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Dependence of adenine isolation efficiency on the chain length evidenced using paramagnetic particles and voltammetry measurements

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ABSTRACT

The main aim of this work was to study the dependence of oligoadenine isolation efficiency on the chain length by using paramagnetic particles covered by homo-deoxythymidines ((dT)25) with subsequent detection by adsorptive transfer technique coupled with square wave voltammetry. For this purpose, the oligonucleotides of the length A5, A10, A15, A20, A25, A30, A35, A40 and poly(A) in various concentrations were chosen. We determined that the isolation efficiency defined as "isolated oligonucleotide concentration"/"given oligonucleotide concentration" was about 55% on average. Sequence A25 demonstrated the best binding onto microparticles surface.

0. Introduction

The reliability of nucleic acids or proteins studies strongly depends on the accuracy of their isolation from native biological material [1]. New materials, technologies and procedures focused on the improving of isolation effectiveness are proposed [2,3]. Some technologies are based on (para)magnetic particles (MPs). These particles with 5 nm–100 μm diameter having metal core (mostly gamma-Fe_2O_3 or Fe_3O_4) covered by chemical shells, which can be modified according to target molecules as specific protein or nucleic acids sequence. The principle of isolation technique is based on physico-chemical properties of MPs that after specific interaction with target molecules can be controlled by outer magnetic field, rinsed and transferred to pure solution. The molecules bonded on MPs surface can be further released by chemical or physical processes, such as higher temperature treatment—denaturation [4]. The main advantage of the isolation process based on MPs is related to the short time duration and missing of laborious and destructive procedures, such as centrifugation or dialysis [5,6].

It is known that nucleic acids provide two redox peaks at mercury electrodes: (i) redox signal of adenine and cytosine and (ii) oxidative signal of guanine [7,8]. The resolution of overlapped adenine and cytosine signals can be carried out by elimination voltammetry [8–12]. Guanine is primarily reduced at negative potential at mercury electrode and the reduction product is oxidized at −0.2 V [13]. To detect guanine, cytosine, adenine and thymine, carbon electrodes can be also used [14–16].

1. Materials and methods

All chemicals of ACS purity and parafilm were purchased from Sigma Aldrich (USA) unless noted otherwise. Synthetic polyadenylic acid (poly(A)) was used. Synthetic homo-oligoadenines, purified by high-performance liquid chromatography, were obtained from Generi Biotech (Czech Republic). Stock oligonucleotide solutions (100 μg/ml) were prepared with water of ACS purity and stored in the dark at −20 °C being spectrophotometrically assayed at 260 nm using spectrometer Spectronic Unicam (England). Deionised water (18 MΩ) was prepared by reverse osmosis using Aqua Osmotic 02 (Czech Republic) and purified using Millipore RG (USA). The pH value and conductivity was measured using inoLab Level 3 (Wissenschaftlich-Technische Werkstätten GmbH; Weilheim, Germany).

Electrochemical measurements were performed with AUTOLAB PGS30 Analyzer (EcoChemie, Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland), using a standard cell with three electrodes; working electrodes: hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2 or carbon paste electrode (CPE) (carbon paste (about 0.5 g) was made of 70% graphite powder and 30% mineral oil [17,18]), reference electrode: an Ag/AgCl/3 M KCl electrode, and auxiliary electrode: glassy carbon electrode. Square wave voltammetric (SWV) measurements were carried out in acetate buffer (pH 4.6). SWV parameters were: potential step 5 mV and frequency 280 Hz [19]. The analysed samples were deoxygenated prior to measurements by

