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Electrochemical response of oligonucleotides on carbon paste electrode

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Abstract

Electrochemical response of synthetic oligonucleotides with different DNA bases sequences was investigated to find relationships between a chain composition and a signal. All DNA mononucleotides present electroactivity at a carbon paste electrode yielding anodic peaks at potentials: 1.00 (GMP), 1.28 (AMP), 1.47 (TMP) and 1.53 V (CMP). Also 15-mer homooligonucleotides show respective anodic peaks. Electrochemical response of 15- and 19-mer oligonucleotides consisting of all four DNA bases in different amounts was determined by the composition of oligonucleotide chain. When the contribution of different bases in oligonucleotide was balanced two anodic peaks were obtained that can be attributed to guanine and adenine moieties. Thymine residue is shown as a separate peak in voltammogram when its content in oligonucleotide chain is close to 50% of the total number of bases. Cytosine also yields a peak at its significant contribution in oligonucleotide chain and both pyrimidinic moieties produce catalytic waves easier when one of them is dominating or when only one pyrimidine derivative is present in a chain. Guanine is the easiest oxidized base and it produces a peak even at its minimal contribution (one guanine residue in 19-mer oligonucleotide). Guanine peak potential is dependent on oligonucleotide concentration and oligonucleotide composition. The lowest oligonucleotide concentration detected by guanine peak was 12.5 nM whereas detected by thymine peak was 90 nM.

Keywords: Oligonucleotides; Square wave voltammetry; Structure/signal relationship

1. Introduction

Nucleic acids are still objects of intensive research and variety of analytical methods have been developed for this aim. Nucleic acid research has a fundamental meaning for the explanation of molecular processes in all living organisms and finds wide practical applications in molecular diagnosis, food analysis and environmental monitoring. Among different methods of nucleic acids analysis electrochemical techniques are quite frequently used as simple, relatively cheap, sensitive and reliable tools [1–6]. One of the most interesting fields of electrochemical analysis applications are electrochemical DNA sensors. They present all positive features of electrochemical analysis and are much faster than traditional methods [4]. DNA sensors are mainly applied in hybridization detection where DNA fragment immobilized on the electrode surface is used as a specific probe. Hybridization event is detected via some indicators but more attracting is label-free detection based on changes of electroactivity of DNA duplex after hybridization [4]. Very promising application domain of DNA biosensors is a detection of DNA damage [5,7]. Increasing attention in DNA research by means of electrochemical methods requires very careful and perfect studies of electrochemical behaviour of nucleic acids. Detailed knowledge of electrochemical response of nucleic acids and their fragments including also an influence of experimental conditions is necessary to understand and perform analytical procedures correctly. Investigation of interactions between nucleic acids and the electrically charged surfaces is of great importance in basic molecular biology research.

Electroactivity of nucleic acids discovered already half century ago comes from electroactivity of bases composing nucleic acid chains [1]. Early electrochemistry of DNA was based on mercury electrode where electrochemical reduction of two bases — adenine and cytosine gave rise to cathodic signals and reoxidation of guanine produced an anodic signal [8]. Thymine is not reducible on mercury electrode according to literature reports [9] but both pyrimidine bases, thymine and cytosine were reported not to show an electrochemical activity at

