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Biophysical Chemistry 112 (2004) 59–67

Biophysical Chemistry

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The binding of zinc (II) to a double-stranded oligodeoxyribonucleotide. A voltammetric study

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Received 21 May 2004; received in revised form 29 June 2004; accepted 2 July 2004
Available online 1 September 2004

Abstract

Binding of zinc to a 19 mer double-stranded oligodeoxyribonucleotide was investigated by anodic stripping voltammetry and cyclic voltammetry in order to understand the roles of zinc in DNA cleavage catalyzed by mung bean nuclease. These methods rely on the direct monitoring of zinc oxidation current in the absence and in the presence of the oligo. Zinc titration curves with the ds-oligodeoxyribonucleotide were obtained in concentrations ranging from 3.62×10^{-9} to 3.62×10^{-8} M and 4.06×10^{-10} to 5.25×10^{-9} M. The acquired data were used to determine the dissociation constant, stoichiometry and zinc binding sites of the complex and to understand the specific changes of ds-oligodeoxyribonucleotide secondary structure by zinc binding. The oxidation–reduction process of zinc was also investigated by cyclic voltammetry through I (oxidation current) versus $v^{1/2}$ (square root of scan rate) curves in the absence and in the presence of the double-stranded oligodeoxyribonucleotide.

Keywords: Anodic stripping voltammetry; Cyclic voltammetry; Zinc; Double-stranded oligodeoxyribonucleotide

1. Introduction

Since the beginning of 1960s, electrochemical methods have been used to study nucleic acids and their components [1–17]. These studies have been particularly directed towards DNA behavior at electrolyte/Hg electrode interface since the charged mercury electrode has been found to be a very good model to simulate the DNA interaction with highly charged membranes and proteins. Nucleic acids are electroactive species that can be reduced and/or oxidized at various electrodes. At mercury electrodes, DNA produces a cathodic peak due to the reduction of cytosine and adenine residues and an anodic peak due to the reoxidation of the reduced guanine moiety [1,2,4,7]. At solid electrodes (glassy carbon, graphite, gold), DNA produces two anodic peaks that were assigned to the oxidation of adenine and guanine residues [3,5,6]. These findings led to a deep understanding of DNA premelting and the polymorphism of the DNA double helix and the development of procedures for trace analysis of DNA [3,8,9]. Recent voltammetric research using DNA-modified electrodes or DNA biosensors has been extended to the detection of DNA damage, analysis and sequencing of specific sequences of DNA bases by hybridization techniques, and interactions between DNA and other molecules (drugs, metal ions) [3,10–17]. Alternating current, differential pulse, linear scan, cyclic, square wave, adsorptive stripping and adsorptive transfer stripping voltammetric techniques have been used in all of applications mentioned above [1–17].

Metal ions, especially Zn^{2+}, Mg^{2+} and Mn^{2+}, play an important role in many enzyme-catalyzed reactions involving nucleic acids, like DNA cleavage by zinc nucleases. Zinc, as an integral component of this class of enzymes, is probably involved in the binding site to the DNA molecule. The presence of Zn^{2+} ions is required for DNA cleavage and it has been suggested that zinc is responsible for a tight

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doi:10.1016/j.bpc.2004.07.003

binding between DNA and the enzyme. Unfortunately, it is often difficult to determine the actual molecular mechanism responsible for the observed effects, since zinc nucleases can act as mediators between the metal ion and nucleic acid in vivo. Before such complex systems can be understood, binding and conformation effects of the components have to be analyzed [18,19]. Thus, the properties of DNA metal ion complexes are of great interest. Mung Bean Nuclease [20] is a zinc metalloenzyme that catalyzes the hydrolysis of DNA and RNA. The detailed mechanism of DNA cleavage catalyzed by this zinc nuclease is currently of significant interest. One of the most important questions is the catalytic and structural roles of Zn^{2+} ions. While is clear that divalent ions play a major role in DNA cleavage, it is uncertain which function such cations have in cleavage and why two or three are needed in some cases and only one in others. A first step in understanding this reaction includes the investigation of the interaction between Zn^{2+} ions and DNA. Zinc-ion binding [21–27] to DNA has been studied by optical rotatory dispersion [23,24], NMR [22,28,29], gel electrophoresis [30–33], quantum chemical methods [34], UV–Vis spectroscopy [23,35,36], circular dichroism [36], ultra centrifugation [36], equilibrium dialysis [37], isotope dilution [37] and voltammetry [13,38] in order to understand specific changes in DNA secondary structure by Zn^{2+} ions binding. However, only electrochemical methods proved to be suitable to determine the quantitative parameters of Zn^{2+}-DNA complexes by studying the voltammetry of zinc in the absence and in the presence of DNA and noting shifts in standard potential caused by the interaction. Recently, the interaction of zinc ions with Lambda Phage DNA was studied by voltammetric techniques [39]. In this study, the stoichiometry and dissociation constant of the complex were successfully determined by the direct monitoring of the reduction and oxidation currents of zinc in the absence and in the presence of this virion DNA. However, with this lambda genome, it was not possible to make an accurate measurement of the number of zinc binding sites due to stereochemical problems caused by overfolding of such a large molecule under the experimental conditions used. Therefore, a smaller double-stranded DNA should be synthesized in order to make an accurate measurement of the quantitative data of Zn^{2+}-DNA system and to investigate the DNA cleavage catalyzed by a zinc nuclease using voltammetric techniques.

