochemical methods, either label-free [8] or involving miRNA labeling [10], have been proposed. Labeling of miRNA offers better selectivity and sensitivity but application of ferrocene or enzyme labels (frequently used in DNA detection) has some limitations, because they cannot be applied for direct labeling of miRNAs either directly in a biological matrix or after the miRNA isolation.

Already in 1981 we invented the first electroactive label of nucleic acids [18–20] based on osmium tetroxide complexes with nitrogenous ligands (L). These complexes bind covalently to pyrimidine bases through addition to the 5,6 double bond in single stranded (ss) or distorted (but not in intact) double stranded DNA structures [21]. Os(VIII)L complexes were applied for end-labeling of DNA and PNA in hybridization sensors using carbon [22–24], gold [25–28] and mercury [22,29,30] electrodes, as well as for electroactive labeling of peptides [31] and proteins [32]. Recently they were applied also for sensitive tRNA voltammetric determination at Hg electrodes [14]. Labeling of miRNA with these Os complexes is feasible but modification of bases (uracil and cytosine) would interfere with the miRNA hybridization necessary for determination of specific miRNAs. Recently we have shown that six-valent Os complexes react specifically with sugar residues in ribosides [33] and in polysaccharides (PSs) [34,35] producing redox couples at carbon and Hg electrodes. In addition, PS adducts with some Os(VI)L complexes yielded an electrocatalytic signal at Hg electrodes, including solid amalgam electrodes [36].

In this paper we show that in short synthetic oligonucleotides the ribose residue at the 3′-end of the molecules is selectively modified by Os(VI)L complexes producing redox couples at carbon and Hg electrodes and electrocatalytic peak at Hg electrodes. Using the latter signal RNAs can be detected at pM concentrations.

2. Experimental

2.1. Material

Potassium osmate dihydrate, Merck; 2,2′-bipyridine (bipy), Sigma; *N,N,N′,N′*-tetramethylethylenediamine (temed), Merck; and oligonucleotides from VBC Biotech GmbH (Wien, Austria):

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$dA_{21}rA$: 5′-AAA AAA AAA AAA AAA AAA AAA rA-3′
ODRN: 5′-CTG TCA GCG TTT ACA AAC GGT rA-3′
ODN: 5′-CTG TCA GCG TTT ACA AAC GGT A-3′.

We chose this set of oligonucleotides for two reasons: (a) signals obtained with ODRN having a single ribonucleotide at the 3′-end can be unequivocally interpreted as due to Os(VI)L modification of the ribose at the 3′-end; and (b) $dA_{21}rA$ and ODRN were much less expensive than the same oligonucleotides composed fully of ribonucleotides. Other chemicals were of analytical grade, and all aqueous solutions were prepared from deionized water.

2.2. Instrumentation

Electrochemical measurements were performed with an Autolab analyzer (Eco Chemie, The Netherlands) in connection with VA-Stand 663 (Metrohm, Switzerland). A three-electrode system with Ag/AgCl/3 M KCl electrode as a reference and platinum wire as an auxiliary electrode was used. The pyrolytic graphite electrode (PGE, 9 mm^2), hanging mercury drop electrode (HMDE, 0.4 mm^2) and mercury meniscus-modified solid amalgam electrode (m-SAE, disc diameter 0.54 mm) were the working electrodes. All measurements were carried out at room temperature using conventional adsorptive stripping (AdS) or adsorptive transfer stripping (AdTS) technique [34].

2.3. Modification of oligonucleotides with Os(VI)L complexes

Modification of oligonucleotides (oligos) was done as described previously [35]. Briefly, 200 μM oligonucleotide and 3 mM Os(VI) temed in 50 mM phosphate, pH 7.0, were mixed and agitated for 16 h at 37 °C. Products were purified using Amicon Ultra 3 K membrane (Millipore Corporation, USA). Os(VI)bipy-modified oligos were prepared by the ligand exchange using previously prepared Os(VI) temed-oligos, because direct modification with Os(VI)bipy is not convenient for work with small volumes. 0.1 mL of 20 mM bipy was added to the purified Os(VI)temed-oligo samples and the reaction mixture was agitated at 37 °C for 24 h. Resulting products were purified using membranes as mentioned earlier.

3. Results and discussion

In order to test the binding specificity of Os(VI)L complexes to ribose at the 3′-end, two oligos with the same sequence were used: ODRN containing 21 deoxyribose residues and one ribose residue at the 3′-end, and an oligodeoxynucleotide (ODN) lacking any ribose. We measured Os(VI)bipy-treated oligos using CV, DPV or SWV with carbon and mercury electrodes.

