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Elimination Voltammetry with Linear Scan as a New Detection Method for DNA Sensors

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Dedicated to Professor Emil Palecek, on the Occasion of His 75th Birthday

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Abstract: The paper describes successful coupling of adsorptive transfer stripping (AdTS) and elimination voltammetry with linear scan (EVLS) for the resolution of reduction signals of cytosine (C) and adenine (A) residues in hetero-oligodeoxynucleotides (ODNs). Short ODNs (9-mers and 20-mers) were adsorbed from a small volume on a hanging mercury drop electrode (HMDE). After washing of the ODN-modified electrode by water and its transferring to an electrochemical cell, voltammetric

curves were measured. The AdTS EVLS was able to determine of C/A ratio of ODNs through the elimination function conserving the diffusion current component and eliminating kinetic and charging current components. This function, which provides the elimination signal in a peak-counterpeak form, increased the current sensitivity for A and C resolution, and for the recognition of bases sequences in ODN chains. Optimal conditions of elimination experiments such as pH, time of adsorption, and scan rate were found. The combination of EVLS with AdTS procedure can be considered as a new detection method in a DNA sensor.

Keywords: Oligodeoxynucleotide, DNA biosensor, linear sweep voltammetry, elimination voltammetry with linear scan, reduction of adenine and cytosine, mercury electrode, sequence of nucleotides

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1. Introduction

Although the structure of DNA was described more than 50 years ago [1], DNA still remains one of the most studied biological molecule. According to Web of Science database more than thirty-nine thousands articles dealing with DNA analysis have been published in 2004. From a number of methods and procedures used, probably agarose and polyacrylamide electrophoresis and capillary electrophoresis [2-5] are the most commonly applied methods for DNA analysis, including DNA separation and DNA sequencing. In addition to these electrophoretic techniques, electrochemical methods based on electroactivity of nucleic acid bases are frequently used as well. Palecek was the first who published papers about electrochemical activity of nucleic acids [6,7], and during the next four decades he and his colleagues found that DNA and RNA produce redox signals at mercury or carbon electrodes due to purine and pyrimidine base residues [7-13]. Recently, it has been shown that the voltammetric techniques can be use not only for the study of redox properties of nucleic acid but also for the analysis of structure and damage of DNA, and for sequence detection in DNA biosensors[13-21]. Research and development of electrochemical sensors for DNA hybridization and damage is closely connected with adsorptive and redox properties of oligodeoxynucleotides (ODNs) that are adsorbed strongly on electrode/electrolyte interfaces. This phenomenon is utilized in adsorptive stripping (AdSV) and/or adsorptive transfer stripping voltammetry (AdTSV) [22,23], reviewed in[13]. In AdTSV, an electrode with adsorbed DNA layer is transferred into a medium not containing DNA in bulk solution.

Generally, DNA biosensors based on nucleic acid recognition processes are aimed at fast, simple and inexpensive testing of genetic and infections diseases. Electrochemical hybridization biosensors rely on the immobilization of a single-stranded DNA probe onto the tranducer surface that converts the duplex formation into a useful electrical signal [24]. For these purposes various modifications of working electrode surfaces have been used [11,13,25-30]. Considerable improvement of the sensitivity of nucleic acids determination has been achieved by applying different electrochemical methods in combination with nucleic acids labelling by redox labels such as osmium and ruthenium complexes [15,16,18,31-33]. In addition, selectivity in electrochemical analysis of DNA has been addressed by a new method using paramagnetic or polymeric beads with modified surface [14,34,35]. The method is also known as double – surface technique [14,16,36-38].

Recently, the elimination voltammetry with linear scan (EVLS) was successfully applied to data analysis of ODNs signal, aiming at better understanding of basic electrochemical processes on working electrode surfaces, and for better resolution of voltammetric signals of adenine (A) and cytosine (C) in ODNs [39-43]. The EVLS, resulting in elimination functions as linear combinations of currents measured at different scan rates provides a significant increase of voltammetric signal sensitivity [39,40].

The present paper deals with the application of EVLS in combination with AdTS technique to the analysis of short synthetic ODNs with 9- and 20-mers (the former ODNs contained only A or C residues, the latter included random sequences) we addressed particularly the following points: (a) to increase the sensitivity in ODN voltammetric responses, (b) to resolve A and C reduction signals, (c) to determine the ratio A/C, and (d) the mutual position of A and C in ODN chain.

2. Materials and methods

2.1. Chemicals

Chemicals used were prepared by Sigma Aldrich Chemical Corp. USA (purity: ACS). Phosphate buffer, of 0.1 M NaH_2PO_4 + 0.1 M Na_2HPO_4, was used. All solutions were prepared using deionized ACS water (Sigma Aldrich). The pH of the phosphate buffer was checked by pH-meter Präcitronic (type MV870, Germany). The pH value was measured using WTW inoLab Level 3 with terminal Level 3 (Weilheim, Germany), controlled by personal computer program (MultiLab Pilot; Weilheim, Germany). The pH-electrode (SenTix-H, pH 0–14/3M KCl) was regularly calibrated by a set of WTW buffers (Weilheim, Germany).

2.2. Oligonucleotides

In our experiments we used synthetic oligodeoxynucleotides (ODNs) purchased from Thermo Electron, Ulm, Germany: **ODN A9** :5´- AAA AAA AAA -3´; **ODN C9:**- 5´- CCC CCC CCC -3´; **ODN A3:** 5´- CCC AAA CCC -3´; **ODN A3a:** 5´- CCC ACA ACC -3´; **ODN A2:** 5´- CCC AAC CCC -3´; **ODN A1:** 5´- CCC ACC CCC -3´; **ODN (20a):** 5´- CCT ICC CCA ATC CCT TTA TT -3´; **ODN (20b):** 5´- GGG AGG TTT CGT ATA GGG AA -3´; **ODN (20c):** 5´- AAT AAA GGG ATT GGG GCA GG -3´. The stock solutions of ODNs were prepared in ACS water (Sigma-Aldrich, USA) and stored in the dark at -20 °C. Working solutions were prepared daily by dilution of the stock solutions.