In this paper, we report the complex formation between a 19 mer double-stranded oligodeoxyribonucleotide and zinc ions using anodic stripping voltammetry and cyclic voltammetry. Zinc amperometric titrations were performed to determine the stoichiometry, dissociation constant and zinc binding sites of a Zn^{2+}-DNA complex and to understand the specific changes of ds-oligodeoxyribonucleotide secondary structure by zinc ions binding. These findings were employed to formulate a mechanism for DNA cleavage catalyzed by mung bean nuclease [40,41].

2. Experimental

The single-stranded oligodeoxyribonucleotides were purchased from Gibco and have 14 (MM=4.47×10^3 g mol^{-1}), 21 (MM=6.63×10^3 g mol^{-1}) and 31 (MM=1.04×10^4 g mol^{-1}) mer, respectively.

The 19 mer double-stranded oligodeoxyribonucleotide (5′ CGATCGACGTCGACGATTA 3′, MM=5.88×10^3 g mol^{-1}) was synthesized by the solid-phase phosphoramidite method [18] using the DNA/RNA synthesizer model 392 (Applied Biosystems) and reagents purchased from Perkin Elmer and Merck. The solid-phase phosphoramidite method [18] is based on the coupling reaction between a 5′-hydroxyl group of a support-bound deoxynucleoside and an alkyl (methyl or 2-cyanoethyl) 5′-DMtr-(*N*-acylated)-deoxynucleoside 3′-O-(*N*,*N*-diisopropylamino) phosphite. Five basic steps are involved in one cycle of nucleotide addition: detritylation (removal of dimethoxytrityl groups) by trichloroacetic acid in methylene chloride; activation of phosphoramidite by tetrazole in acetonitrile solution; addition of activated phosphoramidite to the growing chain; blocking of chains which are not reacted during coupling reaction (capping) and oxidation of the intermediate phosphite to the phosphotriester by iodine and water. Each strand of the 19 mer ds-oligodeoxyribonucleotide was obtained by repeating 18 times the cycle. Deprotection and release from the support was preformed using concentrated aqueous ammonia at 57 °C for 12 h. The purity of each strand was attested by MALDI-TOF/MS measurements using 3.70×10^{-6} M oligo 1 and 4.25×10^{-6} M oligo 2 solutions and Voyager-DE STR (Applied Biosystems) at the following settings: operation on linear mode, accelerating voltage 25,000 V, N_2 laser, pressure at the ion source 1.11×10^{-7} Torr, pressure at detector 8.90×10^{-9} Torr. About 3.59×10^{-9} M 3-hydroxypicolinic acid in 2.21×10^{-9} M ammonium citrate (8:1) was used as matrix. A total of 1.0 μl of the matrix-analyte mixture was applied onto the sample plate and allowed to dry at room temperature. Annealing of complementary strands of the ds-oligo was performed using the MJ research PTC-100 thermal cycler and a solution composed by 7.03×10^{-7} M oligo 1 and 5.65×10^{-7} M oligo 2 in 0.01 M pH 7.4 Tris–HCl buffer. The thermal cycler was programmed to incubate the solution at 95 °C for three minutes and 58 °C (Theoretical T_m value) for five minutes and then reduce the temperature to 23.5 °C at the end of the cycle.