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carbon based electrodes [5,9–11]. Paleček and Fojta [9] reported that in DNA and RNA guanine and adenine residues are oxidizable at carbon electrodes and at these electrodes they can be analyzed whereas cytosine should be investigated only at mercury electrodes. Purine nucleobases have attracted more attention of publication authors [12–14] and only few literature positions were published on electrochemistry of pyrimidine bases [15,16]. The number of guanine residues in oligonucleotide chain influenced both oxidation potential and peak current at HMDE electrode [8]. Peak of guanine from decamer containing four guanine residues was shifted about 20 mV to more negative potentials compared to a chain with two guanine residues. Signals of guanine and adenine are influenced by the DNA structure and this property is useful in DNA damage detection [5,9]. The determination of purine bases contents in nucleic acids by square wave voltammetry (SWV) was used as a method of the determination of nucleic acid concentration [14]. It was also found that at full mercury electrode coverage guanine peak current reflects the content of guanine in the molecule [8]. Nowadays mainly solid electrodes are in use, the most popular electrochemical transducers in biosensors are carbon electrodes (carbon paste, glassy carbon, graphite) and majority of authors perceive only that DNA oxidation at carbon electrodes is associated with irreversible oxidation of guanine and adenine [17,18]. According to some other reports thymine and cytosine derivatives are electrochemically oxidized at carbon electrodes but they give the response at high positive potentials, at high pH values and only in specific conditions, i.e. with the use of sonovoltammetry. Detection of pyrimidine bases was possible only at their high concentration, 10 times higher than the purine bases concentration [15,16,19]. However, using preconditioned glassy carbon electrode the detection of equimolar concentration of all DNA bases and all mononucleotides was achieved. Also not exactly identified anodic peak attributed to pyrimidine residue was obtained in differential pulse voltammogram [16]. Brooks and Richter [20] admitted that electrochemical oxidation in DNA can occur at each of the four bases, however, voltammetric methods do not result in resolution of the adenine and thymine oxidation waves. They also assert that cytosine oxidative wave can not be observed in aqueous solution.

Unfortunately, publications on relationships between electrochemical signals on solid electrodes and base sequences of poly- or oligonucleotides have appeared very rarely [8].

Facing some confusion over nucleic acid electrochemistry on carbon electrodes we have investigated electrochemical response of nucleic acid fragments of different composition at the carbon paste electrode to try to explain their electroactivity and structure/signal relationship. In order to estimate how the composition of nucleic acid chain influences the electrochemical signal we have used simple synthetic single-stranded 15- and 19-mer oligonucleotides, both homooligomers and heterooligomers. Particularly, a contribution of pyrimidine bases present in nucleic acid fragments in electrochemical signal drew our special attention because electroactivity of thymine and cytosine on solid electrodes is still questionable. Selectivity of the electrochemical nucleic acid signal would allow to conclude about their structure and give a chance to use electrochemical methods for nucleic acid composition detection including the detection of nucleic acid damage.

2. Experimental

The synthetic single-stranded oligonucleotides (Table 1) were purchased from Institute of Biochemistry and Biophysics, DNA Sequencing and Oligonucleotides Synthesis Laboratory, Warsaw, Poland and from Tib Molbiol, Poznan, Poland.

Mononucleotides — guanosine-, adenosine-, thymidine-, cytidine monophosphates (GMP, AMP, TMP, CMP), graphite powder, mineral oil were from Sigma, USA. KCl, NaCl were from Fluka, Switzerland. KH_2PO_4, Na_2HPO_4 were from Polskie Odczynniki Chemiczne, Gliwice, Poland. All solutions were prepared using deionized water.

Stock solutions of the single-stranded oligonucleotides and monophosphate nucleosides at various concentrations between 0.01 and 100 μM were dissolved in PBS buffer, pH 7.4 until shaken for 1 h and stored in a freezer for 24 h before use. PBS buffer, pH 7.4 contained 140 mM NaCl, 2 mM KH_2PO_4, 10 mM Na_2HPO_4, 2 mM KCl diluted in 1 L deionized water.

Electrochemical measurements were performed with a potentiostat μAutolab with GPES, version 4.8 software (Eco Chemie B.V., Utrecht, The Netherlands). The experimental conditions for electrochemical analysis were: the three electrode system consisted of a carbon paste working electrode, Ag/AgCl reference electrode and a platinum wire counter electrode. The carbon paste was prepared by mixing graphite powder with mineral oil with the ratio 70:30. The resulting paste was packed into Teflon tube of 0.1 cm internal diameter. Electrical connection was supplied with a copper wire. The surface of the working electrode was always renewed before use by removing outer layer of carbon paste and polishing to a smoothed finish on a weighing paper [21–23].