2.3. Voltammetric measurements

Linear sweep voltammetry (LSV) measurements were performed with an AUTOLAB analyzer (EcoChemie, Netherlands) connected to the VA-Stand 663 (Metrohm, Switzerland). The electrochemical standard cell consisted of three electrodes. The working electrode was a hanging mercury drop electrode (HMDE) with an area of 0.4 mm^2, the reference electrode was Ag/AgCl/3M KCl. Platinum wire was used as an auxiliary electrode. Solutions were deoxygenated prior to analysis by purging with 99.9 % argon gas for at least 7 minutes. Our measurements were performed by adsorptive stripping voltammetry with transfer step - AdTS (Fig. 1).

LSV experimental parameters: start potential 0V, vertex potential –1.8 V, step potential 5 mV, scan rates from 100 to 2 400 mV/s, equilibrium time 5s, scan rates for EVLS: 400, 800, and 1 600 mV/s, reference scan rate 800mV/s with the same step potential. All experiments were carried out at room temperature in phosphate buffer under standard conditions. The raw data were treated using the Savitzky and Golay filter (level 4) integrated to the GPES software (EcoChemie). The data processing and statistic analysis were performing by Excel (Microsoft, USA).

Application of AdTS EVLS as a new detection method in DNA sensors

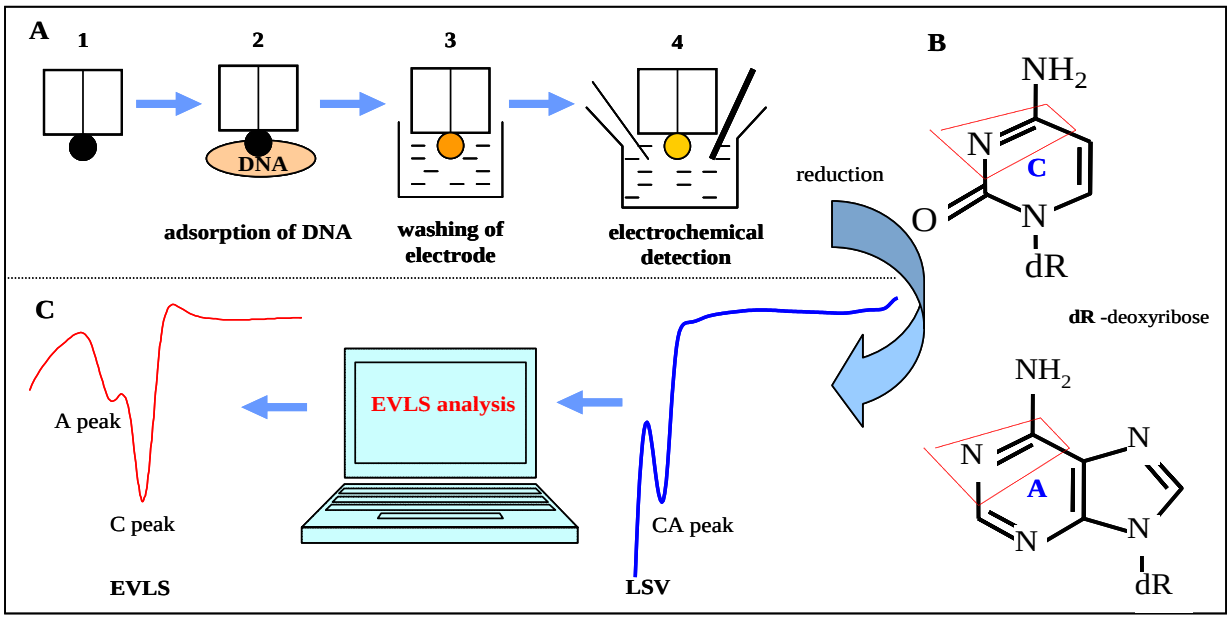

Figure 1. Application of AdTS EVLS as a new detection method in DNA sensors. **A)** Adsorptive transfer stripping voltammetry – AdTSV: (**1**) hanging mercury drop electrode - HMDE; physico-chemical part of the biosensor, (**2**) modification of the working electrode surface by DNA – biological part of the biosensor; (**3**) washing of electrode with water; (**4**) electrochemical detection. **B**) Elimination voltammetry with linear scan – EVLS: linear sweep voltammetric data are treated by elimination procedure. **C**) Cytosine (C) and adenine (A); the functional groups, which are reduced, are marked by red frames.