λ phage DNA and the single and double-stranded oligodeoxyribonucleotides were exhaustively dialyzed against 0.01 M EDTA at 10 °C in order to remove zinc and other metals. The resulting solutions were submitted to an additional dialysis step against triply distilled water to remove all the EDTA and other salts. The concentration of λ phage DNA and single and double-stranded oligodeoxyribonucleotides was determined by UV measurements at 260 nm through the expressions 1.0 $A_{260\ nm}$ SS DNA corresponds to 33 μg/ml and 1.0 $A_{260\ nm}$ DS DNA

corresponds to 50 μg/ml [42] using the U-2001 UV–Vis double beam spectrometer (Hitachi). The purity (freedom from bound protein, carbohydrates and organic solvents) of λ phage DNA and all oligo samples was assessed from the ratio of the absorbances at 260 and 280 nm and at 260 and 230 nm [42].

The integrity of a double-stranded DNA in potassium nitrate solutions was investigated by electrophoresis at 0.8% agarose gel. Nine λ phage DNA samples were prepared in 1000, 100, 10 and 1 m M KNO_3 solutions. Ladder DNA (Marker) and one sample of λ phage DNA were prepared using a solution composed by TAE 1X and 0.1% ethidium bromide as a solvent. TAE 1X (40 m M Tris–acetate+1 m M EDTA) is a medium commonly used by molecular biologists to stock and manipulate DNA samples which does not cause any damage to the structure of DNA. The experiment was performed using a load buffer composed by 0.4% bromophenol blue, 0.4% xylene cyanol and 25% ficoll at room temperature and at 68 °C.

Electrodes were submitted to periodic cleaning with 20% HNO_3 (v/v) followed by a generous wash with triply distilled water in order to avoid measurement interference due to single and double-stranded oligos and zinc adsorption on the working surfaces of the electrode system. All experiments were carried out at room temperature and preceded by a gentle N_2 bubbling to prevent oxygen diffusion into the electrochemical cell (10 min for the supporting electrolyte −0.1 M KNO_3 and 3 min after each oligo addition).

The amperometric titration of zinc ions with the single-stranded oligodeoxyribonucleotides by cyclic voltammetry was carried out on a PAR 173 Potentiostat/Galvanostat connected to a PAR 175 Universal Programmer and a Houston X–Y recorder at the following settings: initial potential E_i=−0.2 V, switching potential E_w=−1.6 V and scan rate range 2 mV $s^{-1} \leq v \leq 100$ mV s^{-1}. A three electrode system with a hanging mercury electrode (Metrohm) as working electrode, a Ag/AgCl (3.0 M KCl) electrode as reference electrode and a platinum wire as the auxiliary electrode were used. Additions of 100 μl of each oligo to the electrochemical cell containing 30 μl or 130 μl of 1.53×10^{-2} M Zn^{2+} ions in 25 ml of 0.1 M KNO_3 were done.

The amperometric titration of zinc ions with the 19 mer double-stranded oligodeoxyribonucleotide by anodic stripping voltammetry (ASV) was carried out on a 646 Metrohm Voltammetric Analyzer Processor connected to a 647 Metrohm electrochemical cell composed of a hanging mercury electrode (working electrode), a Ag/AgCl (3.0 M KCl) electrode (reference electrode) and a platinum electrode (auxiliary electrode). All ASV measurements were performed in the potential range −1.15 V (initial potential, E_i) to 0.18 V (final potential, E_f) at the following settings: accumulation potential E_d=−1.15 V, accumulation time t_d=90 s, equilibration time t_e=20 s, surface area of the mercury drop 0.4 mm^2, pulse amplitude 50 mV and the scan rate v=10 mV s^{-1}. Additions of 2 μl of this ds-oligo to the electrochemical cell containing 6.01×10^{-8} M Zn^{2+} ions in 20 ml of 0.1 M KNO_3 were done.

The amperometric titration of zinc ions with the 19 mer double-stranded oligodeoxyribonucleotide by cyclic voltammetry (CV) was carried out on a PAR 174 polarographic system connected to a PAR 175 Universal Programmer and a Houston X–Y recorder at the following settings: initial potential E_i=−0.4 V, switching potential E_w=−1.4 V and scan rate range 2 mV $s^{-1} \leq v \leq 20$ mV s^{-1}. A three-electrode system with a hanging mercury electrode (Metrohm) as working electrode, a saturated calomel electrode as reference electrode and a platinum wire as the auxiliary electrode were used. Additions of 5 μl of this oligo to the electrochemical cell containing 6.07×10^{-9} M Zn^{2+} ions in 10 ml of 0.1 M KNO_3 were done.