All electrochemical experiments were carried out using square wave voltammetry. Experimental conditions were (unless otherwise stated): frequency 100 Hz, amplitude 0.04 V, step potential 0.015 V. Three electrodes were immersed in 1 ml cylindrical cell. Before each measurement the carbon paste electrode (CPE) was activated by applying a potential of +1.6 V for 2 min in background

Table 1
Base sequences of synthetic oligonucleotides

Code	Sequence	G	A	T	C	Total number of bases
gna	GGGGGGGGGGGGGGG	15				15
aaa	AAAAAAAAAAAAAAA		15			15
ttt	TTTTTTTTTTTTTTT			15		15
cyt	CCCCCCCCCCCCCCC				15	15
btym	CAGACGAGGAAGCAG	6	6	0	3	15
tym	TGTCTCTTACTGTTT	2	1	9	3	15
tym2	TTTCTCTTACTGTTT	1	1	10	3	15
tym2a	TGTCTCAAACTGTTT	2	3	7	3	15
tym3	TGTCCCTTACCGTTT	2	1	7	5	15
tym4	TGTCTCTTTCTGTTT	2	0	10	3	15
tym5	TGTCCCTTGCCGTTT	3	0	7	5	15
bar3	GTCAACTTCCGTACCGAGC	4	4	4	7	19
bar4	GCTCGGTACGGAAGTTGAC	7	4	4	4	19
bar4a	TCTCGGTACGGAAGTTGAC	6	4	5	4	19

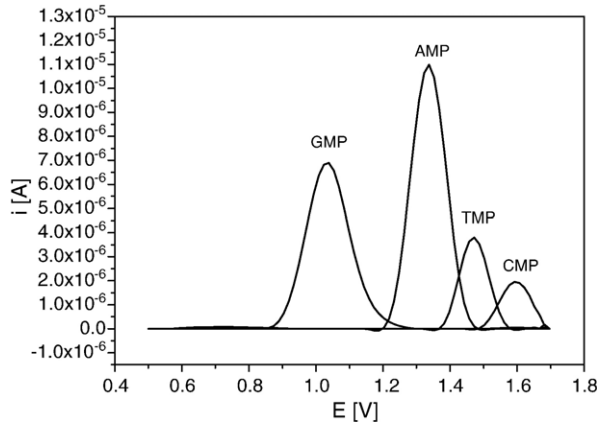

Fig. 1. Square wave voltammogram of 1.4 nM mononucleotides GMP, AMP, TMP, CMP in PBS buffer, pH 7.4. Experimental conditions for SWV: scan range from 0.5 to 1.7 V, frequency of 100 Hz, amplitude of 0.04 V, step potential of 0.015 V, scan rate 1.5 V/s.

electrolyte — PBS buffer, pH 7.4. Then the oligonucleotides were adsorbed on activated CPE by applying a potential of 0.5 V for 2 min in the stirred PBS buffer. The electrode was then rinsed in blank PBS buffer for 30 s and SWV measurement was carried out in the next cell in the same buffer.

The monophosphate nucleoside measurements were performed on pretreated CPE directly in a buffered sample after incubation for 5 min in PBS buffer, pH 7.4.

For the presentation of all experimental voltammograms Origin, version 6.0 (Microcal Software) was used.

All measurements were done in a room temperature.

All potentials are referred to Ag/AgCl electrode.

All voltammograms are presented after base line correction.

3. Results

3.1. Electrochemical response of DNA mononucleotides (GMP, AMP, TMP, CMP)

Guanosine, adenosine, thymidine and cytidine monophosphates irreversibly oxidize on a carbon paste electrode to produce catalytic waves in PBS buffer pH 7.4 at potentials ca 1.00, 1.28, 1.47 and 1.53 V, respectively (Fig. 1). Anodic peaks of mononucleotides shift slightly with pH, i.e. they move to more positive potentials when pH decreases. This is exemplified in Fig. 2 presenting electrochemistry of thymidine mononucleotide in different buffers. Decrease of pH gives also a rise to diminish a peak current of nucleotide, however, even at low pH (pH 2.15) thymidine monophosphate exhibited distinct peak. There is no data in the literature on a pH dependence of DNA mononucleotides peak current, some reports on free bases are inconsistent. Thymine peak obtained at the β-cyclodextrin modified electrode disappeared at acidic pH (<5) [24] but at a glassy carbon electrode it remained almost constant over pH range 3–10 [16]. Anyway, all mononucleotides, not only purine but also pyrimidine derivatives are electroactive on a carbon paste electrode, whereas guanosine monophosphate exhibits the lowest potential and anodic potential of CMP exceeds 1.5 V. Anodic peak of thymidine mononucleotide remains detectable also when mixed with some other DNA fragments. Electrochemical response of thymidine monophosphate added to the solution of 15-mer homoguanine oligomer in a high concentration is very pronounced (Fig. 3), but it is not visible in a solution of 15-mer homocytosine oligomer (not shown). Anodic peaks of both pyrimidine moieties are localized in a short