2.2. Elimination procedure

The function $f(I)$ which eliminates the charging and kinetic currents (I_c, I_k), and conserves the diffusion current (I_d) for the integer 2 (the scan rate of $\frac{1}{2}\nu$, ν and 2ν , where ν is the reference scan rate) is expressed as the linear combination:

$$f(I) = -11.657\ I_{1/2} + 17.485\ I - 5.8284\ I_2 \qquad (1)$$

where I is the reference current measured at the reference scan rate, $I_{1/2}$ and I_2 are the total currents measured at half and double reference scan rate [44-46]. All voltammetric currents were related to this reference current. For better understanding of the elimination procedure, the calculation of the coefficients in linear combination is given. A total current can be expressed as the sum of partial currents:

$$I = I_d + I_c + I_k + ... \qquad (2)$$

The diffusion, charging and kinetic current component at the same potential can be written: $I_d = Y_d(E)\,\nu^{1/2} = const.\nu^{1/2}$, $I_c = Y_c(E)\nu^1 = const.\nu^1$, and $I_k = Y_k(E) = const.\nu^0$. Total currents recorded at three above mentioned scan rates are:

$I_{1/2} = (I_d)_{1/2} + (I_c)_{1/2} + (I_k)_{1/2}$ or $a_1 I_{1/2} = a_1(1/2)^{1/2}(I_d) + a_1(1/2)(I_c) + a_1(I_k)$

$I = (I_d) + (I_c) + (I_k)$ or $a_2 I = a_2(I_d) + a_2(I_c) + a_2(I_k)$

$I_2 = (I_d)_2 + (I_c)_2 + (I_k)_2$ or $a_3 I_2 = a_3(2)^{1/2}(I_d) + a_3(2)(I_c) + a_3(I_k)$

$= I_d$ $= 0$ $= 0$

For the simultaneous elimination of $I_c + I_k$ with I_d conserved the requirements shown below the arrows must be fulfilled. According to these requirements three equations can be written:

$$\begin{aligned} a_1(1/2)^{1/2}(I_d) + a_2(I_d) + a_3(2)^{1/2}(I_d) &= I_d \\ a_1(1/2)(I_c) + a_2(I_c) + a_3(2)(I_c) &= 0 \\ a_1(I_k) + a_2(I_k) + a_3(I_k) &= 0 \end{aligned} \qquad (3)$$

The solution of throse equations yields the coefficients a_1, a_2, and a_3 in the elimination function defined by Eq. 1 :

$$a_1 = -11.657 \qquad a_2 = 17.485 \qquad a_3 = -5.828$$

For adsorbed electroactive substance this elimination function provides an EVLS signal, i.e. a peak (I_p), which passes directly to a large and sharp counter peak (I_{cp}), and creates the characteristic peak-counterpeak signal ($I_p + I_{cp}$) [39-41,43]. The theoretical value of the ratio $I_p/(I_p + I_{cp})$, where I_p and I_{cp} represent the heights of the current peak and counter-peak, is 0.4097 [41,43,46,47].

Linear sweep voltammetric data obtained at the scan rate of $\frac{1}{2}\nu$, ν and 2ν were exported into Microsoft Excel (Microsoft, USA). The particular elimination function was calculated using Microsoft Visual Basic 6.0. The EVLS program for fast calculation of coefficients of the above mentioned elimination function is available from the corresponding author of this publication on request.

3. Results and discussion

The detection of homo- and/or hetero-ODNs on the mercury electrode surface consists in the reduction responses of A and C at negative potentials (about –1.4 V vs. Ag/AgCl/3M KCl) represented by one overlapped voltammetric peak, which cannot be distinguished by common electrochemical methods, such as linear sweep, square wave, and differential pulse voltammetry. Recently we have applied the EVLS to the resolution of reduction signals of A and C in homo-deoxyoligonucleotides A9 and C9, and have shown that this method in connection with adsorptive stripping technique (AdS) is as a useful electroanalytical tool for the ODNs research [41,43]. Our results evoked the idea of coupling of the EVLS and the adsorptive stripping voltammetry with transfer step (AdTS) to perform the ODN analysis with higher ability in A and C resolution in very small sample volumes (units of microliters). Since AdTSV enabled to analyze microliter volumes of ODN samples yielding results very close to those obtained by conventional voltammetry (reviewed in[13]), we could expect improved voltammetric results using the elimination procedure.

Therefore, ODNs were immobilized at a HMDE surface from a small drop of the analyzed solution (5 μL), then the ODN-modified electrode was washed and immersed into the buffer solutions (not containing ODN) to perform the voltammetric measurements. Using LSV we obtained voltammetric data for reduction of A and C at three scan rates. From these data we calculated the EVLS function eliminating kinetic and charging currents and conserving the diffusion current (see equation in section *Elimination procedure*). The whole procedure is schematically shown in Fig.1., where the EVLS analysis contributed to the transformation of the LSV data (overlapped signal) to EVLS data (resolved signal).

3.1. Analysis of homo-oligodeoxynucleotides (A9 and C9)

Recently we have found that EVLS in combination with AdS (without transfer step) enabled the separation of A and C signals of A9 and C9 mixtures in acetate buffer at pH 5.3 [41]. In contrast, in the present paper, we studied the two ODNs using AdTS (i.e, with transfer step) as a function of scan rate and pH (Fig. 2). The pH of phosphate buffer was changed from 5.0 to 7.7, and LSV peak heights were measured at a scan rate of 800 mV/s. While with increasing pH the peak height of C9 increased up to the maximum value at neutral pH and decreased at higher pH, the peak height of A9 dropped already from pH 5.0 to reach only 3% of maximum value at pH 6.0. The peaks of both C9 and A9 were linearly growing with increasing scan rate with slopes of 763 $nA/V.s^{-1}$ and 345 $nA/V.s^{-1}$, respectively (not shown).