The oxidation–reduction process of zinc was studied in the presence and in the absence of the single and double-stranded oligodeoxyribonucleotides by cyclic voltammetry through I (oxidation current of zinc) vs. $v^{1/2}$(square root of scan rate) curves. The oxidation current of zinc was measured in the scan rate range 2 mV $s^{-1} \leq v \leq 100$ mV s^{-1} with and without the addition of 30 μl of 9.96×10^{-6} M ARC5IIC2 ss-oligo, 100 μl of 5.10×10^{-6} M 3′AOX1 ss-oligo, 500 μl of 1.13×10^{-5} M PR1AECO ss-oligo or 10 μl of 8.13×10^{-7} M ds-oligo to the electrochemical cell containing 1.83×10^{-5} or 7.91×10^{-5} M Zn^{2+} ions in 25 ml of 0.1 M KNO_3 or 6.07×10^{-9} M Zn^{2+} ions in 10 ml of 0.1 M KNO_3, respectively.

3. Results and discussion

The interaction of zinc ions with three single-stranded oligodeoxyribonucleotides was studied by cyclic voltammetry [41,43,44] in order to determine optimal experimental parameters (DNA size, base sequence) for the synthesis of a model of double-stranded DNA (oligo).

ARC5IIC2 (MM = 4472 g mol^{-1}) is a single-stranded oligodeoxyribonucleotide 14 mer, whose sequence (ACACCCAACGGAGA) corresponds to part of the coding region of an arcelin gene, a protein of the lectin family, found in seeds of wild bean. The cyclic voltammograms of 30 μl of 1.53×10^{-2} M Zn^{2+} ions in 25 ml of 0.1 M KNO_3 with different concentrations of ARC5IIC2 single-stranded oligo is shown in Fig. 1. Curve 2 (Fig. 1) represents the oxidation of zinc in 0.1 M KNO_3. Curves numbered 3–7 (Fig. 1) show the decrease of the zinc oxidation current during incremental additions (100 μl) of 9.96×10^{-6} M ss-oligo. The resulting changes in this current demonstrate interaction between Zn^{2+} ions and ARC5IIC2. A peak was also observed around −1.3 V in the cyclic voltammograms of ARC5IIC2 (Fig. 1), which can be assigned to adenine and cytosine reduction [1,2,4,7]. The −1.3 V peak is characteristic of a single-stranded DNA because in this case adenine and cytosine are free to reduce at mercury electrode. The nature of this

