Electrochemical genosensor based on disc and screen printed gold electrodes for detection of specific DNA and RNA sequences derived from Avian Influenza Virus H5N1

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Abstract

The genosensors based on thiolated ssDNA probe deposited on the two types of gold electrodes: screen-printed (miniaturized) and disc electrodes destined for determination of specific sequences of DNA and RNA derived from Avian Influenza Virus H5N1 have been proposed. The working principle of genosensor is based on the ion–channel mechanism. The analytical signals generated upon hybridization processes were recorded using electrochemical technique – Osteryoung square wave voltammetry in the presence of a redox active marker $[Fe(CN)_6]^{3-/4-}$ in the sample solution. The miniaturized genosensor was able to detect the 20-mer complementary DNA oligonucleotide sequence as well as ~280-mer RNA sequences containing the complementary 20-mer sequence in various positions: at 3'-terminus, at 5'-terminus and in the middle of the RNA transcript at the 1 pM concentration. The measuring systems were selective. Non-complementary 20-mer oligonucleotide sequence as well as RNA transcript without complementary region generated weak response. The RNA transcripts were also tested with GDEs modified in the same manner. This classical device was able to detect ~280-mer RNA sequences, but at higher concentration of 10 pM. The good discrimination of the position of complementary part in the ~280-mer RNA sequences was observed with using both type of modified electrodes.

1. Introduction

Avian influenza virus (AIV), especially H5N1 has become nowadays a very dangerous pathogen threatening not only for poultry. The H5N1 is the virus which mainly affects the birds and usually does not spread among people. Nevertheless, highly pathogenic H5N1 virus has been registered in about 650 confirmed human infections (with approximately 60% of deaths) in 15 countries [1]. The greatest threat to mankind can be the case when the person who is ill with seasonal flu will be infected with avian flu. Then there is the probability that the H5N1 virus can exchange genetic information with the human flu virus and acquire the ability for transmission from human to human. An easily human-transmissible AIV strain could have disastrous implications. Thus, the development of methods for early diagnosis, as well as for preventions are essential. Highly sensitive, accurate and rapid tests for identification of AIV infection would allow early antiviral therapy.

However, the most commonly used methods of virus detection are laborious, time-consuming, expensive, require specialized equipment and trained personnel. These include, for example: cultivation of viruses in cell culture, immunofluorescence, serological methods, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) [2-7], reverse transcription-polymerase chain reaction (RT-PCR) [8,9], RT-PCR with detection by ELISA [10], real-time reverse transcription-polymerase chain reaction (RRT-PCR) [11,12] and nucleic acid sequence-based amplification (NASBA) [13-15].

Therefore, in order to minimize the social and economic costs, the development of rapid diagnostic tests is indispensable [16]. It is believed that this type of tests should meet the following conditions: high efficiency, the ability to detect multiple targets, accuracy: sensitivity and specificity, speed, ease of use, the appropriateness of the use of research in the field and affordable price. These conditions fulfil the electrochemical biosensors. The biosensors are analytical tools developed for specific and selective detection of analytes such as: nucleic acids, drugs or proteins that are crucial in the field of diagnostics. Electrochemical biosensors offer a sensitive, selective, practical, time-saving and fast data analysis, as well as being suitable for the design of miniaturized portable point-of-care tools. Thus, the area of electrochemical biosensor technology has expanded from day to day [17-22].

The screen-printed electrodes have been designed especially for miniaturization of electrochemical analytical systems. These disposable sensors can be easily modified in various ways. They are also suitable candidates for measuring of multiple biological samples, as they require a small sample volume [23].

Here, we report on a sensitive ion-channel mimetic miniaturized genosensor incorporated mixed monolayer of thiolated DNA probe (SH-NC3) and 6-mercaptohexanol (MCH) deposited onto screen printed gold electrode (SPGE) surfaces for electrochemical detection of specific DNA and RNA sequences derived from AIV H5N1. The electroanalytical signals generated based on the DNA-DNA and DNA-RNA duplexes formed at the electrode surface were explored using Osteryoung square wave voltammetry (OSWV) and cyclic voltammetry (CV) in the presence of $[Fe(CN)_6]^{3-/4-}$ as an electroactive redox marker. As targets short (20-mer) single stranded DNA sequences and long (ca. 280-mer) RNA transcripts with different localization of the 20-mer region complementary to the probe were used. The RNA transcripts responses recorded using modified SPGEs were compared with GDEs modified in the same manner.

2. Materials and Methods

2.1. Reagents and Materials

6-mercaptohexan-1-ol (MCH), potassium ferro- and ferricyanides, phosphate buffer saline (PBS) components (NaCl, KCl, Na_2HPO_4, KH_2PO_4) and sodium azide were obtained from Sigma–Aldrich (Poznań, Poland). Alumina slurries 0.3 and 0.05 μm were purchased from Buehler (USA). Sulphuric acid, potassium hydroxide, hydrogen peroxide, ethanol and methanol were supplied by POCh (Poland).