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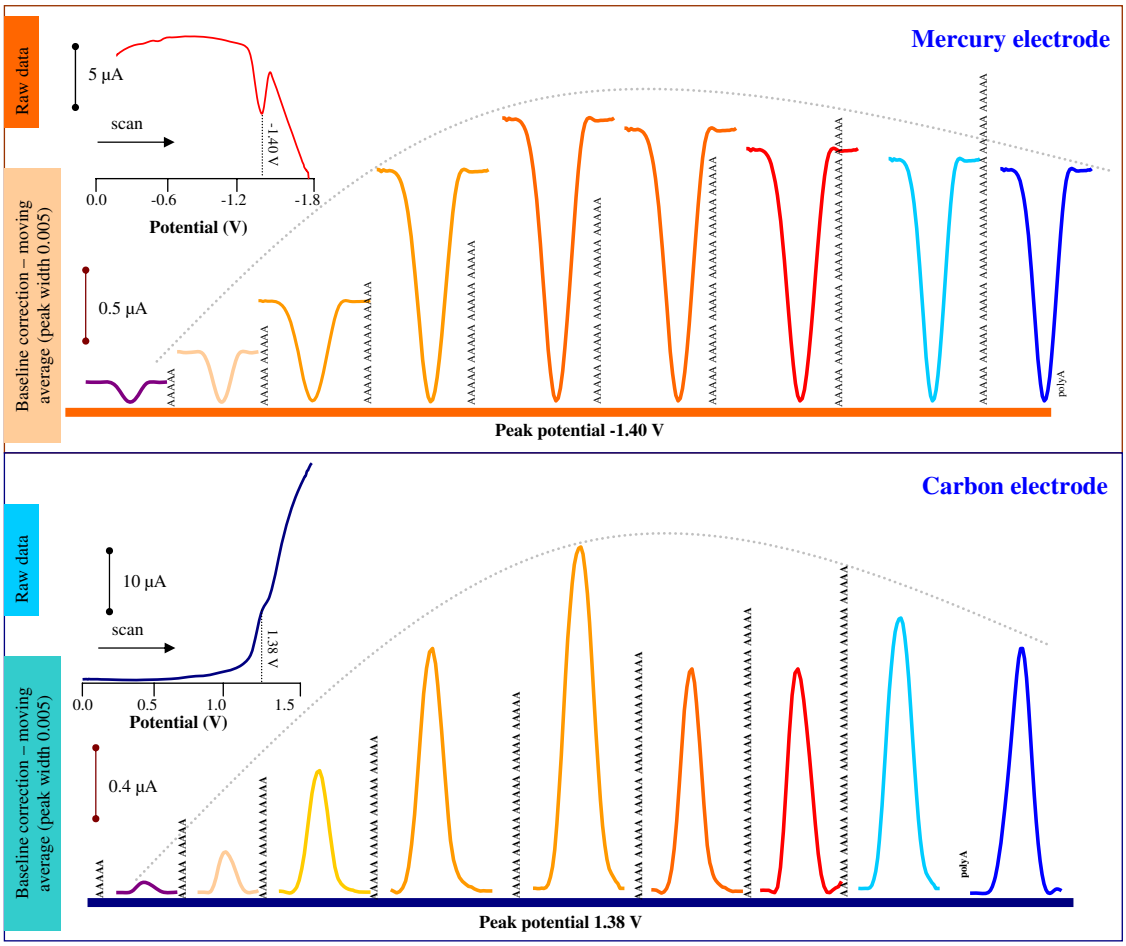

Fig. 1. Typical voltammograms of the studied oligonucleotides (A5, A10, A15, A20, A25, A30, A35, A40 and poly(A)) measured at HMDE and/or CPE. Concentration 40 μg/ml and time of accumulation 120 s.

purging with argon (99.999%), saturated with water for 120 s. All experiments were carried out at room temperature.

Isolation of oligonucleotides was performed with magnetic stand Dynal Magnetic Particle Concentrator-S both supplied by Dynal A.S. (Norway) and paramagnetic particles Dynabeads Oligo (dT)25. All experiments with paramagnetic particles were performed in RNA/DNA UV cleaner box UVT-S-AR Talron Biotech. Ltd.. (Israel). For centrifuging and vortexing of a sample, multi-spin MSC-3000 centrifuge (Biosan, Latvia) was used. Denaturation was carried out at 85 °C using the Thermomixer 5355 Comfort/Compact (Eppendorf, Germany). The buffers were defined—(a) phosphate buffer I: 0.1 M NaCl+0.05 M Na_2HPO_4+0.05 M NaH_2PO_4; (b) phosphate buffer II: 0.2 M NaCl+0.1 M Na_2HPO_4+0.1 M NaH_2PO_4; and (c) acetate buffer: 0.2 M CH_3COOH+0.2 M CH_3COONa. The hybridization process was optimised by Huska et al. [19]. Briefly, the optimal composition of hybridization solution was: 100 mM Na_2HPO_4+100 mM NaH_2PO_4, 0.5 M NaCl, 0.6 M Guanidium thiocyanate, 0.15 M Trizma base adjusted by HCl on pH of 7.5. Length of hybridization was 40 min and room temperature except A5 and A10, at which we carried out this process at 10 °C (Fig. 1).

2. Results and discussion

SWV is one of the most sensitive electrochemical methods for analysis of biomolecules [8,18,20,21]. To prevent interferences [22] and to enable analysis of very low volumes [20,23], voltammetric methods can be coupled with adsorptive transfer stripping technique. The main improvement is based on electrode removing and rinsing after accumulating of target molecules [22,24]. Nevertheless an isolation of target molecules is needed in most cases. One of the most promising techniques used for isolation of biomolecules is based on using of paramagnetic particles [4,19,25,26].