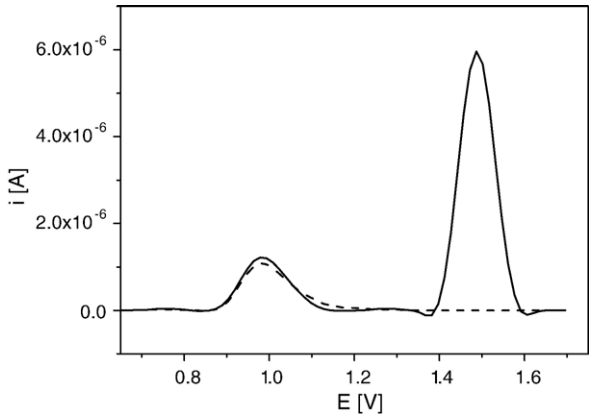

Fig. 3. Square wave voltammogram of 124 mM TMP in the solution of gna oligonucleotide (8 μM), PBS buffer, pH 7.4. Experimental conditions for SWV as in Fig. 1.

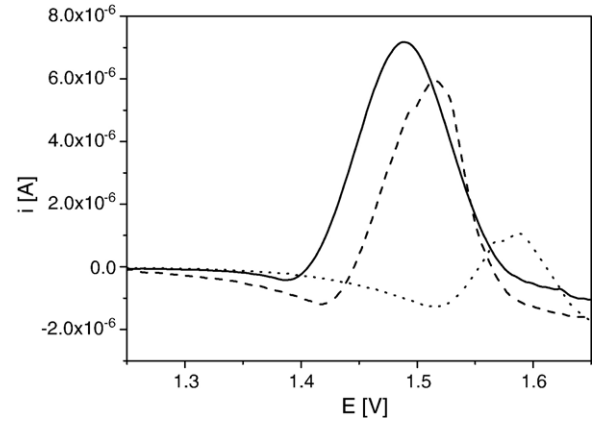

Fig. 2. pH dependence of electrochemical response of 1.4 mM TMP in PBS buffer, pH 7.4 (–), acetate buffer, pH 4.7 (—) and HCl–KCl buffer, pH 2.15 (···). Experimental conditions for SWV: scan range from 0.5 to 1.7 V, frequency of 100 Hz, amplitude of 0.04 V, step potential of 0.0015 V, scan rate 0.15 V/s.

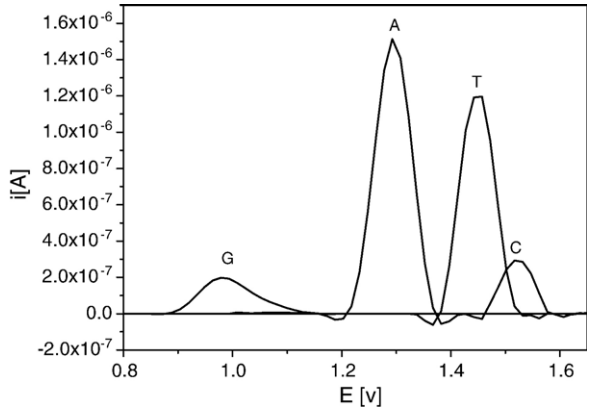

Fig. 4. Electrochemical response of 15-mer 1.4 μM homooligomers of guanine, adenine, thymine and cytosine in PBS buffer, pH 7.4. Experimental conditions for SWV as in Fig. 1.