The modified oligonucleotide SH-ss-DNA (5'-HS–$(CH_2)_6$-CCT CAA GGA GAG AGA AGA AG-3') was used as a probe (named NC3) for immobilization on a surface of gold electrodes, while two unmodified oligonucleotides, c-NC3 (5'-CTT CTT CTC TCT CCT TGA GG-3') and nc-NC3 (5'-GGA GTT CCT CTC TCA TCA TC-3') served as complementary and non-complementary hybridization targets, respectively. The oligonucleotides were supplied by Biomers (Germany).

The region complementary to the probe is located in the region of 83–102 nt, 242-261 nt and 160–179 nt from the 5'-terminus of the RNA1, RNA2 and RNA3, respectively. The

RNA4 has no complementary sequence to the probe at all and was used for demonstration of the genosensor selectivity. DNA and RNA oligonucleotides were derived from the HA gene of Polish isolate of the HPAIV H5N1 (A/swan/Poland/305-135V08/2006) – Supplementary data.

For DNA immobilization and hybridization 0.1 M PBS, pH 7.4 (consisting of 137 mM NaCl, 2.7 mM KCl, 10 mM KH_2PO_4, 1.8 mM Na_2HPO_4) was used. RNA hybridization buffer has the same composition and was prepared with sterile, nuclease free water from Sigma–Aldrich (Poznań, Poland).

All aqueous solutions were prepared using autoclaved Milli-Q water, resistivity 18.2 MΩ·cm (Millipore Corporation, USA). Reagents and solvents were of analytical grade and used without further purification. All experiments were carried out at room temperature.

2.2. Fabrication of Genosensors – Successive Steps of Gold Electrode Modification

2.2.1. Gold disc electrodes

The elements of genosensors are schematically shown in Figure 1. Gold disc working electrodes with a diameter of 2 mm were obtained from Bioanalytical Systems (BAS), West Lafayette, IN.

GDEs were initially cleaned mechanically by polishing with alumina slurries (Alpha and Gamma Micropolish; Buehler, Lake Bluff, IL) with particles sizes of 0.3 and 0.05 μm on microcloth pad (BAS) for 5 minutes each. Afterwards, they were carefully rinsed with Milli-Q water. The polished electrodes were further cleaned electrochemically by cyclic voltammetry (CV). At first they were dipped in 0.5 M KOH solution and swept with the potential between –0.4 V and –1.2 V against the Ag/AgCl reference electrode and the platinum wire counter electrode with scan rate of 0.1 Vs^{-1}, number of cycles: 3, 50 and 10. Next, the electrodes were cleaned in 0.5 M H_2SO_4 in the potential window between -0.3 V and +1.5 V, number of cycles: 3, 10 and 3. Before modification, the surfaces of electrodes were refreshed in 0.5 M KOH solution for 10 cycles. After finishing of the electrochemical cleaning, each electrode was washed with MiliQ water and stored in water (for several minutes, until the next step) to avoid contaminations from air. All solutions were deoxygenated by purging with nitrogen (ultra pure 6.0, Air Products, Poland) for 10 minutes.

Directly after cleaning, the electrodes were rinsed repeatedly with water and PBS buffer pH 7.4. Then, 10 µL droplets of the mixture containing 1 µM SH-ssDNA and 10 µM MCH (backfiller) in PBS buffer pH 7.4 were placed on the surface of each electrode. The electrodes were covered with tubes and stored for 3h at room temperature. After self-assembled monolayer formation and oligonucleotide immobilization, 10 µL of 1 mM MCH was spotted on each electrode and incubated for 1h to reduce non-specific DNA binding. The modified electrodes were rinsed profusely with 0.1 M PBS, pH 7.4 and stored overnight at room temperature.

2.2.2. Screen-printed gold electrodes

The electrochemical measurements were also performed with miniaturized system – µStat 400 bipotentiostat/galvanostat (DropSens, S.L., Spain). For these experiments disposable SPGEs (DropSens, S.L., Spain) were applied. They include a three electrode configuration printed on the same strip. The dimensions of the strips are 34 x 10 x 0.5 mm (length x width x height). They are composed of gold working electrode, silver reference electrode and gold counter electrode. The working electrode has the diameter of 1.6 mm.

Before the electrochemical cleaning, each SPGE was washed with Milli-Q water. Afterwards, the SPGES were cleaned electrochemically by CV. 40 µL droplets of 0.5 M sulphuric acid were placed on the three electrodes surfaces, and swept with the potential window between -0.2 V and +1.1 V, with the scan rate of 0.1 Vs^{-1}, number of cycles: 6, 10 and 6. Before modification, the surfaces of electrodes were carefully rinsed with Milli-Q water and buffer.