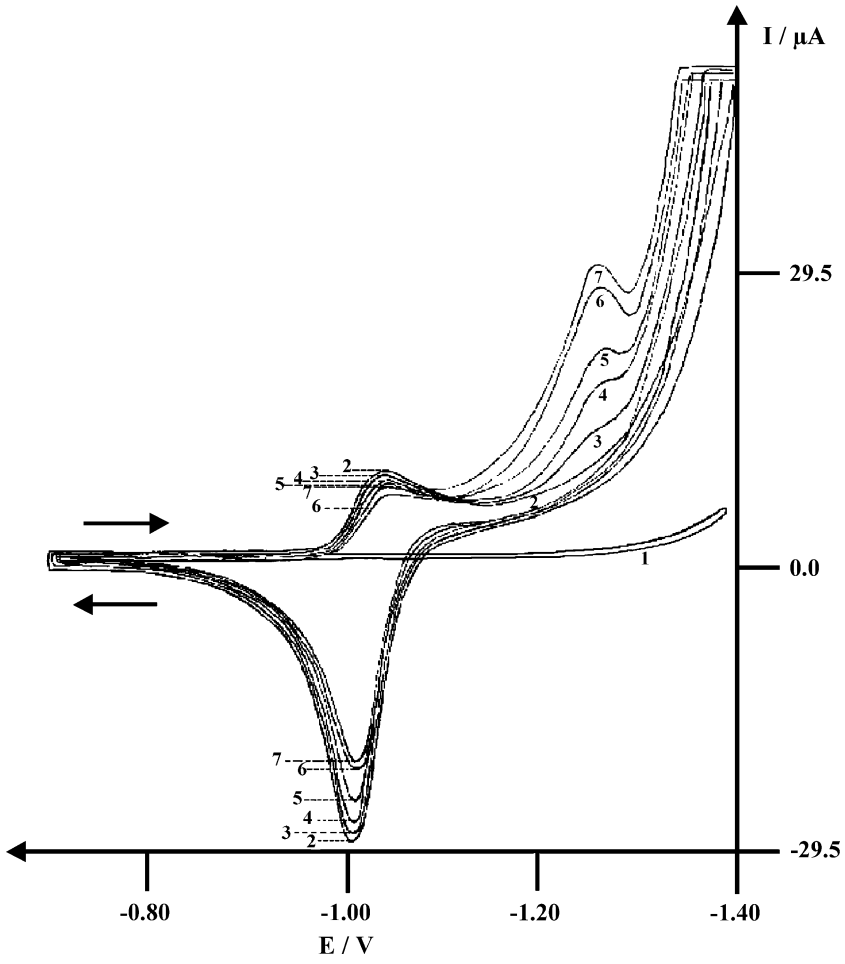

Fig. 1. Cyclic voltammograms of 30 µl of 1.53×10^{-2} M Zn^{2+} ions in 25 ml of 0.1 M KNO_3 with different concentrations of ARC5IIC2 single-stranded oligo. (1) KNO_3, no Zn^{2+} ions and ARC5IIC2 single-stranded oligo; (2) 1.83×10^{-5} M Zn^{2+} ions, no ARC5IIC2 single-stranded oligo; (3) Zn^{2+} ions + 3.96×10^{-8} M ARC5IIC2 single-stranded oligo; (4) Zn^{2+} ions + 7.90×10^{-8} M ARC5IIC2 single-stranded oligo; (5) Zn^{2+} ions + 1.18×10^{-7} M ARC5IIC2 single-stranded oligo; (6) Zn^{2+} ions + 1.57×10^{-7} M ARC5IIC2 single-stranded oligo; (7) Zn^{2+} ions + 1.95×10^{-7} M ARC5IIC2 single-stranded oligo;. E_i=−0.2 V, E_w=−1.6 V, scan rate=10 mV s^{-1}, working electrode: HMDE, reference electrode: Ag/AgCl (3 M KCl).

peak was also confirmed by an experiment in which a 31 mer ss-oligo (AAGAATTCGCIAAYCGTGYAAYCCIGCICAA) produced a cathodic peak near −1.4 V, at mercury electrode, in the absence of zinc.

As described by the Randles-Sevcik equation, the oxidation current of zinc varies linearly with $v^{1/2}$ in the absence of ARC5IIC2. A plot of this equation yields a straight line; the slope can be used to determine the diffusion coefficient of the system. However, in the presence of the ss-oligo the electrochemical behavior of zinc changed and its oxidation–reduction process was no longer controlled by diffusion [41,43,44].

Similar voltammetric experiments were carried out with a 21 (GCAAATGGCATTCTGACATCC) and a 31 mer (AAGAATTCGCIAAYCGTGYAAYCCIGCICAA) single-stranded oligodeoxyribonucleotides. The interaction of Zn^{2+} ions with these two ss-oligos was also confirmed by the decrease in the zinc oxidation current after incremental additions of each oligo. The reduction of adenine and cytosine residues was also verified by the appearance of the cathodic peak near −1.3 V after the addition of each ss-oligo. After these experiments, we concluded that: all the three single-stranded oligodeoxyribonucleotides interact with zinc ions, by the same mechanism [41,43,44]. The size and base sequence of the oligo do not interfere in the signals of the interaction, although the smaller ones produced better results. All the single-stranded oligos produce well defined peaks at −1.3 V, which represent the reduction of adenine and cytosine at mercury electrodes [1,2,4,7].

A 19 mer double-stranded oligodeoxyribonucleotide, consisting of 60% of GC residues and 40% of AT residues (5′ CGATCGACGTCGACGATTA 3′), was synthesized according to the experimental data obtained in the study of the interaction between zinc ions and three single-stranded oligodeoxyribonucleotides and its susceptibility to the cleavage by mung bean nuclease through its ends containing AT residues. The high efficiency of the synthesis was attested by the recovery rates obtained for each step that ranged from 97% to 100% [41,44,45].