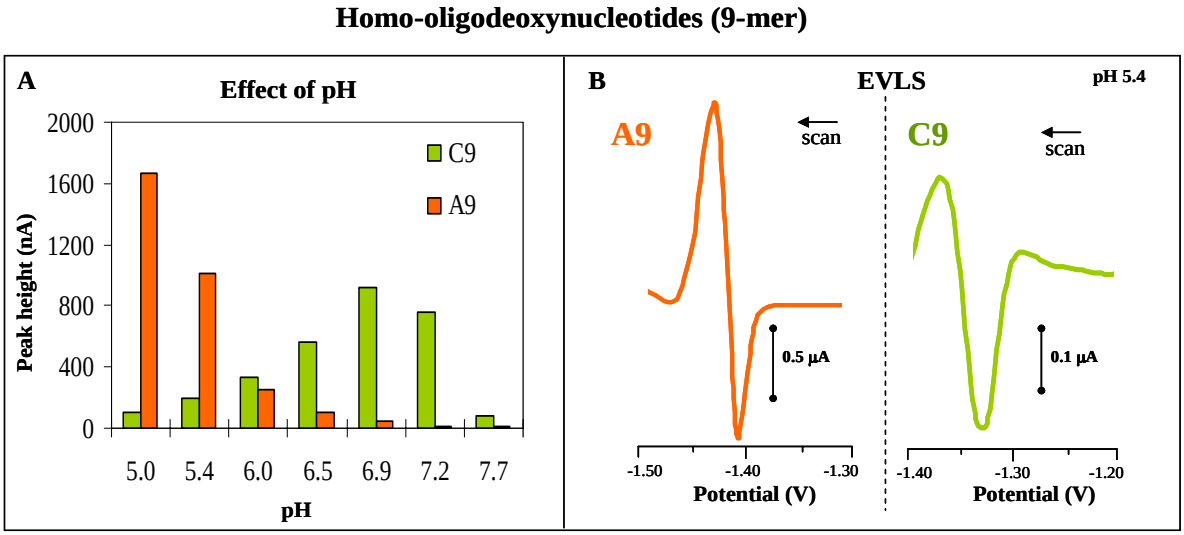

Figure 2. Analysis of homo-oligodeoxynucleotides A9 and C9. **A**) Influence of pH (0.1 M phosphate buffer) on peak heights of ODNs. **D**) Elimination voltammograms of A9 and C9 suitable for the function eliminating kinetic and charging currents, and conserving the diffusion current (reference scan rate of 800 mV/s, pH 5.4). LSV experimental parameters: start potential 0 V, vertex potential –1.7 V, step potential 5mV, adsorption time 120s.

If we simultaneously eliminated the charging and kinetic currents and maintained the diffusion current, we observed elimination signals in the peak-counterpeak form indicating strongly adsorbed electroactive substances for both ODNs (Fig.2B). It was found that the C9 elimination curve is smaller and less pronounced then the A9 elimination curve. While the ratio $I_p/(I_p + I_{cp})$ (I_p and I_{cp} are the heights of the current peak and counter-peak, respectively) for A9 (0.409) agreed with the theoretical value of 0.4097 [41,46] the ratio $I_p/(I_p + I_{cp})$ it indicated a more complex electrode process for cytosine (0.612). For more details see Trnkova et al. [39,41,48].

3.2. Analysis of hetero-oligodeoxynucleotides (9-mers)

Besides the homo-ODNs, four hetero-ODNs (9-mers, A3, A3a, A2 and A1) with various numbers and sequences of A in the primary ODN structure have been analyzed using AdTS in connection with the elimination procedure. Typical LS voltammograms of the studied ODNs, which were analyzed in the presence of 0.3 M ammonium formate and 0.05 M phosphate buffer (pH 6.9), are shown in Fig. 3A.