Directly after cleaning, the electrodes were rinsed repeatedly with water and 0.1 M PBS buffer pH 7.4. Then, 4 µL droplets of the mixture containing 1 µM SH-ssDNA and 10 µM MCH (backfiller) in PBS buffer pH 7.4 were placed on the surface of each working electrode. Then each electrode were closed in cells (vials) and stored for 3h at room temperature. After self-assembled monolayer (SAM) formation, 10 µL of 1 mM MCH was spotted on each electrode and incubated for 1 h to reduce non-specific DNA binding. Then, the modified electrodes were rinsed profusely with 0.1 M PBS, pH 7.4 and stored overnight in 0.1 M PBS + 0.01 % NaN_3 at 4°C.

2.3. Hybridization Processes

2.2.1. Gold disc working electrodes

The GDEs surfaces modified with a SH-NC3/MCH SAM were covered with 10 µL of a solution of target oligonucleotides (RNA1, RNA2, RNA3 and RNA4) of one concentration in 0.1 M PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM KH_2PO_4, 1.8 mM Na_2HPO_4) pH 7.4, for 30 minutes at room temperature. Then the electrodes were rinsed with 5 mL of PBS, pH 7.4 in order to remove the unbound targets.

RNA transcripts (RNA1, RNA2, RNA3, RNA4) were diluted with the 0.1 M PBS hybridization buffer (pH 7.4) to the concentration of 10 pM.

The hybridization processes were monitored using OSWV with a potential from +0.4 V to −0.2 V, a step potential of 0.001 V, a square-wave frequency of 25 Hz and amplitude of 0.05 V in 0.1 M PBS. The electrode responses were expressed as: $(I_n - I_0) / I_0$, where I_n is the peak current measured in the presence of the analyte and I_0 the peak current before applying the analyte (in buffer without analyte).

2.3.2. Screen-printed gold electrodes

Nucleic acid targets, either oligonucleotides (c-NC3, nc-NC3) or RNA transcripts (RNA1, RNA2, RNA3, RNA4) were diluted with the 0.1 M PBS hybridization buffer (pH 7.4) to the concentration of 1 pM for DNA and RNA targets.

Hybridization reactions based on SPGE modified with a SH-NC3/MCH SAM were performed by dropping of the 4-µL aliquots of the respective dilutions of the targets (c-NC3, nc-NC3, RNA1, RNA2, RNA3 and RNA4). After 30 minutes at room temperature, the electrodes were rinsed with 5 mL of 0.1 M PBS, pH 7.4 in order to remove the unbound targets.

2.4. Cyclic Voltammetry (CV) and Osteryoung Square Wave Voltammetry (OSWV) Measurements

2.4.1. Gold disc working electrodes

The electrochemical measurements were performed with a potentiostat–galvanostat AutoLab (Eco Chemie, Utrecht, Netherlands) with a conventional three-electrode configuration. The voltammetric experiments were carried out in an electrochemical cell of 5 mL volume containing the modified gold working electrode, an Ag/AgCl electrode and a platinum wire as an auxiliary electrode. The measurements were performed in the presence of 0.1 M PBS buffer and 1.0 mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ purged with nitrogen for 10 minutes, in order to control electrode modification and to record the hybridization reaction.

OSWV was performed with potential from + 0.6 V to −0.2 V and with a step potential of 0.001 V, a square-wave frequency of 25 Hz, and amplitude of 0.05 V for $[Fe(CN)_6]^{3-/4-}$. In CV, potentials were cycled from +0.6 V to –0.2 V with the scan rate of 0.1 V/s.

The dependence of the sensor response on the concentration of analytes was expressed as the currents at the peak potential in OSWV measured in a solution containing no analyte. The electrode responses were expressed as: $(I_n − I_0) / I_0$ where I_n is the peak current measured in the presence of the analyte and I_0 the peak current before applying analyte (in buffer without analyte).

2.4.2. Screen-printed gold electrodes

The electrochemical measurements were also performed with miniaturized system – µStat 400 bipotentiostat/galvanostat. For these experiments disposable SPGEs were applied. They include a three electrode configuration printed on the same strip – gold working electrode, silver reference electrode and gold counter electrode. Electrochemical measurements using SPGEs were carried out by dropping of 40 µL of 0.1 M PBS with 1 mM of $[Fe(CN)_6]^{3-/4-}$ solution.

OSWV was performed with potential from +0.3 V to −0.2 V and with a step potential of 0.001 V, a square-wave frequency of 25 Hz, and amplitude of 0.05 V for $[Fe(CN)_6]^{3-/4-}$. In CV, potentials were cycled from +0.6 V to –0.2 V with the scan rate of 0.1 V/s.