We analysed homo-oligoadenines with the different lengths A5, A10, A15, A20, A25, A30, A35, A40 and poly(A). For isolation of the oligonucleotides, paramagnetic microparticles covered by oligo-deoxythymidines ((dT)25) were used. Experimental conditions and procedure of isolation were published elsewhere [19]. Solution containing isolated nucleic acids was analysed by the adsorptive transfer technique in connection with SWV using HMDE and/or carbon paste electrode as working electrode. Fig. 3 shows raw voltammograms measured at mercury and/or carbon electrode. We detected a redox signal at −1.40 V (HMDE) and oxidative signal at +1.38 V (CPE). To process experimental data, GPES 4.9 software was used (Fig. 3). Peaks measured both at HMDE and CPE had similar dependency on the length of the chain. They enhanced up to A25, and then slightly decreased.

Further, we investigated the isolation efficiency of six oligonucleotide concentrations by using paramagnetic particles. Fig. 2 shows the experimental data obtained. We detected that the electrochemical signal of nucleic acid enhanced with its increasing concentration. For A5, A10, A15 and A20, the electrochemical

signal height linearly increased with oligonucleotide concentration (R^2 between 0.9 and 0.98). Higher concentrations of longer adenine chains did not result in proportional increase in electrochemical signal height. It clearly follows that the length of isolated chain plays a very important role in the process of its linkage to the thymine chain anchored on the surface of