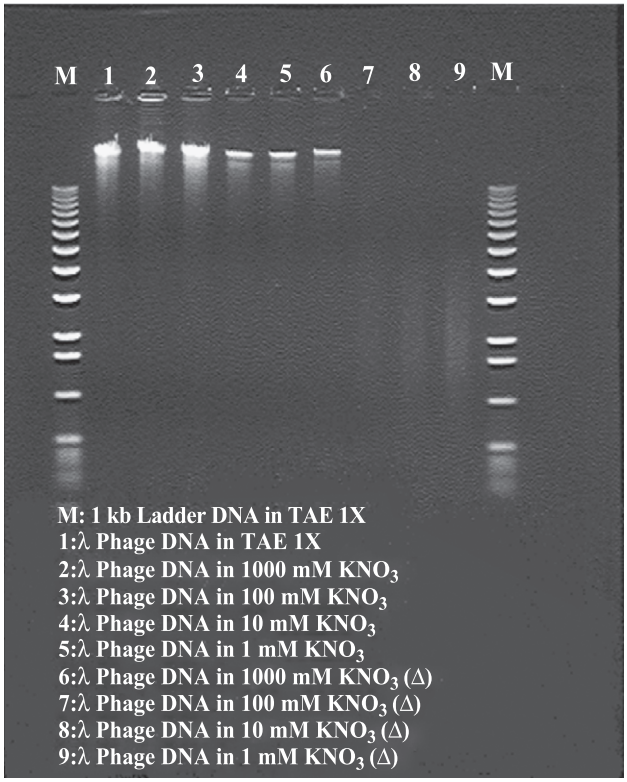

Fig. 2. Agarose gel obtained in the study of the integrity of λ phage DNA in KNO_3 solutions of different concentrations. M=Ladder DNA (molecular mass marker) in a solution composed by TAE 1× (40 mM Tris-acetate + 1 mM EDTA) and 0.1% ethidium bromide; (1) λ phage DNA in a solution composed by TAE 1X and 0.1% ethidium bromide; (2) λ phage DNA in 1000 mM KNO_3 solution; (3) λ phage DNA in 100 mM KNO_3 solution; (4) λ phage DNA in 10 mM KNO_3 solution; (5) λ phage DNA in 1 mM KNO_3 solution; (6) λ phage DNA in 1000 mM KNO_3 solution (T=68 °C); (7) λ phage DNA in 100 mM KNO_3 solution (T=68 °C); (8) λ phage DNA in 10 mM KNO_3 solution (T=68 °C); (9) λ phage DNA in 1 mM KNO_3 solution (T=68 °C). [λ phage DNA]=1.5×10^{-8} M.

MALDI-TOF/MS experiments attested the purity of the two complementary strands of the 19 mer double-stranded oligodeoxyribonucleotide [41,44,45]. For both strands, only one peak was observed in their mass spectra, whose experimental masses (1=5869.99 Da and 2=5877.58 Da) were very similar to the theoretical values calculated for each oligo (1=5867.71 Da and 2=5876.71 Da).

Prior to voltammetric experiments, the annealing of the 19 mer double-stranded oligodeoxyribonucleotide was demonstrated by measuring its UV absorption (λ=260 nm) as a function of temperature [41,44,45]. Two steps were observed in its melting curve, which attested that the ds-oligo is denatured at 95 °C and annealed at room temperature. The melting temperature (T_m) was found to be 54 °C and this result agrees with the theoretical value (58 °C) calculated through the expression: T_m (°C)=[2 °C×(number of AT base pairs)+4 °C×(number of GC base pairs)] [18].

KNO_3 was chosen as the supporting electrolyte because it simulates a medium with conditions (pH, ionic strength) near to physiological and does not react with DNA, since it is a neutral salt. Gel electrophoresis was used to study the integrity of a double-stranded DNA in potassium nitrate solutions at room temperature and at 68 °C. Fig. 2 shows that λ phage DNA did not denature or degrade in 1000, 100, 10 and 1 m M KNO_3 solutions at room temperature, since the bands that were observed in gel are very similar to those that are usually obtained for ds-DNA in TAE 1X. However, when the λ phage DNA KNO_3 solutions were heated (T=68 °C), it was verified that 100, 10 and 1 m M KNO_3 solutions affected the integrity of the DNA molecule, since only light tracks were observed in gel (absence of bands). These results demonstrated that potassium nitrate (1000, 100, 10 and 1 m M solutions) can be used as supporting electrolyte in voltammetric experiments, at room temperature, without causing any damage to the structure of a ds-DNA.