3. Results and Discussion

3.1. Characterization of electrochemical genosensors based on screen printed gold electrodes (SPGEs) and gold disc electrodes (GDEs) modified with SH-NC3/MCH monolayer

Figure 1 illustrates the scheme of the genosensor fabrication. The SH-NC3 probe and MCH has been covalently attached to the gold electrode surface via Au-S bonds. After self-assembling the mixed SH-NC3/MCH SAM, the electrode was treated with 1 mM solution of MCH to avoid non-specific adsorption and eliminate direct contact between the redox marker and the gold electrode surface. This step of genosensor preparation using SH-NC3 probe is widely applied [24-30].
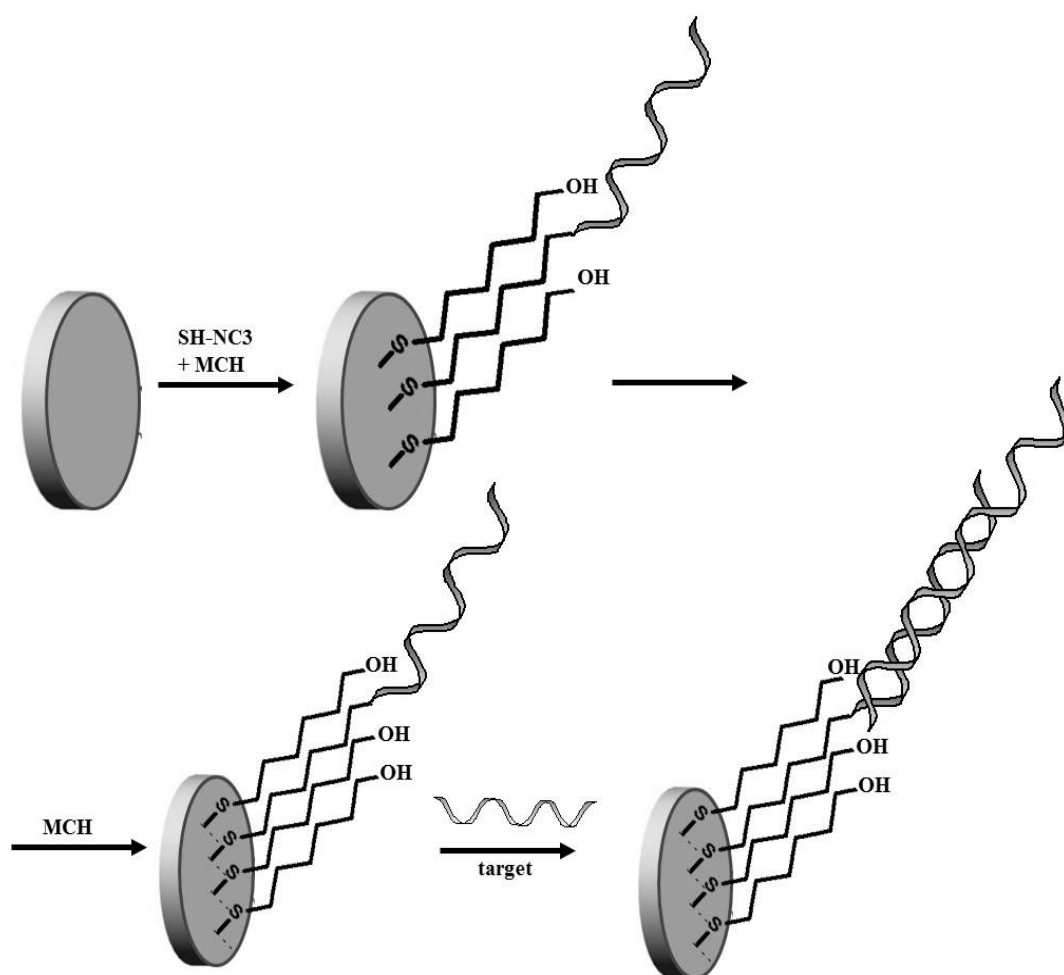

Fig. 1. The scheme of the genosensor preparation on the gold electrodes.

The immobilization of SH-NC3/MCH SAM was confirmed by CV and OSWV in the presence of $Fe(CN)_6^{3-/4-}$ as a redox marker. As expected, in the CV, the redox marker shows

