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Electrochemical impedance spectroscopy of polynucleotide adsorption

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Abstract

The dependence of the impedance of the electrode double layer of mercury electrode on frequency around the potentials of the tensammetric peaks of single-stranded and double-helical polynucleotides and DNA was studied. From the frequency dependence of the impedance of the electrode double layer represented in a complex plane impedance plot, the electric equivalent circuit of the electrode covered with adsorbed DNA layer was determined. It was concluded that the desorption of denatured ssDNA is accompanied by higher dielectric losses than the desorption of native dsDNA. This can be explained by the higher flexibility of ssDNA compared to the dsDNA. The capacitance peak of single-stranded polyadenylic acid (poly A) observed at pH 8 around − 1.3 V splits at low frequencies in two peaks.

Keywords: Electrochemical impedance spectroscopy; DNA adsorption; Poly A adsorption; Differential capacitance

1. Introduction

Since 1961 when Miller [1] has published his work on differential capacitance of the mercury electrode double layer in the solutions of nucleic acids, the measurement of the impedance of electrified interfaces started to be widely used for investigation of the interactions of nucleic acids and their components with electrode surface [2–11]. Differential capacitance C of the electrode double layer is a sensitive indicator of adsorption.

Usually, the dependence of C on electrode potential U (C–U curves) or the dependence of the electrode double layer impedance **Z** on frequency (electrochemical impedance spectroscopy, EIS) is measured.

From the measurement of C–U curves, we have found that bases, nuclesides and nucleotides can form by a two-dimensional condensation on a compact, self-assembled layer at the mercury electrode surface [2–7,11]. On the C–U curves of DNA and synthetic polynucleotides, adsorption–desorption (tensammetric) peaks are observed [2,7–9]. The native double helical DNA (double stranded, dsDNA) usually yields just only one tensammetric peak at about − 1.1 V corresponding to the desorption of the sugar/phospahte backbone and denoted as peak 1 [9]. The denatured DNA (single stranded, ssDNA) yields besides peak 1 another tensammetric (peak 3) at about − 1.4 to − 1.6 V corresponding to the desorption of bases. If dsDNA contains some distorted regions in which hydrophobic bases can come into contact with the electrode, another tensammetric (peak 2) appears between peaks 1 and 3 [7–10].

Polyadenylic acid (poly A) may have (depending on pH and ionic strength) either ss or ds form [12,13]. We have found that at the frequency of 22 Hz the peak observed on C–U curves around − 1.4 V of the ss poly A (pH 8, 0.1 M NaCl) is higher and more asymetric than the peak of the ds poly A (pH 5, 0.1 M NaCl).

With increasing frequency the height of the tensammetric peaks decreases. The decrease is more pronounced with the ssDNA and ss poly A than with the double stranded conformations [14].

Frequency dependence of the impedance of the electrode double layer (electrochemical impedance spectroscopy, EIS) [15–17] yields useful information about the adsorption kinetic and mobility of the adsorbed polynucleotide segments. The impedance measurements performed with DNA solutions earlier [17] were at low frequencies disturbed by noise, the impedance values were scattered and the difference between impedance spectra of ssDNA and dsDNA were not quite convincing.