The anodic stripping voltammograms in Fig. 3 show the dependence of the diffusion current at constant Zn^{2+} ions concentration on the added ds-oligo concentration. Curve 1 (Fig. 3) represents the oxidation of 6.01×10^{-8} M zinc ions in 20 ml of 0.1 M KNO_3. Curves numbered 2–10 (Fig. 3) represent the effect of 3.62×10^{-9}, 7.24×10^{-9}, 1.09×10^{-8},

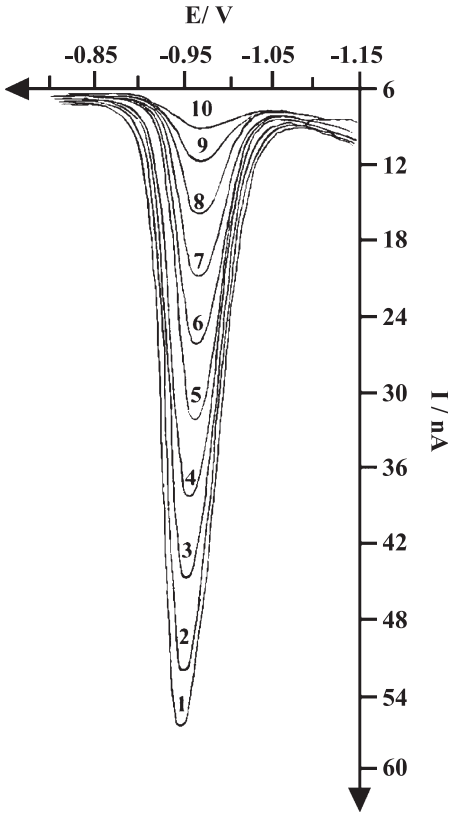

Fig. 3. Anodic stripping voltammograms of 6.01×10^{-8} M Zn^{2+} ions in 20 ml of 0.1 M KNO_3 with different concentrations of the double-stranded oligo. (1) Zn^{2+} ions, no double-stranded oligo; (2) Zn^{2+} ions + 3.62×10^{-9} M double-stranded oligo; (3) Zn^{2+} ions+7.24×10^{-9} M double-stranded oligo; (4) Zn^{2+} 1.09×10^{-8} M double-stranded oligo; (5) Zn^{2+} ions + 1.45×10^{-8} M double-stranded oligo; (6) Zn^{2+} ions+1.81×10^{-8} M double-stranded oligo; (7) Zn^{2+} ions+2.17×10^{-8} M double-stranded oligo; (8) Zn^{2+} ions + 2.53×10^{-8} M double-stranded oligo; (9) Zn^{2+} ions + 2.89×10^{-8} M double-stranded oligo; (10) Zn^{2+} ions + 3.25×10^{-8} M double-stranded oligo. E_d=−1.15 V, t_d=90 s, E_i=−1.15 V, E_f=0.180 V, scan rate=10 mV s^{-1}, working electrode: HMDE, reference electrode: Ag/AgCl (3 M KCl).

1.45×10^{-8}, 1.81×10^{-8}, 2.17×10^{-8}, 2.53×10^{-8}, 2.89×10^{-8} and 3.25×10^{-8} M added ds-oligo to the electrochemical cell. The resulting changes in oxidation current attest the interaction between Zn^{2+} ions and the 19 mer ds-oligodeoxyribonucleotide [45]. The nature of this current is supported by three evidences: a new mercury drop was used for each measurement (after each ds-oligo addition); consequently, the electrode surface could not be blocked by the 19 mer ds-oligodeoxyribonucleotide. Second, after the complete complexation of zinc ions with a ds-DNA (λ phage DNA), another peak was observed at 65 mV, which represents the interaction between mercury and λ phage DNA [44]. The height of this peak (current) is proportional to the DNA concentration. If DNA was adsorbed onto Hg surface, it could not interfere in the oxidation process of mercury electrode when in excess. Third, the calculation of $E^{0'}$ of Zn^{2+}-ds-oligo complex offers a value of −1.18 V, which means that the complex is hidden by the solvent reduction current and this is the reason that its signal is not seen in the voltammograms. Such evidences demonstrate that the decrease in the oxidation current of zinc in the presence of 19 mer ds-oligodeoxyribonucleotide is due to its interaction with this macromolecule and not due to the ds-oligo adsorption onto Hg electrode surface. Similar voltammetric results were obtained with a 3.86×10^{-8} M Zn^{2+} ions solution.

The amperometric titration curve of zinc ions with the ds-oligodeoxyribonucleotide is shown in Fig. 4. It was found a complete decrease in the zinc oxidation current after the addition of 16.9 μl of 3.62×10^{-5} M ds-oligo to the electrochemical cell. The volume of oligo used in the zinc ions complexation was determined through the inflection point (I.P.) of the titration curve and was employed to calculate the stoichiometry of the reaction. By this method, the stoichiometry rate was found to be one 19 mer ds-oligodeoxyribonucleotide per 2 Zn^{2+} ions.