reversible behaviour on bare SPGE, with a peak-to-peak separation $\Delta E_p = 0.071 \pm 0.003$ V (Figure 2A). After the covalent attachment of the SH-NC3/MCH SAM on the SPGE the peaks current decreased and increased peaks separation to 0.449 ± 0.013 V (Figure 2B). This indicates decreasing the reversibility of the system.
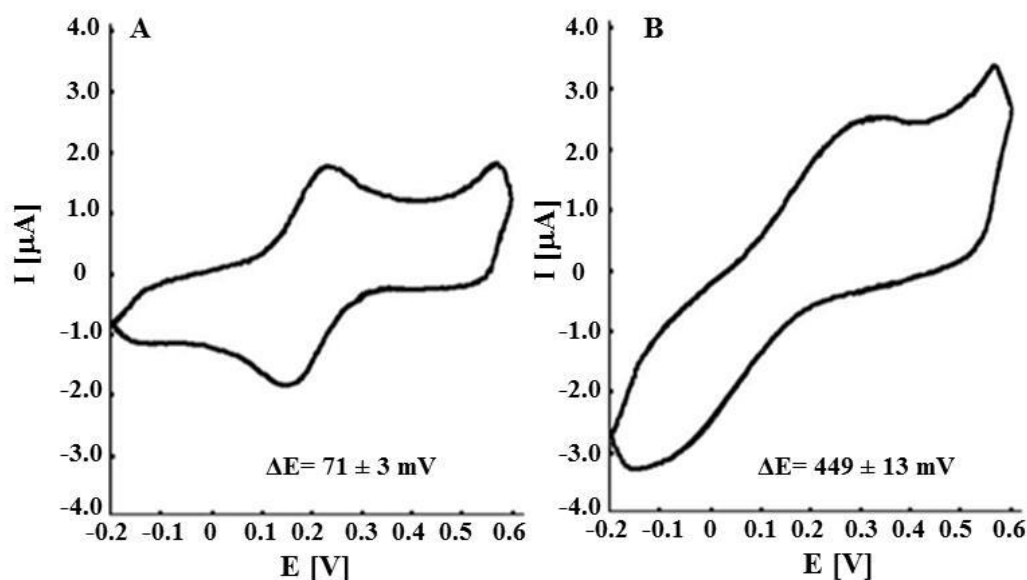

Fig. 2. Cyclic voltammograms (scan rate 0.1 V/s) of: (A) bare SPGE; (B) SH-NC3/MCH SAM modified electrode; Solution composition: 1 mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$, 0.1 M PBS buffer, pH 7.4. The measuring conditions: screen printed electrode with three electrode configurations – gold working electrode, silver reference electrode and gold counter electrode.

In OSWV on bare SPGE the values of peak potential and peak current were as follows: $E = 0.111 \pm 0.007$ V, $I = 2.0 \pm 0.1$ µA, respectively (Figure 3A). After immobilization of the SH-NC3/MCH SAM the peak current decreased to $I = 0.15 \pm 0.01$ µA, and the peak potential shifted towards the positive potential to $E = 0.130 \pm 0.007$ V (Figure 3B). The results obtained by OSWV are in good accordance with CV measurements.
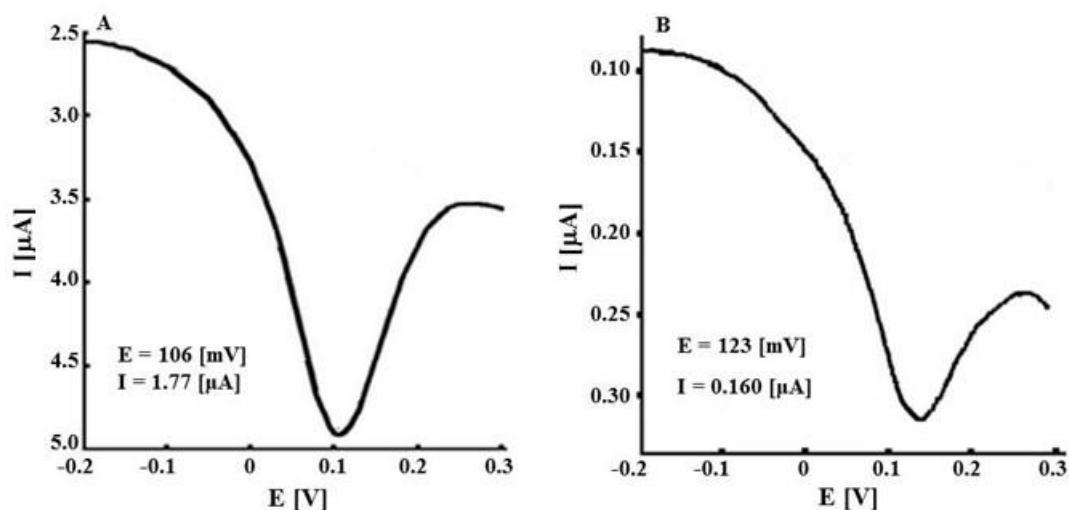
Fig. 3. Square wave voltammograms (frequency 25 Hz) of: (A) bare SPGE; (B) SH-NC3/MCH SAM modified electrode; Solution composition: 1 mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$, 0.1 M PBS buffer, pH 7.4. The measuring conditions: screen printed electrode with three electrode configurations – gold working electrode, silver reference electrode and gold counter electrode.