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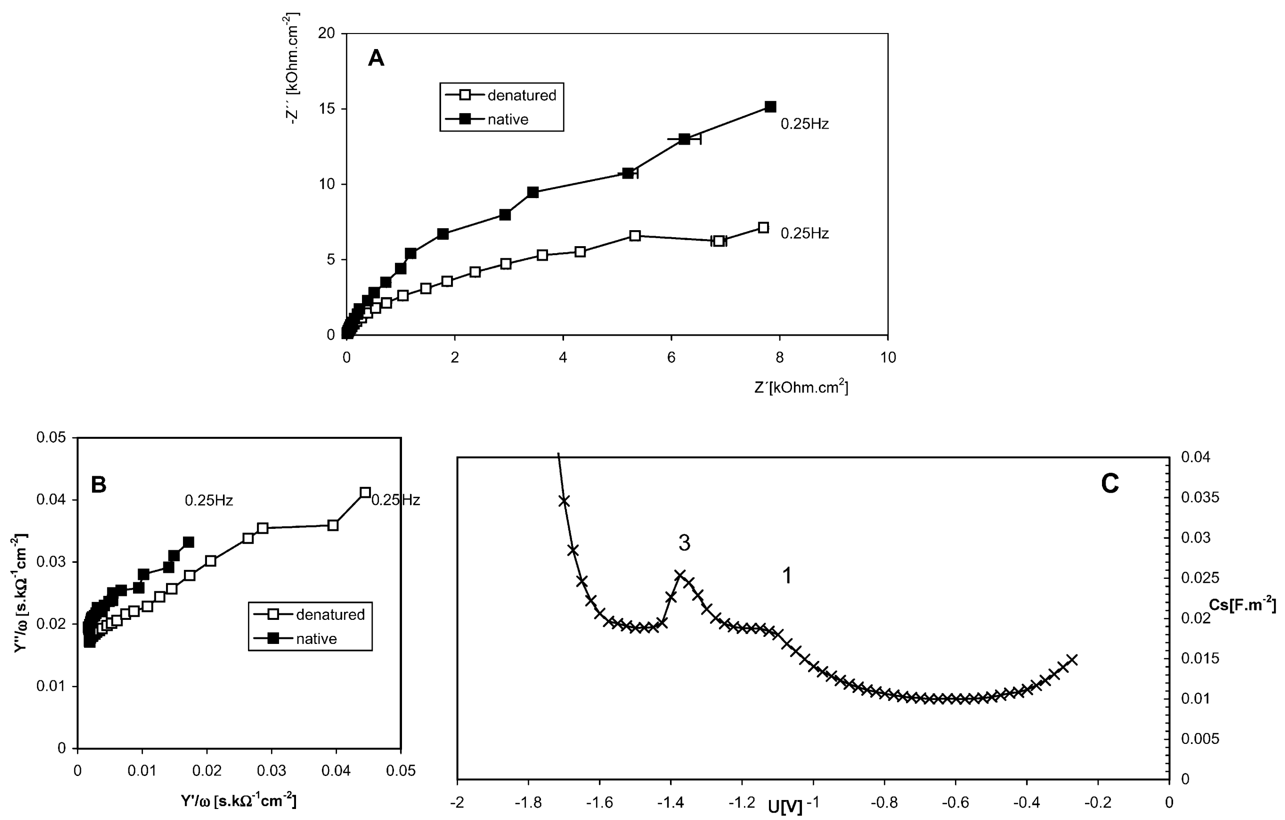


Fig. 1. (A) Complex impedance $\mathbf{Z} = \mathbf{Z}' + iZ''$ plot of 180 μg/ml native and denatured calf thymus DNA in 0.3 M NaCl + 0.05 M Na_2HPO_4, pH 8.5, measured at − 1.14 V. Frequency range 0.25 – 100 Hz in. . .steps. Temperature 20 °C. Waiting time $t_1 = 1200$ s at − 0.6 V. (B) Complex capacitance $\mathbf{Y}/\omega = Y'/\omega + iY''/\omega$ plot of the same sample. (C) $C–U$ curves of 180 μg/ml denatured calf thymus DNA in 0.3 M NaCl + 0.05 M Na_2HPO_4, pH 8.5. Temperature 20 °C.

In this paper, we have studied the adsorption behaviour of ssDNA and dsDNA by impedance measurements in order to find out if the difference in impedance parameters between ssDNA and dsDNA are statistically significant and can be used for sensing hybridization by DNA biosensors.

2. Experimental

Calf thymus DNA isolation, thermal denaturation and determination of DNA concentration were carried out as previously described in Ref. [18]. Polyadenylic acid, potas-