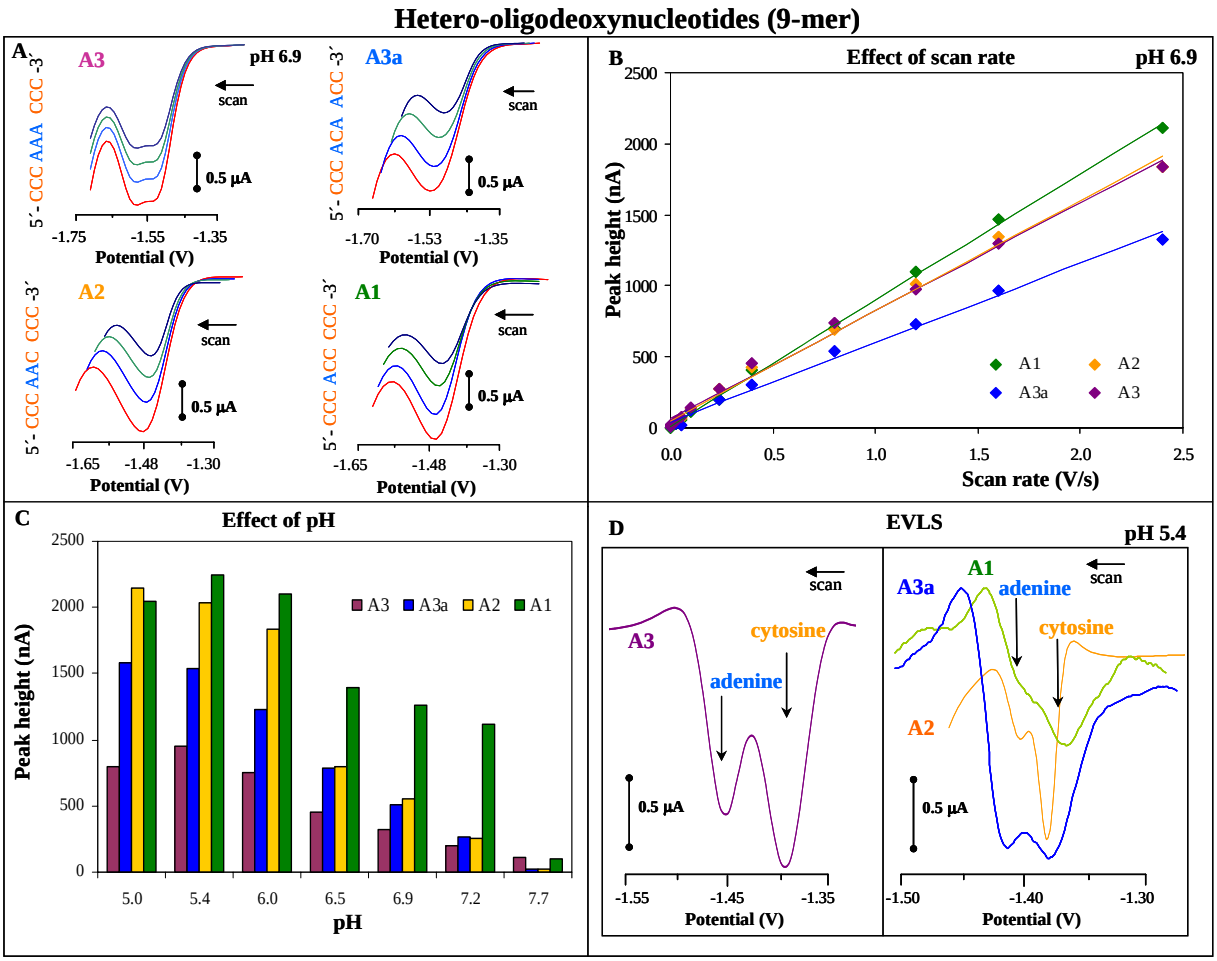

Figure 3. Analysis of hetero-oligodeoxynucleotides (9-mers): A3, A3a, A2 and A1. **A**) Voltammograms of ODNs (1µM) in the presence of 0.3 M ammonium formate and 0.05 M phosphate buffer (pH 6.9) at different scan rates (200, 400, 800, and 1 600 mV/s). **B**) Dependence of ODNs current responses on scan rate. **C**) Influence of pH (0.1 M phosphate buffer) on heights of ODNs signals. **D**) Elimination voltammograms of ODNs suitable for the function eliminating kinetic and charging currents, and conserving the diffusion current (reference scan rate of 800 mV/s, pH 5.4) For other details see Fig. 2.

While A3a, A2 and A1 yielded only one common peak corresponding to the reduction of A and C, the A3 provided a wide signal with a small plateau indicating the presence of two reduction processes. In addition, we studied the dependence of LSV ODNs signals on different scan rates (5, 10, 25, 50, 100, 200, 400, 800, 1200, 1600 and 2400 mV/s)(Fig.3B). The plots of peak height vs. scan rate were linear for all four hetero-ODNs (1μM), and ODNs peaks shifted to more negative potentials with increasing scan rate (not shown).

It is known that due to the protonization of A and C [49] pH values of the supporting electrolyte markedly influences the electrochemical behaviour of ODNs. The study of the effect of pH on the height of ODNs signals (Fig 3C) showed that the highest current responses were observed at lower pH values (5.0 and 5.4). Moreover, the highest current response was observed for A1 at all studied pH values except the lowest pH, where the A2 provided the highest response. In the range of pH from 6.5 to 7.7 the heights of A3a and A2 signals are almost similar. From these results it follows that adenine was responsible for the observed changes in the peak heights. The peak potentials shifted with increasing pH to more negative potentials, similarly as in the case of homo-ODNs.

The elimination voltammograms of these 9-mers are shown in Fig. 3D. At pH 5.4 two well-developed and separated signals of A and C were observed. With increasing number of A in the ODN chain the EVLS adenine signals increased from A1 (11%) to A3a (33%). The best resolution between signals of adenine and cytosine were observed for ODN A3 (three successive adenines in the middle of ODN chain).

Table 1. Characterization of ODNs (20-mers) by the ratio of A:C in ODNs (pH 5.4).

ODNs	Content of adenine	Content of cytosine	True Ratio value[1]	EVLS experimental Ratio value[2]	Difference between experimental and true Ratio value[3]	Difference between experimental and true Ratio value (%)[4]
ODN(20a)	3	9	0.33	0.39	0.06	18
ODN(20b)	5	1	5	4.65	-0.35	7
ODN(20c)	7	1	7	6.34	-0.66	1

[1] … True ratio value = Content of adenine/Content of cytosine

[2] … EVLS experimental Ratio value = EVLS adenine peak height/EVLS cytosine peak height

[3] … Difference between true and experimental Ratio value = EVLS experimental Ratio value - True ratio value

[4] … Difference between experimental and True Ratio value (%) = (|Difference between true and experimental Ratio value|*100)/True ratio value

3.3. Analysis of hetero-oligodeoxynucleotides (20-mers)

In order to test the possibility to resolve the reduction signals of A and C in hetero-ODNs with different nucleic bases including guanine, thymine and inosine, the EVLS was applied. Voltammograms of ODNs (20a), (20b) and (20c), which were analysed in the presence of 0.3 M ammonium formate and 0.05 M phosphate buffer (pH 6.9), are shown in Fig. 4A.

