MIR-150 electrochemical detection connected with specific isolation based on magnetic particles

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Since the establishment of association between microRNA (miRNA) abnormal expression profiles and diseases development the miRNAs become a very significant group of molecules. Due to their potential in diagnostics and therapy the considerable effort to develop sensitive, simple and low-cost detection method exists. For these reason was the sensitive electrochemical detection combined with selective magnetic particle-based separation.

In this work, the square wave voltammetric detection (SWV) of miRNA was optimized. The limit of detection of miR-150 by SWV was 1 nM. This detection method was subsequently connected with magnetic separation when the magnetic particles (MPs) were modified by probe specific to miR-150. The sensitivity of coupled SWV with MPs-based separation was established by nanomolar concentration (9 nM) limit of detection.

1. Introduction

The microRNA (miRNA) belongs to short noncoding RNA molecules (generally 20-25 nt in length) which are able of specific gene regulation expression[1]. These regulatory molecules have significant influence on different biological processes like cell proliferation, development, differentiation, apoptosis and metabolism[2].

The aberrant expression of various miRNAs is involved in the cell pathological status formation. The abnormal miRNA expression levels were found at number diseases like inflammatory and autoimmune diseases, diabetes, cardiovascular and neurodegenerative diseases and cancer [3-6]. Specific miRNAs expression profiles were identified at various tumors so miRNA are interesting diagnostic biomarkers. Moreover their detection might be helpful to find more about cancer staging, prognosis and/or response to treatment [7]. Different studies have shown that miR-150 is upregulated in lung cancer tissue and lupus nephritis, downregulated at patients with heart failure, acute myeloblastic leukemia, colorectal cancer and systemic sclerosis[8-14].

Because of miRNAs importance the simple and fast detection method is needed. Therefore, the sensitive electrochemical analysis was combined with specific magnetic particles-based isolation.

2. Material and Methods

2.1 Chemicals

All chemicals of ACS purity were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. The 1× binding and washing (BW) buffer (5 mM Tris-HCl, 0.5 mM EDTA and 1.0 M NaCl, pH 7.5), solution A (0.1 M NaOH and 0.05 M NaCl) and solution B (0.1 M NaCl) were employed for MPs washing. For biotinyled anti-miR-124 immobilization the 2× BW buffer (10 mM Tris-HCl, 1 mM EDTA and 2.0 M NaCl, pH 7.5) was utilized. The phosphate buffer I for washing MPs with immobilized oligonucleotide was composed of 0.1 M NaCl, 0.05 M Na_2HPO_4

and 0.05 M NaH_2PO_4, pH 7.8. All solutions were treated with DEPC or prepared using DEPC treated water.

The composition of hybridization solution was as follows: 0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4, 0.6 M guanidinium thiocyanate (Amresco, Solon, OH, USA), 0.15 M Tris-HCl and 0.5 M NaCl (pH 7.5). The elution solution composition was as follows: 0.2 M NaCl, 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4. Acetate buffer (0.2 M CH_3COOH and 0.2 M CH_3COONa, pH 5) was used for electrochemical analysis.

The hsa-miR-150-5p (5´-UCUCCCAACCCUU-GUACCAGUG-3´) and complementary biotinylated oligonucleotide (ODN) antisense miR-150 (5´-Btn- CACTGGTACAAGGGTTGGGAGA-3´), both synthesized by Sigma-Aldrich (St. Louis, MO, USA), were used for magnetic separation optimization.

2.2 Electrochemical analysis

Electrochemical measurements were performed with AUTOLAB PGS30 Analyzer (Eco-Chemie, Utrecht, Netherlands) connected to VA-Stand 663 (Metrohm, Zofingen Switzerland) using a standard cell with three electrodes. A hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2 was employed at the working electrode. An Ag/AgCl/3M KCl electrode served as the reference electrode. Pt electrode was used as the auxiliary electrode.

Adsorptive transfer technique was used for the RNA electrochemical determination. The adsorptive transfer technique is based on the sample (5 µl) accumulation (120 s) onto the working electrode surface and consequently on the electrode washing and square wave voltammetric (SWV) measurement. All experiments were carried out at room temperature (21ºC). SWV measurements were carried out in the presence of acetate buffer pH 5.0. SWV parameters: start potential 0 V, end potential -1.8 V, potential step 5 mV, frequency 280 Hz, and amplitude 25.05 mV. For smoothing and baseline correction the software GPES 4.9 supplied by EcoChemie was employed.