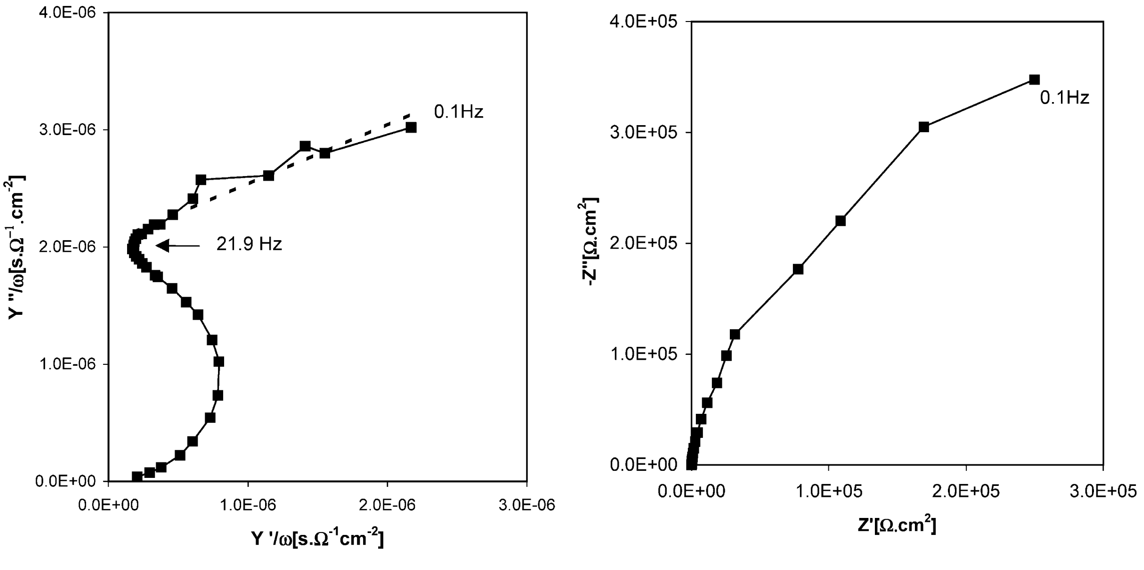


Fig. 2. Complex impedance $\mathbf{Z} = \mathbf{Z}' + iZ''$ and complex capacitance $\mathbf{Y}/\omega = Y'/\omega + iY''/\omega$ plots of 200 μg/ml denatured calf thymus DNA in 0.3 M NaCl + 0.05 M Na_2HPO_4, pH 8.5, measured at − 1.16 V. Temperature 20 °C. Waiting time $t_1 = 10$ s at − 0.6 V. Frequency range 0.1 – 50 000 Hz in 38 frequency steps.