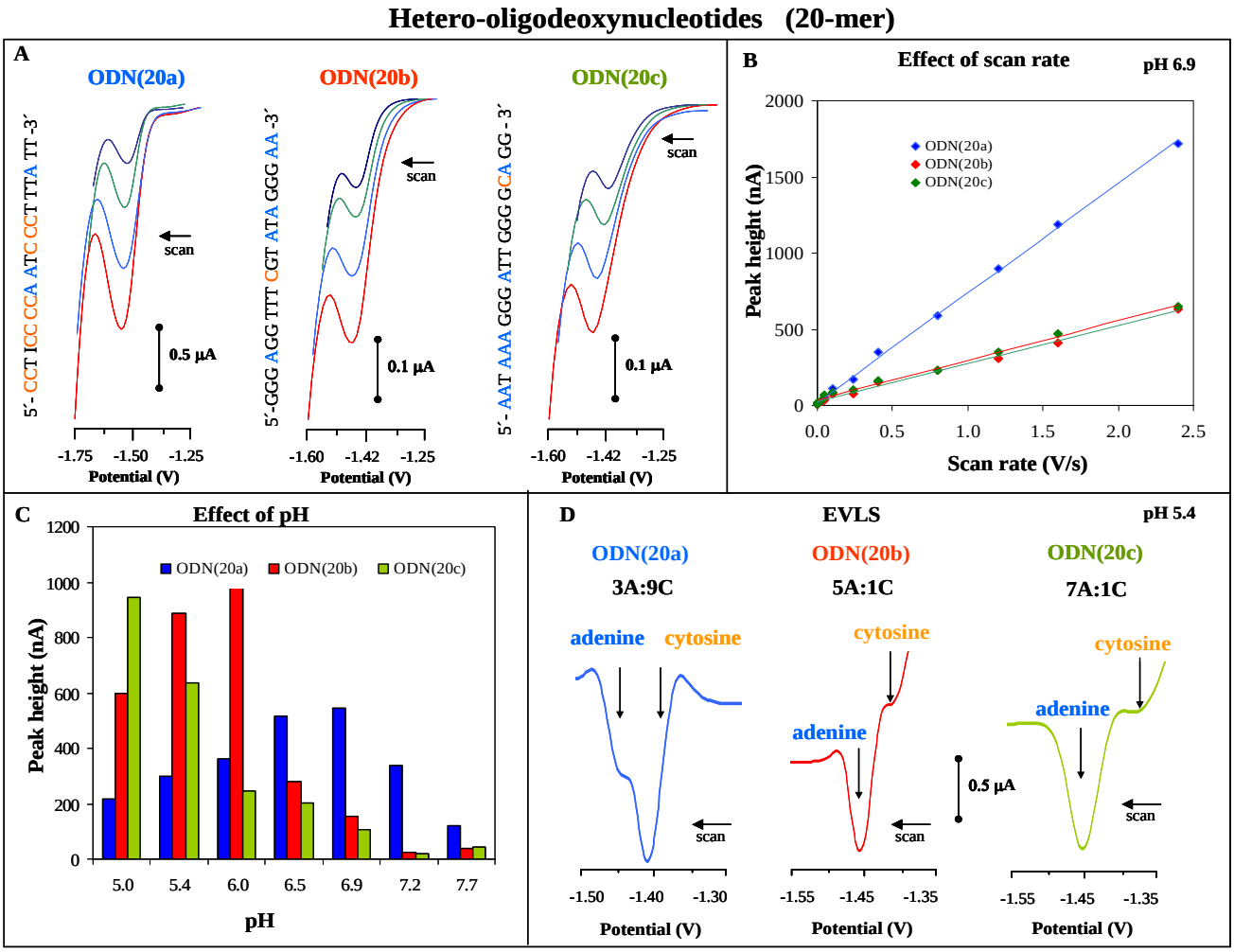

Figure 4. Analysis of hetero-oligodeoxynucleotides (20-mers): ODN(20a), ODN(20b), and ODN(20c) with the A:C ratio the in primary structure of ODNs of 3:9, 5:1, and 7:1, respectively. **A**) Voltammograms of ODNs (1µM) in the presence of 0.3 M ammonium formate and 0.05 M phosphate buffer (pH 6.9) at different scan rates (200, 400, 800 and 1 600 mV/s). **B**) Dependence of ODNs current responses on scan rate. **C**) Influence of pH (0.1 M phosphate buffer) on the height of ODNs signals. **D**) Elimination voltammograms of ODNs suitable for the function eliminating kinetic and charging currents, and conserving the diffusion current (reference scan rate of 800 mV/s, pH 5.4). For other details see Fig. 2.

From the dependence of LSV signals on different scan rates (5, 10, 25, 50, 100, 200, 400, 800, 1 400 and 2 400 mV/s) it follows that dependences were linear at two different ODNs concentrations (1 and 0.5 μM) with R^2 values close to 0.99. In Fig 4B the plot of peak height versus scan rate is shown for these ODNs (1 μM) at pH 6.9. All observed ODNs signals shifted to more negative potentials with increasing scan rate. It was found that the ODNs with higher content of adenine – ODN(20b) and ODN (20c) gave the highest electrochemical response at pH 5 - 5.5. While their signals markedly decreased with increasing pH, the signal of ODN (20a), containing the highest number of C residues of all studied hetero-ODNs, increased and reached the maximum at pH 6.9 (Fig 4C). We were able to observe the change of one nucleotide in the primary ODNs structure and to estimate the content of A and C in the analysed ODNs by the technique used (Fig. 4D).

It was possible to determine the ratio between the number of A and C in the primary DNA structure (Tab.1).

It followed from these results that the precision of A/C ratio determination was higher as the contain of A increased. The minimal concentration of ODN required for the determination of the A/C ratio was different and dependent strongly on the sequences of A and C. The EVLS detection limits of ODNs are shown in the Table 2.

Table 2. EVLS detection limits of ODNs.

ODNs	LD[a] (nM)
A9	0.55
C9	1.6
A3	1.2
A3a	0.24
A2	0.19
A1	0.18
ODN (20a)	0.47
ODN (20b)	0.47
ODN (20c)	0.56

[a] … Limit of detection LD was expressed as 3 S/N ratio.