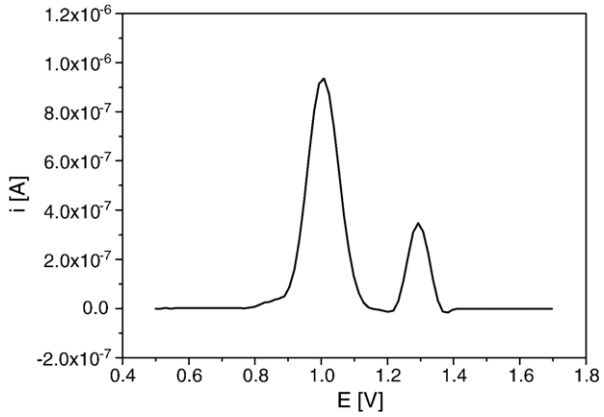

Fig. 5. Electrochemical response of 19-mer 1.8 μM heterooligomers "bar4a" in PBS buffer, pH 7.4. The sequence of "bar4a" is shown in Table 1. Experimental conditions for SWV as in Fig. 1.

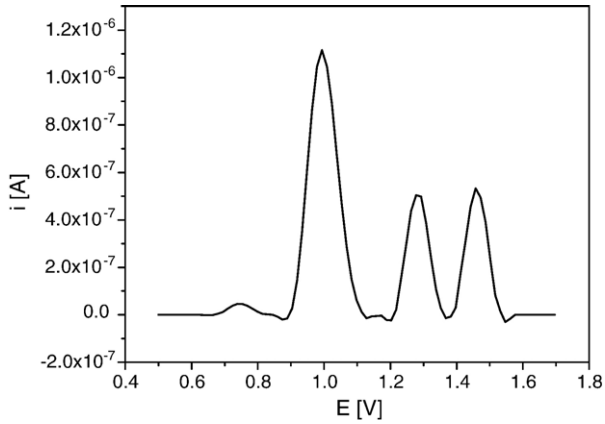

Fig. 7. Electrochemical response of 15-mer 13 μM heterooligomers "btym", in PBS buffer, pH 7.4. The sequence of "btym" is shown in Table 1. Experimental conditions for SWV as in Fig. 1.

potential distance (ca 60 mV, Fig. 1) and their discrimination is not always possible. However, the presented results demonstrate that both pyrimidine bases undergo oxidation on carbon paste electrode contrary to some previous reports [9,22].

3.2. Electrochemical response of homooligomers

Homooligomers of all four DNA nucleobases present a significant electrochemical response (Fig. 4). Anodic peaks of purine and pyrimidine oligonucleotides were observed at potentials almost identical to those obtained at an oxidation of mononucleotides at carbon paste electrode. The presented data confirm again electroactivity of all DNA nucleobases at carbon paste electrode where respective anodic peaks can be observed also when bases are coupled in homooligonucleotides.

3.3. Electrochemical response of heterooligomers

Electrochemical experiments were continued with some synthetic heterooligomers. They were composed of 15 or 19 nucleotides, a number of particular nucleobases were differentiated although in most oligonucleotides pyrimidine bases were in majority (Table 1). The number of guanine varied from 1 to 7 whereas the number of thymine reached even 10. In any case square wave voltammetry has not shown peaks of all four nucleobases. When a contribution of all four nucleobases was balanced an electrochemical response of oligonucleotide yielded 2 anodic peaks associated with an oxidation of guanine and adenine like as in DNA voltammograms [1]. Fig. 5 presents a typical example of square wave voltammogram of synthetic oligonucleotide containing all four bases in similar amounts.

It was found that electrochemical response of thymine moiety could be visible only when its contribution in oligonucleotide chain draws near 50% of the total number of bases. Fig. 6 presents a square wave voltammogram of oligonucleotide "tym2a" containing 7 thymine residues in the 15-mer oligomer and this composition is sufficient to generate 3 anodic peaks that can be attributed to guanine, adenine and thymine. The DNA fragment "tym2a" contains also 3 residues of cytosine, but this is not reflected in voltammogram. Cytosine hardly appears in voltammograms, it reveals easier when thymine is lacking in oligonucleotide chain (Fig. 7). Heteronucleotide "btym"