Optimized parameters were accumulation time (0, 30, 60, 90, 120, 150, 180, 210 s); purge time (0, 30, 60, 120, 180, 240, 300, 360, 420, 480, 540 s) and pH of acetate buffer (3, 4, 5, 6, 7). 25 nM miR-150 was used for the optimization steps.

2.3 The miR-150 isolation by magnetic particles

The magnetic microparticles Dynabeads M-270 Streptavidin (Life Technologies, Invitrogen, Oslo, Norway) and magnetic separation rack MagnaRack (Life Technologies, Invitrogen, Oslo, Norway) were used for miRNA isolation. The miRNA experiments were performed in RNA/DNA UV-cleaner box UVT-S-AR (Biosan, Riga, Latvia).

The biotinylated antisense miR-150 immobilization on MPs surface was done according to the manufacturer's recommendations. A microcentrifuge tube with 50 µl of resuspended MPs was placed on the magnetic rack and the supernatant (storage solution) was removed. The MPs were 3 times washed by 50 µl of 1× BW buffer. Subsequently the MPs were washed twice by 50 µl of the solution A and once with the solution B (50 µl). Thus, washed MPs were ready for immobilization of biotinylated ODN. MPs were resuspended in 100 µl of 2× BW buffer and the 1.02 µg of biotinylated probe in water (final volume 100 µl) was added. The mixture with MPs and biotinylated ODN was incubated for 10 minutes on rotator-mixer (multi RS-60, Biosan, Riga, Latvia) at 60 rpm at the room temperature. After incubation, probe-coated MPs were separated on the magnetic rack and twice washed with 50 µl of 1× BW buffer and once with 100 µl of phosphate buffer I.

The hybridization step was performed according to Huska et al.[15]. 50 µl of hybridization solution and 50 µl of sample (miR-150 diluted in water) were added to the probe-coated MPs. The hybridization process took placed on rotator-mixer at 60 rpm for 40 minutes at the room temperature. After incubation the MPs with coupled miR-150 were three times washed with 100 µl of phosphate buffer.

In the next step the MPs were resuspended in 50 µl of the elution solution. The miR-150 elution was done in Thermomixer 5355 Comfort/Compact (Eppendorf, Hamburg, Germany) for 5 minutes at 70 ºC and 350 rpm. The tube was

placed on magnetic stand after heating-up. The solution with eluted miR-150 was pipetted to a new tube. The miR-150 amount was determined by electrochemistry.

3. Results and Discussion

3.1 The electrochemical detection optimization

For the maximal miRNA electrochemical signal achievement was the SWV method optimized. At first the time required for miRNA accumulation on HMDE surface was determined (**Fig. 1A**). The tested times were $0 - 210$ s. With increasing accumulation time the signal height significantly grew to the value 120 s. Then the signal increase was not so significant and for shortening the analysis time the accumulation for 120 s was selected.

Next, the influence of electrolyte (acetate buffer) pH on electrochemical signal was investigated. The tested pH range was $3 - 7$. From the Fig. 1B is clear that pH 5 is optimal for miRNA SWV detection.

3.2 MIR-150 and antisense miR-150 calibration curves

With optimized parameters of AdTS SWV method was detected influence of miRNA and biotinylated probe concentration on peak height and calibration curves were set down. In the **Fig. 2A** the dependence of peak height on miR-150 concentration is shown. The limit of detection was calculated as 1 nM and limit of quantification as 5 nM. The dependence of antisense miR-150 peak height on its concentration is shown in the **Fig. 2B**. The limit of detection was established as 0.6 nM and limit of quantification was determined as 2 nM.