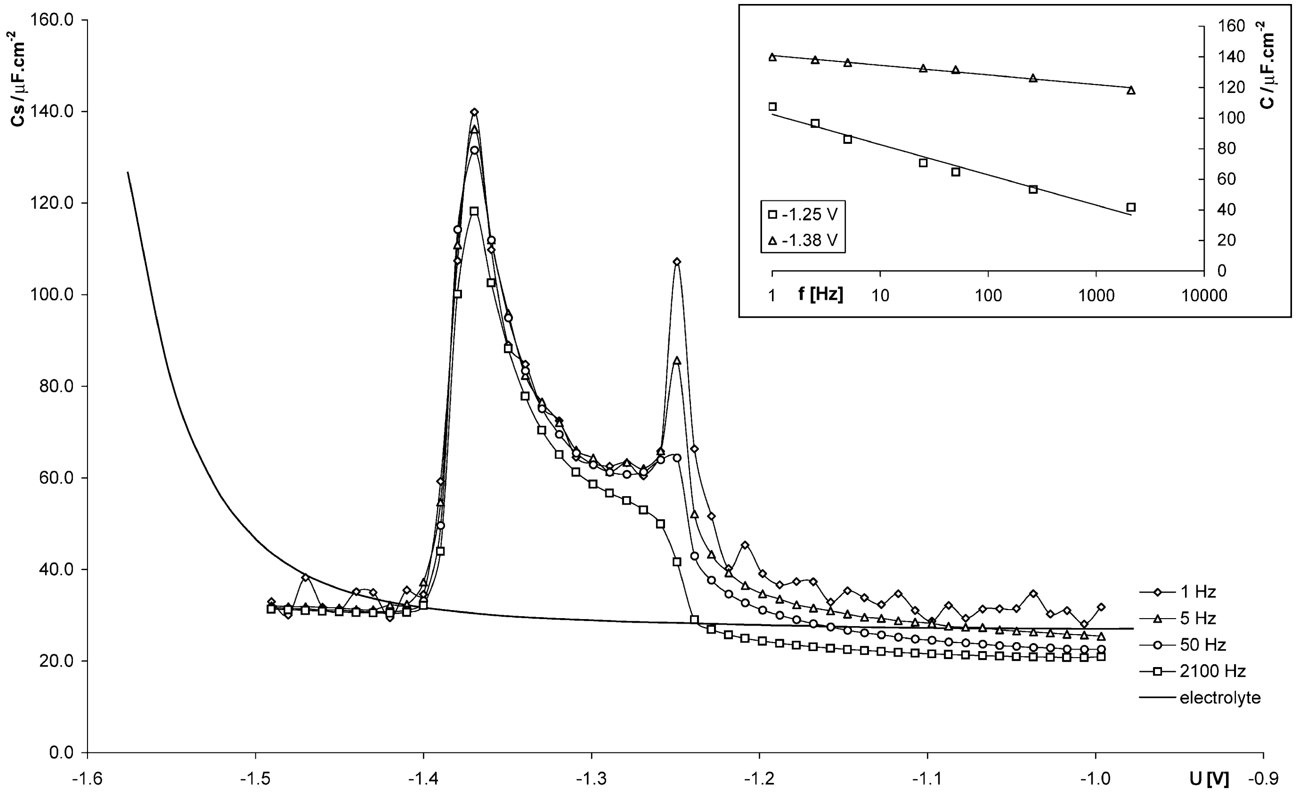


Fig. 3. $C-U$ curves of 180 μg/ml poly A in 0.3 M NaCl + 0.05 M Na_2HPO_4, pH 8.5. Temperature 20 °C. Waiting time $t_1 = 30$s at − 0.6 V. Potential scan from − 1.0 to − 1.5 V with a potential step 0.1 V every 2 s (corresponding to the potential scan rate 50 mV s^{-1}). Insert shows the dependence of peaks at − 1.25 and − 1.38 V on frequency Frequencies: ◇ 1 Hz, Δ 5 Hz, O 50 Hz, □ 2100 Hz.

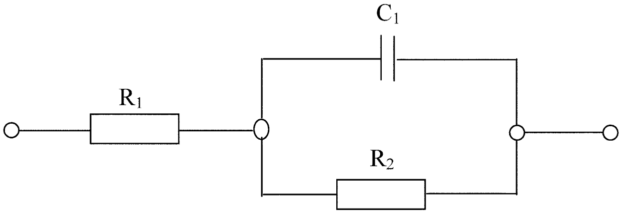
sium salt, was supplied by Sigma. Chemicals for preparation of the supporting electrolyte (NaCl, Na_2HPO_4 and NaH_2PO_4) were of analytical grade purchased from Aldrich. All the measurements were carried out in 0.3 M NaCl with phosphate buffer pH 4.5 (ds poly A) and pH 8.5 (ss poly A, DNA) at 20 °C, pH was measured by pH-meter OP208/1 Radelkis (Hungary). The solutions were deaerated using 99.5% argon saturated with triple distilled water. The water used was distilled in a glass electrodistillator followed by double distillation in quartz.

The measurement of the frequency (or potential) dependence of the impedance and/or admittance of the electrode double layer was performed with a hanging mercury drop electrode (HMDE) using Metrohm VA 663 Stand. The surface of the mercury drop calculated from the mercury flow rate by weighing of hundred droplets was $S=(0.33 \pm 0.01)$ mm^2 corresponding to position 2 on the Metrohm 663 VA Stand, and the diameter of the drop was $r = 0.16$ mm. Counter electrode was a Pt wire, reference electrode was Ag/AgCl in 3 M KCl.

The impedance of the electrode double layer of the mercury electrode was measured using AUTOLAB/FRA impedance measurement system with the potentiostat PGSTAT 20 and FRA-DSG, FRA-ADC modules (Eco Chemie, Utrecht, The Netherlands), the a.c. voltage amplitude was either 2 or 5 mV, the equilibration time (the time between a new potential and/or frequency step and actual impedance measurement) was 1 s ($C-U$ curves, Fig. 1C), 2 s ($C-U$ curves, Fig. 3) and 10 s (frequency dependence, Figs. 1A,B,C and 2). Before the impedance measurements the electrode was kept at − 0.6 V (second conditioning potential) for 10 s (Fig. 2), 30 s (Fig. 3) or 1200 s (Fig. 1A,B,C).

3. Results and discussion

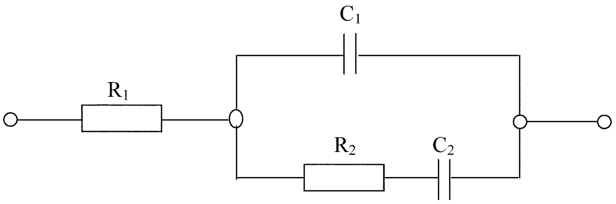
Fig. 1A shows the frequency dependence of the impedance of the electrode double layer $\mathbf{Z} = Z' + iZ''$ in the complex plane impedance plot. Impedance was measured in the solution of 180 μg/ml native and denatured calf thymus DNA in 0.3 M NaCl + 0.05 M Na_2HPO_4, pH 8.5 at the



Scheme 1. Equivalent circuit of the mercury–solution interface for a simple irreversible electrode reaction or for tensammetric process. C_1—double layer capacitance, R_1—solution resistance, R_2—charge transfer resistance (faradayic process) or resistance representing dielectric losses (tensammetric process — reorientation, relaxation).