The same characteristics were also done on the GDEs. In order to confirm the attachment of the SH-NC3/MCH layer on gold surface, the CV and OSWV curves were recorded using a redox active marker $[Fe(CN)_6]^{3-/4-}$. The representative curves have been shown in Figure S1 (Supplementary data). In the CV a peak-to-peak separation registered on bare GDE was 92 ± 7 mV, and after the immobilization of the SH-NC3/MCH layer increased to 350 ± 13 mV. It correlates with the decreasing of the oxidation and reduction peaks current. In the case of OSWV we observed the same trend. Peak current values after deposition of the SH-NC3/MCH layer decreased from 9.9 ± 0.2 µA to 2.7 ± 0.1 µA. So, it means that formation of the SH-NC3/MCH layer on both kinds of electrodes was successful.

3.2. Application of genosensor based on gold screen printed gold electrodes (SPGEs) and gold disc electrodes (GDEs) for monitoring of hybridization processes with 20-mer DNA target derived from AIV H5N1

The working principle of the biosensor proposed is based on the ion-channel mechanism originally developed by Umezawa [31] and precisely described in our previous work [26].

The hybridization reaction with ssDNA and RNA targets sequences were detected using $[Fe(CN)_6]^{3-/4-}$ as a redox marker present in the sample solution. The advantage of this approach is that oligonucleotides labeling is not necessary. In order to eliminate the capacitive current, the hybridization processes performed with genosensor proposed were controlled using OSWV.

The applicability of genosensor proposed was tested by exploring the hybridization processes with specific 20-mer sequences of DNA related to the H5N1 using OSWV. The representative square wave voltammograms recorded in the presence of c-NC3 and nc-NC3 are presented in Figure 4. Upon hybridization with c-NC3 target, the decrease of peak current was observed. The concentration of 1 pM of c-NC3 target caused 39.6 ± 4.6 % (n=7) decreasing of peak current. 1 pM of nc-NC3 generated weak response - oxidation/reduction peak current decreased 0.42 ± 5.0 %. The system was selective. The selectivity of the genosensor confirms the lack of response in the case of nc-NC3 sequence.
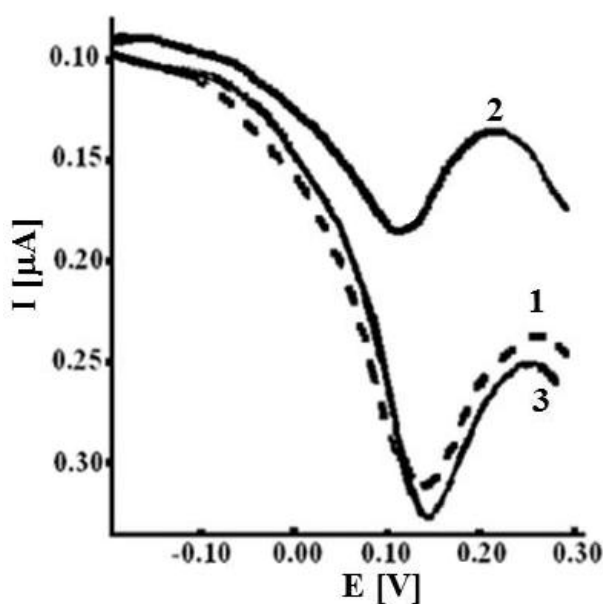

Fig. 4. An example of square wave voltammograms obtained for SPGEs modified with SH-NC3 and MCH upon hybridization with c-NC3 and nc-NC3 at the concentration of 1 pM. Solution composition: 1 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$, 0.1 M PBS (pH 7.4). The measuring conditions: screen printed electrode with three electrode configurations – gold working electrode, silver reference electrode and gold counter electrode.

The detection of 20-mer DNA target derived from AIV H5N1 (c-NC3) with using classic gold electrodes modified with SH-NC3/MCH has been already reported [26]. This system was selective. The decrease of reduction/oxidation peak current of 15.1 ± 2.86 % recorded in presence of the fully complementary c-NC3 and 0.67 ± 1.65 % in presence of

the non-complementary nc-NC3 target were observed [26]. These data indicated that measuring system based on SPGEs in the comparison to GDEs is more sensitive.

3.3. Application of genosensor based on screen printed gold electrodes (SPGEs) and gold disc electrodes (GDEs) for monitoring of hybridization processes with 280-mer RNA transcripts derived from AIV H5N1

The aim of this work was find the answer whether, in a given RNA sample the sequence of avian influenza is present. That was a reason to test the genosensor in one point of concentration .