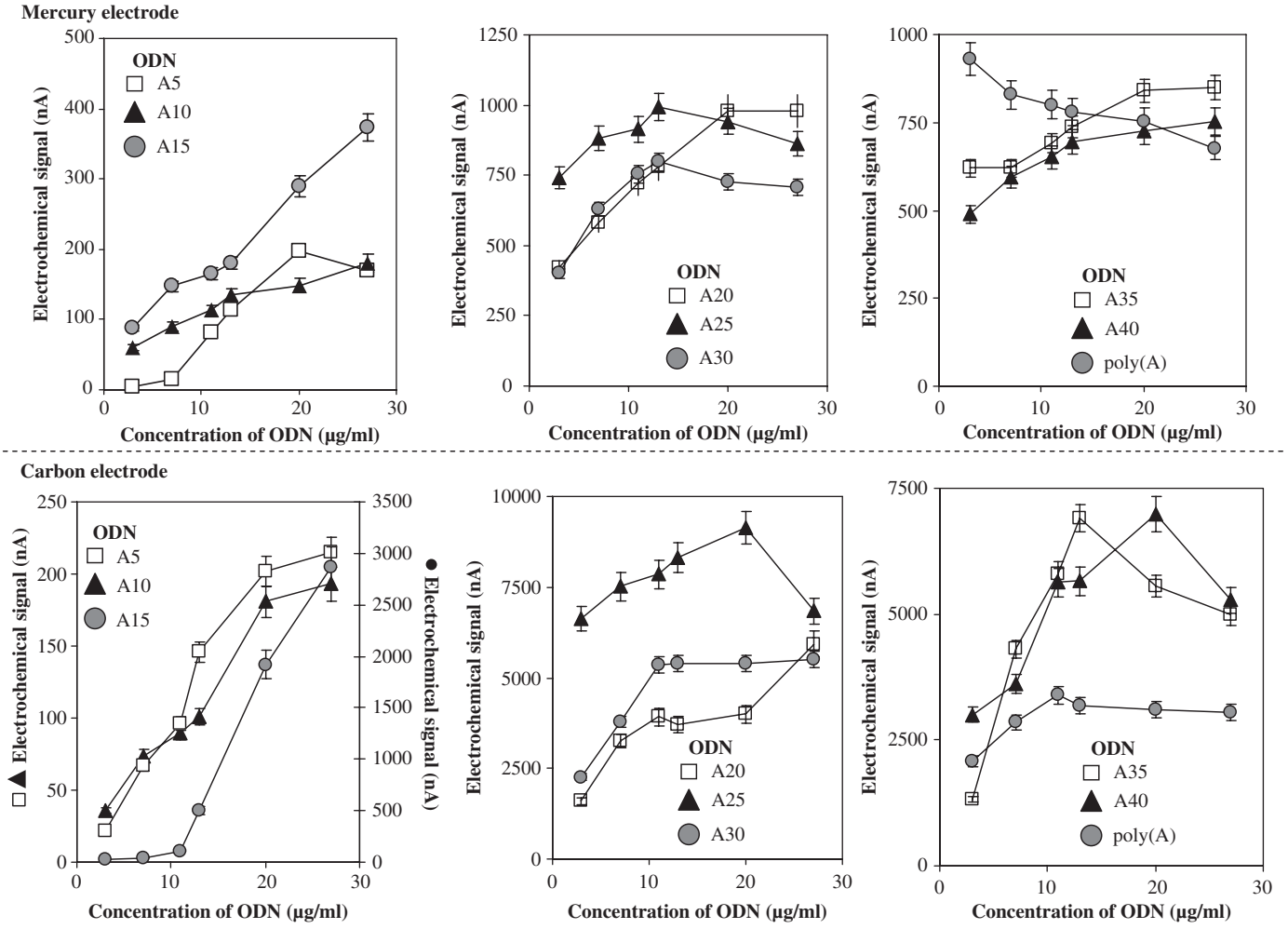

Fig. 2. Dependence of signal height of isolated oligonucleotides (A5, A10, A15, A20, A25, A30, A35, A40 and poly(A)) on their concentration measured by using of HMDE and/or CPE. Time of accumulation 120 s.

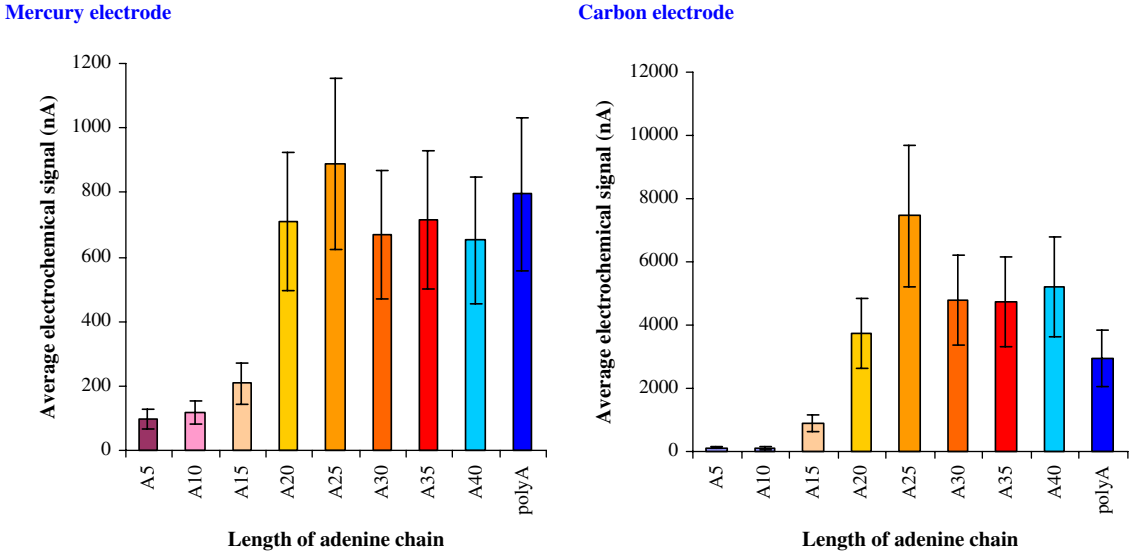

Fig. 3. Mean of height of electrochemical signals presented in Fig. 2.

paramagnetic microparticles. The presumption that higher amount of shorter chains as A5, A10 and A15 could be bound, was not confirmed. On the other hand chains longer than A15 can be better bound on the surface of the particles (Fig. 2). But particles longer than A25 may form different three-dimensional structures, such as loops, pin loops or cross structures, which may cause the decrease in the peak height. Chains longer than A20 probably form less-organized structures. Therefore their binging onto sequence anchored on the surface of paramagnetic microparticle can be spatially inhibited. This presumption is supported by the results obtained in experiments with long chains of poly(A), when higher concentrations resulted in slight decrease of the signal. We also determined that the isolation efficiency defined as "isolated concentration of oligonucleotide"/"given concentration of oligonucleotide" was about 55% at average. With increasing concentration of certain oligonucleotide the isolation efficiency decreased, which was detected at both working electrodes.

To evaluate the effect of the length of oligonucleotide chains on their isolation and electrochemical detection, the results shown in Fig. 2 were summarised and averaged (Fig. 3). It follows that A5, A10 and A15 bind onto paramagnetic microparticles with lower efficiency than we expected. Only longer molecules A20, A25, A30, A35, A40 and poly(A) are isolated with higher efficiency. ODN A25 demonstrated the highest efficiency, which is in good agreement with the fact that A25 is the best complementary to T25 anchored on the surface of microparticles. This fact is very interesting and may enhance the efficiency of isolation of exactly known nucleic acids sequences, because we confirmed that longer or shorter molecules with similar sequence bind onto microparticles surface much less effectively.

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