Table 1
Parameters of the equivalent circuit (Scheme 1) simulating the complex impedance plot of DNA (Fig. 1)

DNA	E (V)	R_1 (Ω cm^2)	R_2 (kΩ cm^2)	C_1 (μF cm^{-2})	n
Denatured	− 1.14	100	154	2.2	0.976
Native	− 1.14	46	363	2.1	0.957



Scheme 2. Debye equivalent circuit. C_1—high frequency capacitance, (C_1+C_2)—low frequency capacitance, R_1—solution resistance, R_2—relaxation resistance [16].

potential of the tensammetric peak 1 (− 1.14 V) corresponding to the adsorption/desorption of sugar-phosphate backbone.

The complex impedance plots of DNA in Fig. 1A exhibited arc shapes. Providing that the mercury–solution interface can be simulated by a parallel combination of C_1 (double layer capacitance) and R_2 (representing dielectric losses by tensammetric processes and/or charge transfer resistance by redox reactions; lower R_2 means higher dielectric losses) in series with the supporting electrolyte resistance R_1 (Scheme 1), the complex impedance plot should be a semicircle [16,19,20,21] the radius of which is smaller with lower R_2 values. Providing that the arc in Fig. 1A can be regarded as a part of the semicircle representing by equivalent circuit shown in Scheme 1, we have evaluated the circuit parameters R_1, R_2 and C_1 (Table 1). It can be seen that denatured ssDNA has a smaller radius (lower R_2) than the native one. Thus, desorption of denatured DNA is accompanied by a higher dielectric losses than the desoprtion of native dsDNA. Dielectric loss is energy lost as heat, which arises due to the friction of charged DNA segments forced to move in a viscous solvent by a.c. electric field. Higher dielectric losses accompanying the desorption of denatured ssDNA can be explained by the higher flexibility of denatured DNA compared to the dsDNA resulting in the faster movement of adsorbed DNA segments by their desorption from the electrode surface.

The **Z** values given in Fig. 1A are the mean values calculated from four measurements. The confidence interval is shown by a length of abscissa at the corresponding point and was at 0.25 Hz higher than at 98.6 Hz (Table 2). Differences between parameters of the electric equivalent circuits corresponding to native and denatured DNA are statistically significant.

Another model of the interface at which adsorption–desorption process takes place is a Debye equivalent circuit [16] (Scheme 2), where C_1 is the high frequency capacitance, C_1+C_2 is the low frequency capacitance, R_1 is the solution resistance and R_2 is the charge transfer resistance. In the case of redox reactions, the mass transfer Warburg term W appears instead of R_2 [15]. In complex capacitance plot (Y''/ω versus Y'/ω), such a circuit is represented at low frequencies by a straight line and at high frequencies by a circle above the vertical Y''/ω axis crossing the vertical axis at the capacitance values C_1+C_2 and C_1 [15]. Fig. 1B is the complex capacitance plot of native and denatured DNA measured in the same low frequency range 0.25–100 Hz as impedance plots shown in Fig. 1A and thus only the low frequency linear part of the complex capacitance spectrum is presented. Fig. 2 shows the frequency dependence of the complex capacitance plot of denatured DNA measured in the frequency range 0.1–50 000 Hz. In this plot, the high frequency circular part of the complex capacitance plot can be seen.

Table 2
The mean values of the electrode double layer impedance and the confidence interval for native and denatured DNA at 0.25 and 98.6 Hz

DNA	f (Hz)	$\mathbf{Z}'$ (Ω)	Z'' (Ω)
Denatured	0.25	7.7 ± 0.1	7.13 ± 0.06
Native	0.25	7.8 ± 0.5	15.1 ± 0.3
Denatured	98.6	$(1.020 \pm 0.007) \times 10^{-2}$	$(9.34 \pm 0.05) \times 10^{-2}$
Native	98.6	$(8.16 \pm 0.01) \times 10^{-3}$	$(8.440 \pm 0.006) \times 10^{-2}$

The capacitance peak of single-stranded poly A observed on $C-U$ curves around the potential of − 1.3 to − 1.4 V is asymmetrical and at low frequencies splits in two peaks (Fig. 3). The height of the more negative peak at − 1.38 V decreases with increasing frequency, less strongly than the height of the peak at − 1.25 V. It means that the adsorption–desorption process giving rise to the tensammetric peak at − 1.25 V is slower than the adsorption–desorption process giving rise to the more negative peak. Thus, the peak at − 1.25 V obviously results from the reorientation and/or desorption of the sugar-phosphate backbone, and at potentials more negative then − 1.25V is poly A adsorbed at the Hg surface only via bases. The peak at − 1.38 V corresponds to the complete desorption of poly A from the Hg surface.

4. Conclusion

We have shown that electrochemical impedance spectroscopy is a useful tool for sensing differences between single-stranded and double-helical conformation of nucleic acids and synthetic polynucleotides.

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