The sensitivity and selectivity of the genosensor constructed on SPGEs and GDEs were tested using four 280-mer RNA transcripts, with (RNA1, RNA2, RNA3) or without (RNA4) the 20 nucleotide (nt) region complementary to the SH-NC3 probe. The representative Osteryoung square-wave voltammograms recorded on SPGEs upon hybridization with RNA1 and RNA4 at the concentration of 1 pM were illustrated in Figure 5. The concentration of 1 pM caused 45 ± 1 (n=8), 10.0 ± 1 % (n=6) and 20.0 ± 1 % (n=7) decreasing of peaks current for RNA1, RNA2 and RNA3, respectively. RNA4 transcript generated weak response 1.0 ± 1 % (n=5) decreasing of peak current (Table 1).
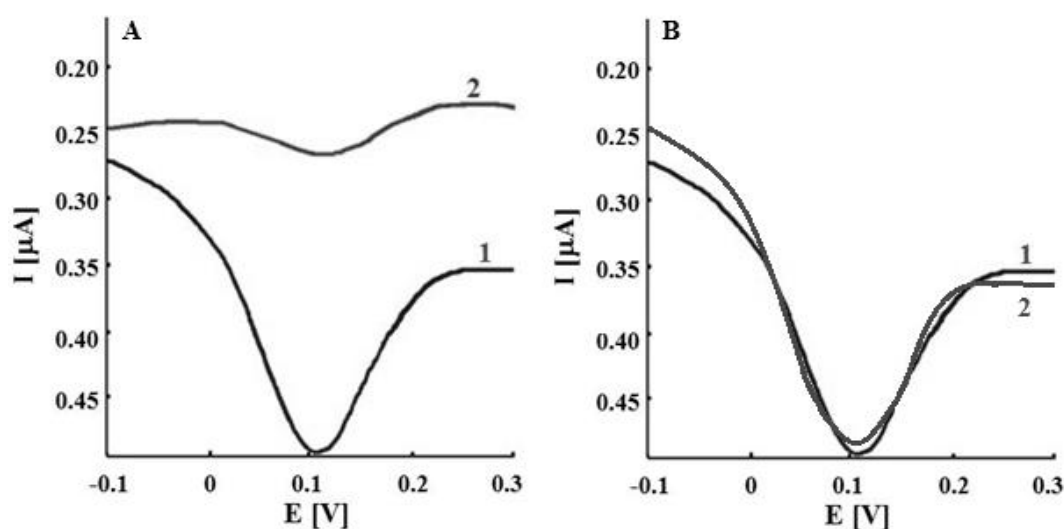

Fig. 5. Typical square wave voltammograms obtained for SPGEs modified with SH-NC3/MCH SAM upon hybridization. Curve 1 – before hybridization and next curve 2 upon hybridization with: (A) RNA1; (B) RNA4 at concentration of 1 pM. Solution composition: 1 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$, 0.1 M PBS (pH 7.4). The measuring conditions: screen

printed electrode with three electrode configurations – gold working electrode, silver reference electrode and gold counter electrode.

The representative Osteryoung square-wave voltammograms recorded on GDEs upon hybridization with RNA1 and RNA4 at the concentration of 10 pM were illustrated in Figure 6. The concentration of 10 pM caused 63.0 ± 6 % (n=7), 11.0 ± 1 % (n=12) and 20.0 ± 2 % (n=7) decreasing of peaks current for RNA1, RNA2 and RNA3, respectively. RNA4 transcript generated weak response: 8.0 ± 1 % (n=5) decreasing of peak current (Table 1).

It is important to underline that genosensor prepared on GDEs and SPGEs recognizes complementary RNA transcripts dependently on the target fragment contextual position in polynucleotide.
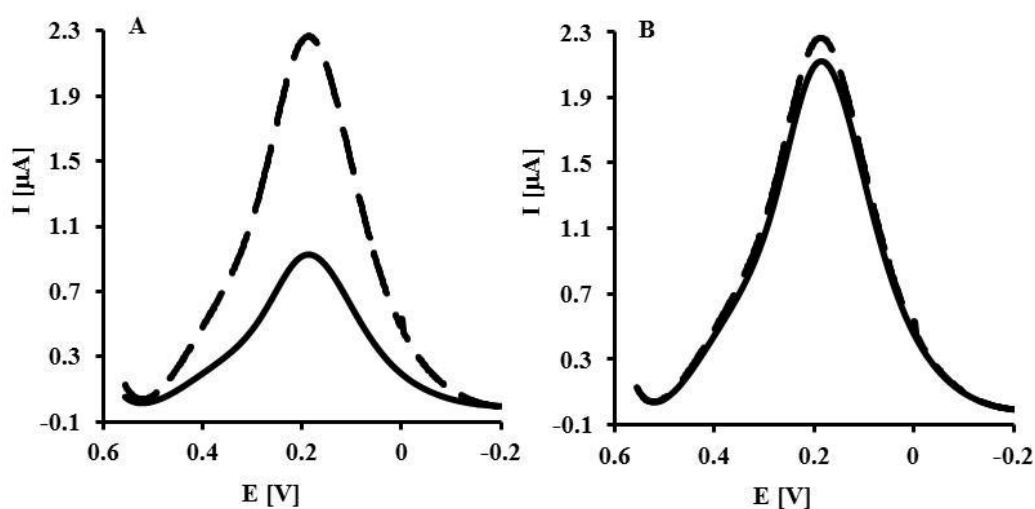

Fig. 6. Typical square wave voltammograms obtained for GDEs modified with SH-NC3/MCH SAM upon hybridization. Dashed curve – before hybridization and next curve upon hybridization with: (A) RNA1; (B) RNA4 at concentration of 10 pM. Solution composition: 1 mM $K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$, 0.1 M PBS (pH 7.4). The measuring conditions: gold disc – working electrode, Ag/AgCl - reference electrode, Pt wire – counter electrode.