3.1. Carbon electrodes

We used AdTS (ex situ) method to adsorb oligos on PGE from 8 μl drops. Fig. 1A shows that Os(VI)bipy-modified ODRN produced well-developed anodic peaks α and β, with potentials (E_P) ~−0.5 V and −0.1 V for α and β, respectively and their cathodic counterparts on CV (Fig. 1A, inset). No such peaks were observable with Os(VI)bipy-modified ODN, in accordance with the selectivity of Os(VI)bipy for ribose. Lack of redox peaks in ODN suggested that the ODN was not modified and that virtually all unreacted Os(VI)bipy was removed during purification. Moreover, all studied oligos yielded oxidation peaks of guanine (G_{ox}) and adenine (A_{ox}) at more positive potentials [11]. Their heights were comparable to those of peaks α and β and did not significantly change as a result of Os(bipy) modification, suggesting that purine bases remained intact during the Os(VI)bipy treatment. As expected, unmodified ODRN and ODN produced no

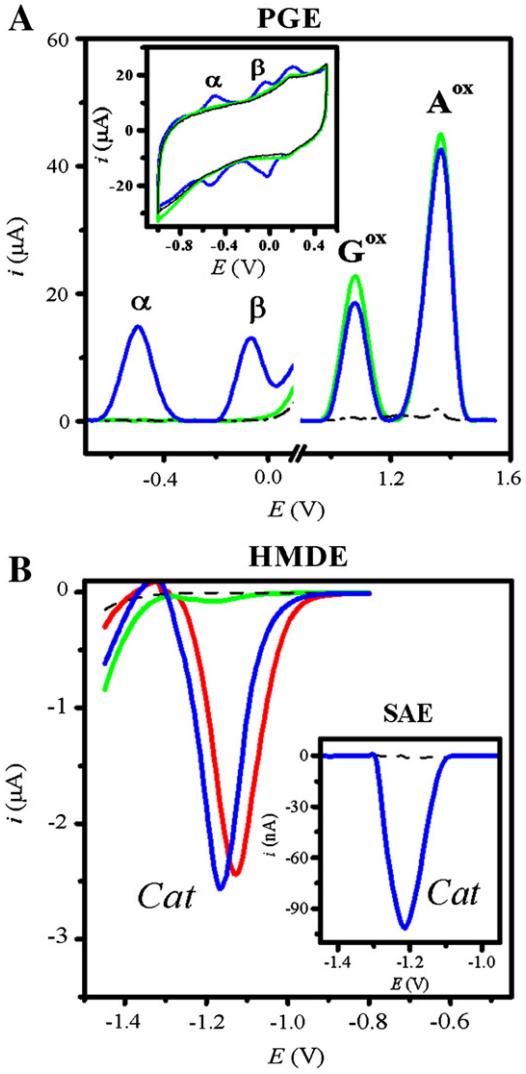

Fig. 1. Voltammetric peaks of three oligonucleotides treated with Os(VI)bipy: ODRN (blue) and $dA_{21}rA$ (red) and ODN containing no ribose residue (green); note that only ribose-containing oligos (ODRN and $dA_{21}rA$) were modified and their adducts produced peaks α and β at carbon and peak *Cat* at Hg electrodes. (A) Pyrolytic graphite electrode (PGE). Baseline-corrected square wave voltammogram (SWV); 20 μM oligos; AdTS (ex situ); SWV: t_A 60 s, E_A open current potential (OCP), frequency 200 Hz, initial potential −1.2 V, and end potential +1.55 V. Inset: Cyclic voltammogram; 110 μM oligos; AdTS, t_A 60 s, E_A OCP, start potential −1 V, first vertex potential +0.5 V, second vertex potential −1 V, scan rate 2 V/s. Background electrolyte 0.2 M acetate buffer, pH 5.0 (dashed). (B) Hanging mercury drop electrode (HMDE). Differential pulse voltammetric (DPV) *Cat* peak of 20 nM Os(VI)bipy-oligos. Inset: m-SAE, 400 nM Os(VI)bipy-ODRN; moving average baseline-corrected. Background electrolyte 0.1 M Britton–Robinson buffer, pH 4.65 (black dashed); AdS, DPV: t_A 60 s, E_A −0.8 V, interval time 0.5 s, initial potential −0.8 V, end potential −1.45 V, and step potential 5 mV.

peaks α and β (not shown). Concentrations of oligos used in CV were rather high (110 μM). PGE is convenient for sensors, but higher sensitivity of the determination would be beneficial. Therefore we used SWV in combination with efficient baseline correction, providing better sensitivity than CV [37]. We chose an anodic direction of potential scanning to obtain oxidation peaks α and β. SWV measurements were done not only with Os(VI)bipy-modified ODRN and ODN, but also with $dA_{21}rA$, which produced almost the same oxidation peaks as ODRN (not shown), suggesting that Os(VI)bipy treatment was independent of the nucleotide sequence. At a relatively short accumulation time (t_A) of 60 s, peak α height increased linearly from 500 nM up to 5 μM ODRN, leveling off at around 20 μM. Even lower concentrations could be detected at longer t_A. For example, at t_A