3.3 The electrochemical detection of magnetic separation yield

For the specific miRNA isolation the magnetic separation was used. The targeted miRNA was miR-150 and for specific isolation streptavidin--coated MPs modified with antisense probe complementary to targeted miR-150 sequence were used. The magnetic separation process was performed according to Smerkova et al. [16]. The calibration curve for the specific isolation connected with optimized electrochemical detection was obtained (**Fig. 3**). The SWV response is in that range linear with determination coefficient $R^2 = 0.9958$. The voltammograms of miR-150 separated by MPs are shown in inset of **Fig.3**. The limit of detection was determined as 9 nM and limit of quantification was established as 30 nM. The average isolation yield of miR-150 by magnetic separation was 17 %.
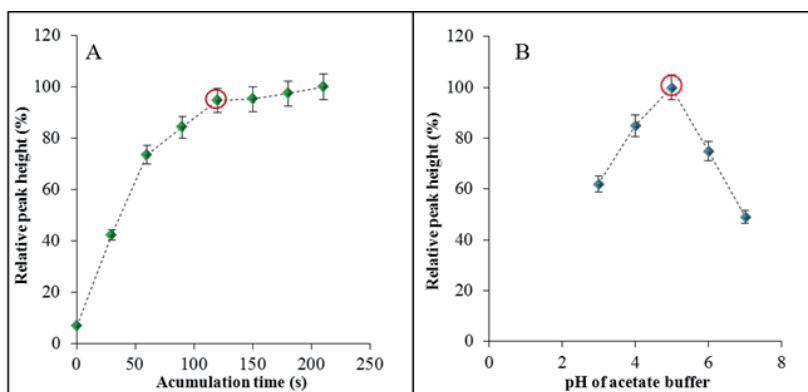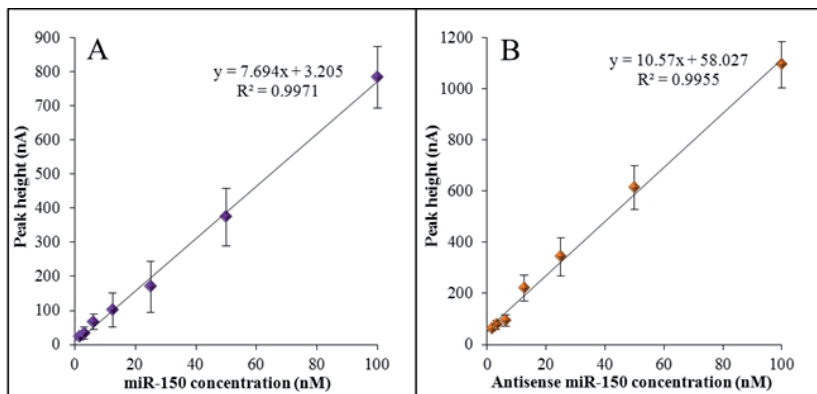

Figure 1. Electrochemical detection parameters optimization. (A) Optimization of accumulation time. (B) Optimization of acetate buffer pH. Selected parameters were highlighted. Results are expressed as average ± standard deviation (number of measurement n = 3).

Figure 2. SWV peak height dependence on concentration of (A) miR-150 and (B) antisense miR-150. SWV parameters were as follows: time of accumulation 120 s, start potential 0 V, end potential -1.8 V, potential step 5 mV, frequency 280 Hz, amplitude 25.05 mV. Results are expressed as average ± standard deviation (number of measurement n = 3).
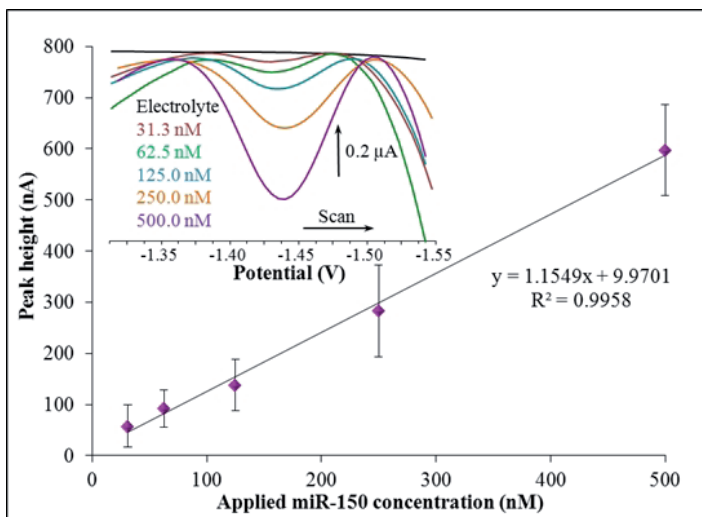

Figure 3. (A) Dependence of the peak height on applied miR-150 concentration using the MPs-based isolation. Inset: the isolated miR-124 voltammograms (SWV conditions as in Fig. 2). Results are expressed as average ± standard deviation (number of measurement n = 3).

4. Conclusion

The miRNAs are very important diagnostic and prognostic biomarkers at various diseases. Therefore, the easy and sensitive detection method is required. The combination of magnetic separation with electrochemical detection is very advantageous. Because of modifiable surface of magnetic particles by probe the specificity is guaranteed and the electrochemical method contributes by the sensitivity. In this work, the possibility of connection the magnetic particles-based separation with sensitive square wave voltammetric detection was demonstrated.

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The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study.

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