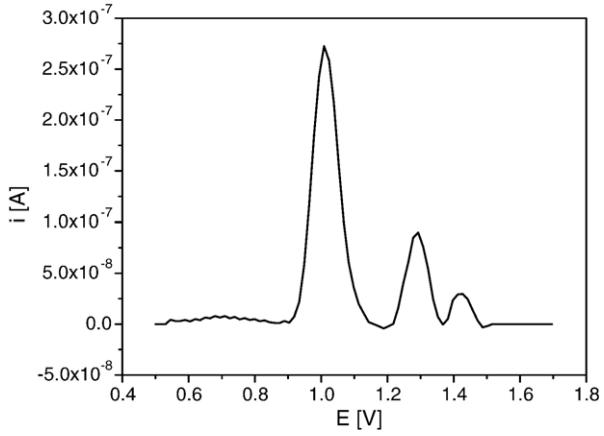

Fig. 6. Electrochemical response of 15-mer 0.5 μM heterooligomers "tym2a", in PBS buffer, pH 7.4. The sequence of "tym2a" is shown in Table 1. Experimental conditions for SWV as in Fig. 1.

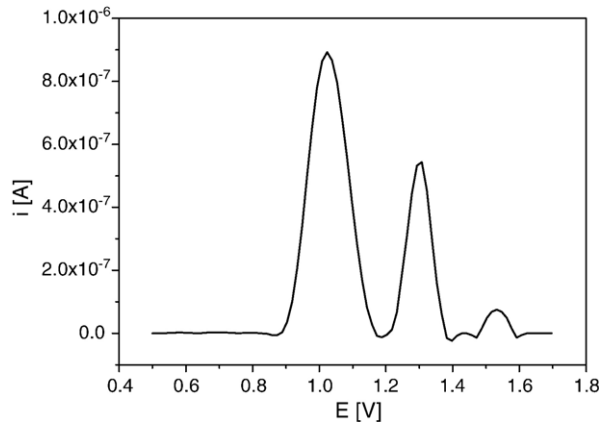

Fig. 8. Electrochemical response of 19-mer 75 μM heterooligomers "bar3", in PBS buffer, pH 7.4. The sequence of "bar3" is shown in Table 1. Experimental conditions for SWV as in Fig. 1.

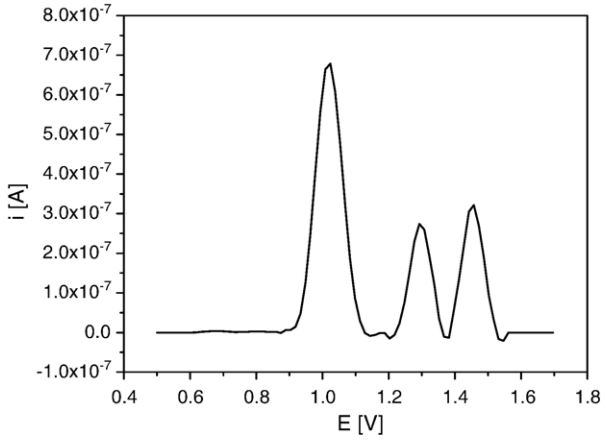

Fig. 9. Electrochemical response of 15-mer 13 μM heterooligomers "tym2" in PBS buffer, pH 7.4. The sequence of "tym2" is shown in Table 1. Experimental conditions for SWV as in Fig. 1.

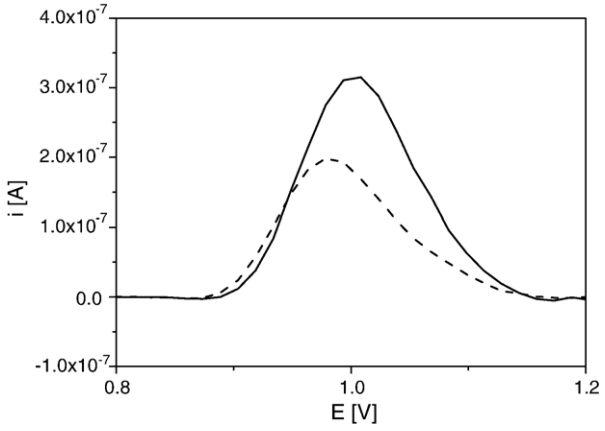

Fig. 11. Guanine oxidation peaks in voltammograms of guanine homooligomer (15-mer oligonucleotide "gna") and heterooligomer (19-mer oligonucleotide "bar4a) containing 6 guanine residues. Experimental conditions for SWV as in Fig. 1.