Conclusion

Electrochemical analysis of DNA still represents a suitable tool for the design of simple, dependable and low cost devices. In the paper we suggested a new, original detection method for DNA biosensors consisting in the combination of adsorptive transfer stripping (AdTS) technique and elimination voltammetry with linear scan (EVLS). We were able to analyze the primary DNA structure, to resolve changes in the primary DNA structure at one nucleotide level, and to determine the ratio between content of A and C (A/C). This was demonstrated in experiments where (i) ODNs (5 μl) were adsorbed (120 s) on a surface of the working electrode (HMDE), (ii) low-molecular and non-adsorbed molecules were removed by rinsing the surface with distilled water and supporting electrolyte, (iii) the ODN modified electrode was placed into electrochemical cell containing phosphate buffer at various pH. The linear sweep voltammograms measured at suitable pH were treated by EVLS procedure.

The AdTS EVLS offers a new tool for very good resolution of A and C in ODNs, and for a qualitative and quantitative sensing device for changes in the primary structure of oligodeoxynucleotide chains. It can be expected that in case of identical number of reducible bases the technique will even make it possible to distinguish between neighbouring and non- neighbouring bases.

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References

1. Watson, J.D.; Crick, F.H.C. A Structure for Deoxyribose Nucleic Acid. *Nature* **1953**, *171*, 737-738.

2. Hartley, J.A.; Souhami, R.L.; Berardini, M.D. Electrophoretic and chromatographic separation methods used to reveal interstand cross-linking of nucleic acids. *J. Chromatogr .Biomed.Appl.* **1993**, *618*, 277-288.

3. Townsend, K.M.; Dawkins, H.J.S. Field alternation gel electrophoresis-status quo. *J. Chromatogr. Biomed. Appl.* **1993**, *618*, 223-249.

4. Guttman, A.; Ronai, Z. Ultrathin-layer gel electrophoresis of biopolymers. *Electrophoresis* **2000**, *21*, 3952-3964.

5. Brody, J.R.; Kern, S.E. History and principles of conductive media for standard DNA electrophoresis. *Anal. Biochem.* **2004**, *333*, 1-13.

6. Palecek, E. Oszillographiche Polarographie der Nucleinsauren und ihrer Bestandteile. *Naturwiss.* **1958**, *45*, 186-187.

7. Palecek, E. Oscillographic polarography of highly polymerized deoxyribonucleic acid. *Nature* **1960**, *188*, 656-657.

8. Palecek, E. New trends in electrochemical analysis of nucleic acids. *Bioelectrochem. Bioenerg.* **1988**, *20*, 179-194.

9. Palecek, E. From polarography of DNA to microanalysis with nucleic acid-modified electrodes. *Electroanalysis* **1996**, *8*, 7-14.

10. Palecek, E.; Fojta, M. Detecting DNA hybridization and damage. *Anal.Chem.* **2001**, *73*, 74A-83A.

11. Palecek, E. Past, present and future of nucleic acids electrochemistry. *Talanta* **2002**, *56*, 809-819.

12. Palecek, E.; Jelen, F. Electrochemistry of nucleic acids and development of DNA sensors. *Crit. Rev. Anal. Chem.* **2002**, *32*, 261-270.

13. Palecek, E.; Fojta, M.; Jelen, F.; Vetterl, V. Electrochemical analysis of nucleic acids. In *The Encyclopedia of Electrochemistry;* Vol. 9 Bioelectrochemistry; Bard, A. J., Stratsmann, M. Eds.; Wiley-VCH: Weinheim, 2002, pp. 365-429.

14. Palecek, E.; Billova, S.; Havran, L.; Kizek, R.; Miculkova, A.; Jelen, F. DNA hybridization at microbeads with cathodic stripping voltammetric detection. *Talanta* **2002**, *56*, 919-930.

15. Fojta, M.; Havran, L.; Kizek, R.; Billova, S. Voltammetric microanalysis of DNA adducts with osmium tetroxide,2,2 '-bipyridine using a pyrolytic graphite electrode. *Talanta* **2002**, *56*, 867-874.

16. Fojta, M.; Havran, L.; Billova, S.; Kostecka, P.; Masarik, M.; Kizek, R. Two-surface strategy in electrochemical DNA hybridization assays: Detection of osmium-labeled target DNA at carbon electrodes. *Electroanalysis* **2003**, *15*, 431-440.

17. Fojta, M. Electrochemical sensors for DNA interactions and damage. *Electroanalysis* **2002**, *14*, 1449-1463.

18. Fojta, M.; Havran, L.; Kizek, R.; Billova, S.; Palecek, E. Multiply osmium-labeled reporter probes for electrochemical DNA hybridization assays: detection of trinucleotide repeats. *Biosens. Bioelectron.* **2004**, *20*, 985-994.

19. Wang, J. Carbon-Nanotube Based Electrochemical Biosensors: A Review. *Electroanalysis* **2005**, *17*, 7-14.

20.Drummond, T.G.; Hill, M.G.; Barton, J.K. Electrochemical DNA sensors. *Nature Biotech.* **2003**, *21*, 1192-1199.

21. Masarik, M.; Kizek, R.; Kramer, K.J.; Billova, S.; Brazdova, M.; Vacek, J.; Bailey, M.; Jelen, F.; Howard, J.A. Application of avidin-biotin technology and adsorptive transfer stripping square-wave voltammetry for detection of DNA hybridization and avidin in transgenic avidin maize. *Anal.Chem.* **2003**, *75*, 2663-2669.

22. Palecek, E.; Fojta, M.; Tomschik, M.; Wang, J. Electrochemical biosensors for DNA hybridization and DNA damage. *Biosens. Bioelectron.* **1998**, *13*, 621-628.

23. Palecek, E.; Postbieglova, I. Adsorptive stripping voltammetry of biomacromolecules with transfer of the adsorbed layer. *J. Electroanal. Chem.* **1986**, *1986*, 359-371.