Table 1. The relative redox marker changes current $\Delta I=(I_n - I_0)/I_0$ [%] measured with gold electrodes modified Au/SH-NC3/MCH after hybridization with RNA transcripts on GDEs and SPGEs. (I_n is the current measured in the presence on a particular RNA transcripts concentration, I_0 is the current measured in buffer with no analyte).

RNA transcript	SPGE	GDE
	ΔI [%] C = 1 [pM]	ΔI [%] C = 10 [pM]
RNA1	-45 ± 1 (n=8)	-63 ± 6 (n=7)
RNA2	-10 ± 1(n=6)	-11 ± 1 (n=12)
RNA3	-20 ± 1 (n=7)	-20 ± 2 (n=7)
RNA4	-1 ± 1 (n=5)	-8 ± 1 (n=5)

The literature examples relating to biosensors for a microRNA (miRNA) detection are collected in a Table 2 [32-41]. Although the examples of sensors given in the Table 2 have lower detection limits, they are able to detect the RNA sequences up to 30-mer. An undoubted advantage of the biosensor proposed is the possibility of determining the 280-mer sequence containing 20-mer fragment complementary to the NC3 probe.

The possibility for direct detection RNA in biological samples, without the necessity of transcription process of viral RNA to DNA is the main advantage of using RNA transcripts for sensing application. To our knowledge, there are no papers regarding determination of long RNA transcripts, excluding our previous publication about an application of a redox-active monolayer designed for construction of biosensor for the detection of specific DNA and RNA oligonucleotide sequences related to the AIV type H5N1 [41]. The hybridization processes were monitored using the OSWV, and as the targets 20-mer DNA and ca. 280-mer RNA oligonucleotides were applied. The lowest detectable RNA concentration based on this device was 10 pM. The miniaturized genosensor proposed in this work is not only able to detect 1 pM of RNA transcripts, and additionally discriminate the different position of the 20-mer sequence complementary to the probe.

Table 2: Comparison of genosensor for detection RNA transcripts presented with those already published.

Electrode modification	Measuring technique	Target	Detection limit	Ref.
SPCE or PGE/S-MB/ biotin-ssDNA	LSV	30-mer miRNA	SPCE 0.114 µg/mL PGE 0.992 µg/mL	32
Au/ SH-ssDNA/MCH/BSA	Am	22-mer miRNA	3 fM	33
Au/ SH-ssDNA-biotin/MCH	CV		0.2 fM	34
PGE/ssDNA	DPV		0.67 µg/mL	35
Au/SH-ssDNA/TGA	EIS		1 fM	36
Au/cpDNA/MCH	DPV		600 fM	37
SPGE/SH-cpDNA/MCH	DPV		0.1 fM	38
Au/tetra-DNA	Am		10 fM	39
GCE/Graphene/DenAu/LNA+MPA	ChA		60 fM	40
Au/AET/Phen-Epoxy/ FeCl₃/Phen-Epoxy/NH₂-ssDNA	OSWV	ca. 280-mer RNA	870·fM	41
Au/ SH-ssDNA/MCH		ca. 280-mer RNA	not determined	This work

Abbreviations: SPCE – screen printed carbon electrode, PGE – pencil graphite electrode, MCH- 6-mercaptohexanol, s-MB – streptavidin coated magnetic beads; Au – gold electrode, ssDNA – single stranded DNA, LSV – linear sweep voltammetry, Am – amperometry, TGA - thioglycolic acid, cpDNA- capture DNA probe, SPGE – screen printed gold electrode, tetra-DNA - Tetrahedral nanostructure-based capture probes, DenAu - dendritic gold nanostructure, LNA - integrated molecular beacon probe, MPA - Mercaptopropanoic acid, ChA - chronoamperometry

4. Conclusions

The genosensor presented is fabricated based on self-assembling mixture of thiolated ssDNA probe and MCH on the two types of gold electrode surfaces – GDEs and SPGEs. The genosensor displayed good sensitivity and selectivity. Both, the non-complementary 20-mer oligonucleotide and the RNA transcripts without complementary sequence generated weak responses. It is worth to emphasize that one of the main advantages of the genosensor presented here is its simple fabrication, the suitability for determination of the single stranded RNA transcripts and distinguishing of the different positions of the complementary parts.

Considering the above analytically important parameters, the presented genosensor could be successfully applied for detection of the H5N1 virus. We believe miniaturized genosensor proposed has a great potential as a point-of-care device for the early diagnosis of AIV H5N1.

Acknowledgements

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