contains both 6 guanine and 6 adenine residues and also 3 cytosine residues and 0 thymine (oligonucleotides sequence in Table 1). Anodic peak of cytosine is shifted here to less positive potential, i.e. it appears at the potential inherent for thymine residues. When the number of cytosine residues significantly exceeded thymine contribution in DNA fragment, anodic peak of cytosine was shown at the potential typical for cytosine and the response of thymine was hardly visible (Fig. 8). Overlapping of pyrimidine anodic peaks makes their identification difficult, however, when the contents of a particular residue are significant, suitable peak is obtained at the typical potential position. It was reported that ssDNA gave at a glassy carbon electrode apart from guanine and adenine oxidation peaks also a third peak that was attributed to pyrimidine residues [16]. It is now evidenced that both pyrimidinic residues can generate separate peaks at carbon paste electrode provided that they are present in oligonucleotide in a significant number or only one pyrimidine is present in oligonucleotide.

It is well known that guanine is the easiest oxidized nucleobase [2,4,9]. This was also confirmed in the presented experiments. Even one guanine residue gave rise to a distinct anodic peak at ca 1.00 V and this was exemplified on Fig. 9 presenting voltammogram of oligonucleotide "tym2" containing one guanine, also one adenine, ten thymine and three cytosine residues.

The lowest electrochemical response of guanine in synthetic oligonucleotides was detected at the sample concentration 12.5 nM. At this concentration only guanine peak was present in voltammograms and the lowest oligonucleotide concentration detected by thymine peak was 90 nM. The oxidation potential of guanine was slightly dependent on oligonucleotide concentration. Guanine anodic peak from oligonucleotide "tym2a" moved from +0.979 V to +1.024 V, i.e. 45 mV when nucleotide concentration increased from 0.5 to 100 μM (Fig. 10). Tomschik et al. [8] observed a shift of a guanine peak potential at a hanging mercury drop electrode to more negative potentials with an increase of guanine residues number in oligonucleotide. Some shifts of guanine peak potential in oligonucleotides of different compositions were also observed in our research. Guanine homooligomer ("gna") produced guanine peak at the potential of 0.979 V, whereas guanine oxidation peak in

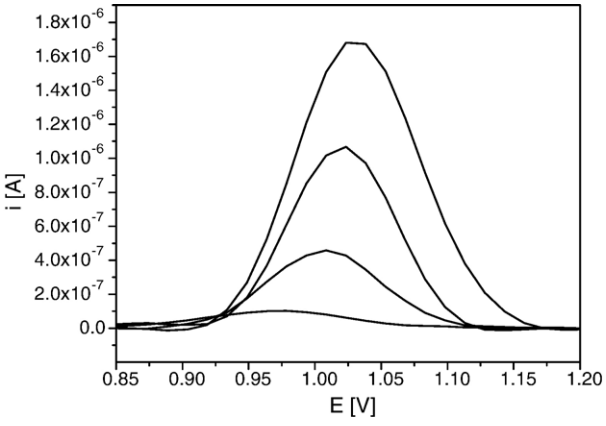

Fig. 10. Effect of oligonucleotide ("tym2a") concentration on guanine peak potential. Oligonucleotide concentrations: 0.025, 0.50, 10.00 and 100.00 μM. Experimental conditions for SWV as in Fig. 1.

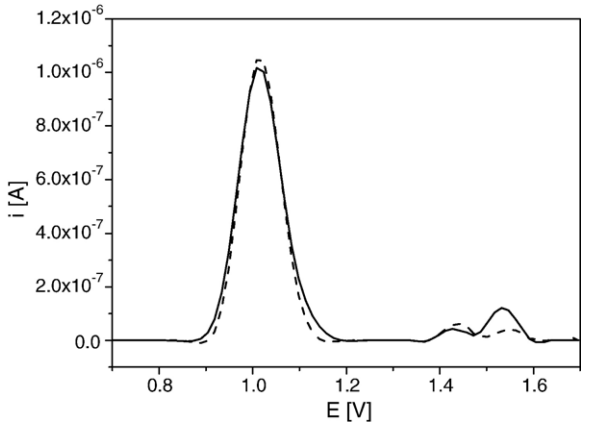

Fig. 12. Alteration of electrochemical response of pyrimidinic bases as a result of removing adenine from oligonucleotides. (—) tym3 (containing 1 adenine residue). (–) tym5 (devoid of adenine). Concentrations 2 μM. Experimental conditions for SWV as in Fig. 1.