24. Gooding, J.J. Electrochemical DNA hybridization biosensors. *Electroanalysis* **2002**, *14*, 149-156.

25. Wang, J. Electrochemical nucleic acid biosensors. *Anal. Chim. Acta*. **2002**, *469*, 63-71.

26. Wang, J.; Rivas, G.; Cai, X.; Palecek, E.; Nielsen, P.; Shiraishi, H.; Dontha, N.; Luo, D.; Parrado, C.; Chicharro, M.; Farias, P.A.M.; Valera, F.S.; Grant, D.H.; Ozsoz, M.; Flair, M.N. DNA electrochemical biosensors for environmental monitoring. A review. *Anal.Chim. Acta* **1997**, *347*, 1-8.

27. Wang, J.; Cai, X.H.; Rivas, G.; Shiraishi, H.; Farias, P.A.M.; Dontha, N. DNA electrochemical biosensor for the detection of short DNA sequences related to the human immunodeficiency virus. *Anal. Chem.* **1996**, *68*, 2629-2634.

28. Thorp, H.H. Cutting out the middleman: DNA biosensors based on electrochemical oxidation. *Trends Biotechnol.* **1998**, *16*, 117-121.

29. Thorp, H.H. Reagentless detection of DNA sequences on chemically modified electrodes. *Trends Biotechnol.* **2003**, *21*, 522-524.

30. Kizek, R.; Havran, L.; Kubicarova, T.; Yosypchuk, B.; Heyrovsky, M. Voltammetry of two single-stranded isomeric end-labeled -SH deoxyoligonucleotides on mercury electrodes. *Talanta* **2002**, *56*, 915-918.

31. Gupta, N.; Grover, N.; Neyhart, G.A.; Liang, W.G.; Singh, P.; Thorp, H.H. $[RuO(DPPZ)(TPY)]^{2+}$ - A DNA cleavage agent with high DNA affinity. *Angew. Chem. Int. Edit.* **1992**, *31*, 1048-1050.

32. Havran, L.; Fojta, M.; Palecek, E. Voltammetric behavior of DNA modified with osmium tetroxide 2,2 '-bipyridine at mercury electrodes. *Bioelectrochem.* **2004**, *63*, 239-243.

33. Kizek, R.; Havran, L.; Fojta, M.; Palecek, E. Determination of nanogram quantities of osmium-labeled single stranded DNA by differential pulse stripping voltammetry. *Bioelectrochem.* **2002**, *55*, 119-121.

34. Kawde, A.N.; Wang, J. Amplified electrical transduction of DNA hybridization based on polymeric beads loaded with multiple gold nanoparticle tags. *Electroanalysis* **2004**, *16*, 101-107.

35. Wang, J.; Polsky, R.; Merkoci, A.; Turner, K.L. "Electroactive beads" for ultrasensitive DNA detection. *Langmuir* **2003**, *19*, 989-991.

36. Fojta, M.; Havran, L.; Vojtiskova, M.; Palecek, E. Electrochemical detection of DNA triplet repeat expansion. *J. Am. Chem. Soc.* **2004**, *126*, 6532-6533.

37. Jelen, F.; Kourilova, A.; Pecinka, P.; Palecek, E. Microanalysis of DNA by stripping transfer voltammetry. *Bioelectrochem.* **2004**, *63*, 249-252.

38. Jelen, F.; Yosypchuk, B.; Kourilova, A.; Novotny, L.; Palecek, E. Label-free determination of picogram quantities of DNA by stripping voltammetry with solid copper amalgam or mercury electrodes in the presence of copper. *Anal. Chem.* **2002**, *74*, 4788-4793.

39. Trnkova, L.; Kizek, R.; Dracka, O. Application of elimination voltammetry to adsorptive stripping of DNA. *Electroanalysis* **2000**, *12*, 905-911.

40. Trnkova, L. Electrochemical behavior of DNA at a silver electrode studied by cyclic and elimination voltammetry. *Talanta* **2002**, *56*, 887-894.

41. Trnkova, L.; Jelen, F.; Postbieglova, I. Application of elimination voltammetry to the resolution of adenine and cytosine signals in oligonucleotides. I. Homooligodeoxynucleotides dA(9) and dC(9). *Electroanalysis* **2003**, *15*, 1529-1535.

42. Trnkova, L.; Kizek, R.; Vacek, J. Square wave and elimination voltammetric analysis of azidothymidine in the presence of oligonucleotides and chromosomal DNA. *Bioelectrochem.* **2004**, *63*, 31-36.

43. Trnkova, L.; Postbieglova, I.; Holik, M. Electroanalytical determination of d(GCGAAGC) hairpin. *Bioelectrochem.* **2004**, *63*, 25-30.

44. Dracka, O. Theory of current elimination in linear scan voltammetry. *J. Electroanal. Chem.* **1996**, *402*, 19-28.

45. Trnkova, L.; Dracka, O. Elimination voltammetry. Experimental verification and extension of theoretical results. *J. Electroanal. Chem.* **1996**, *413*, 123-129.

46. Trnkova, L. Electrochemical elimination methods. *Chem. Listy* **2001**, *95*, 518-527.

47. Trnkova, L.; Kizek, R.; Dracka, O. Elimination voltammetry of nucleic acids on silver electrodes. *Bioelectrochem.* **2002**, *55*, 131-133.

48. Trnkova, L.; Friml, J.; Dracka, O. Elimination voltammetry of adenine and cytosine mixtures. *Bioelectrochem.* **2001**, *54*, 131-136.

49. Dryhurst, G. *Electrochemistry of biological molecules;* Academic Press: New York, 1977.