heterooligomer "bar4a" (19 bases, 6 guanine residues) was observed at 1.009 V (Fig. 11). Some changes in guanine peak potential were observed also in voltammograms of all investigated synthetic oligonucleotides. It is difficult to say definitely whether guanine peak potential is moving with guanine content in the molecule and its concentration on the electrode surface or a structure of oligomer that make guanine oxidation easier or more difficult. It seems anyway that guanine peak is reflecting sequence composition, i.e. guanine oxidation potential is influenced by a sequence of investigated oligonucleotide. The results presented above evidenced that the presence of some particular bases in oligonucleotide and their number influences electrochemical response of other bases (relation of T and C, Figs. 7 and 8).

Removing adenine from oligonucleotide chain "tym3" enhanced a cytosine peak in oligonucleotide "tym5" as it is shown in voltammogram in Fig. 12. However, the presence of adenine in oligonucleotide "tym3" is not reflected in voltammogram. Adenine presenting slightly lower oxidation potential than guanine gave usually an anodic peak in most of the investigated oligomers, however, the peak was not always visible when oligonucleotide contained only one residue of adenine. This was observed in oligonucleotide "tym3" shown in Fig. 12.

The presented results show that electroactivity of DNA fragment is dependent on its composition. The electrochemical signal of a particular base is sensitive to its environment. It means that a damage in DNA fragment is reflected in its electrochemical response.

4. Conclusions

The data presented in this work evidenced clearly that all DNA bases are electroactive and give a pronounced electrochemical response on a carbon paste electrode both as nucleoside monophosphates and as monomers in oligonucleotide chains. However, only guanine and to some extent adenine give always distinct responses at the electrode even at the lowest contribution in an oligonucleotide chain. Guanine anodic peak is present in voltammogram even at one guanine moiety in oligonucleotide chain. The presence of both pyrimidine moieties is reflected in voltammogram providing their significant contribution in oligonucleotide chain. Thymine gave an electrochemical signal only when its number reached almost 50% of all bases. Cytosine competes with thymine and gives a response easier when thymine is lacking or when it significantly exceeds a number of the second pyrimidine base in a chain. Both pyrimidine moieties reveal peaks easier when adenine is not present in oligonucleotide chain.

Guanine anodic peak is sensitive to both the concentration of oligonucleotide sample and to its composition.

The presented data confirm that electrochemical oxidation of deoxyribonucleic acid can occur not only at guanine but also at adenine and both pyrimidine bases. Electroactivity of all DNA bases can be monitored at a carbon paste electrode by a square wave voltammetry. Using this experimental protocol a mechanism of oxidative damage of all DNA constituents can be traced. Up to now oxidative damage of DNA induced by any physical or chemical interactions has been monitored only on guanine, the easiest oxidized DNA base whereas a damage or mutation can occur also on both pyrimidine bases. Also detection of pyrimidine damaging agents and a type of damage is possible with the presented procedure embracing the use of DNA fragment of high pyrimidine contribution.

Further intensive study of electrochemical properties of nucleotides and nucleic acids is requested as it is a source of good knowledge useful in DNA analyses but also in understanding some nucleic acid behaviours including interactions between nucleic acids and different charged surfaces. The determinations of relationships between poly- or oligonucleotide sequences or sequence mutations and their electrochemical response are of basic meaning for DNA analyses useful both in a basic research and in DNA-based biosensing applied in different fields. Oligonucleotides presenting thymine and cytosine peaks in voltammograms can be very useful in DNA analysis to monitor interaction between deoxyribonucleic acids and some compounds specifically reacting with pyrimidinic residues like for example quercetin [25].

Contrary to expectations there are still a lot of issues that need to be clarified to understand electrochemical and electronic properties of DNA and therefore intensive studies are required to explain DNA behaviour. Some of these still unsolved problems present a significant meaning for molecular biology for example the question if DNA is insulator, semiconductor, conductor, and even a superconductor [26].

Acknowledgement

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