For a complex of the electro-active substance Zn^{2+} with ds-oligo, the electrochemical reactions can be divided in two steps:

$$Zn^{2+} - \text{dsoligo} \underset{\leftarrow}{\rightarrow} Zn^{2+} + \text{dsoligo} \quad (1)$$

$$Zn^{2+} + 2e^{-} \underset{\leftarrow}{\rightarrow} Zn^{0} \quad (2)$$

The dissociation constant (K_d) of the Zn^{2+}-oligo complex was obtained using the following equation [46,47]:

$$i_p^2 = \frac{K_d}{[\text{oligo}]}\left(i_{p0}^2 - i_p^2\right) + i_{p0}^2 - [\text{oligo}] \quad (3)$$

where K_d=dissociation constant of Zn^{2+}-ds-oligo complex, i_{p0}^2=oxidation current of zinc in the absence of ds-oligo, i_p^2=oxidation current of zinc in the presence of ds-oligo, [oligo]=concentration of added ds-oligo in solution.

According to Eq. (3), i_{p0} and different values of i_p are determined by holding the concentration of Zn^{2+} ions constant and varying the concentration of ds-oligo.

The curve i_p^2 versus ($i_{p0}^2 - i_p^2$/[oligo]) (Fig. 4) for the complex Zn^{2+}-ds-oligo was plotted and a straight line was obtained. From the slope, the dissociation constant was determined as 4.71×10^{-8} M.

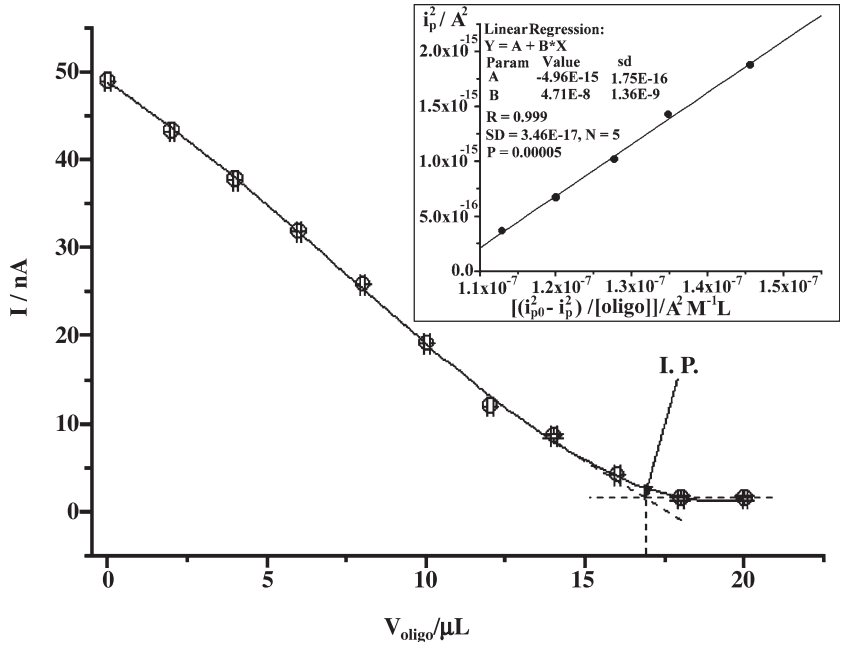

Fig. 4. Zinc ions binding to the 19 mer ds-oligodeoxyribonucleotide studied by anodic stripping voltammetry. The main figure shows the amperometric titration graph of 6.01×10^{-8} M Zn^{2+} ions in 20 ml of 0.1 M KNO_3 with the 19 mer ds-oligodeoxyribonucleotide. Successive additions of 2.0 μl of 3.62×10^{-5} M ds-oligo were made. I.P.=inflection point. (Inset) The plot i_p^2 vs. ($i_{p0}^2 - i_p^2$/[oligo]) used to calculate the dissociation constant of Zn^{2+}-ds-oligo complex. R=correlation coefficient; SD=standard deviation; P=probability.

Binding of zinc ions to the double-stranded oligodeoxyribonucleotide was also demonstrated by cyclic voltammetry [41,44,48]. In this case, the peak near −1.3 V was not observed in the cyclic voltammograms because adenine and cytosine are linked with thymine and guanine by hydrogen bonds, which block their reduction. Fig. 5 shows the amperometric titration curve of Zn^{2+} ions with 19 mer ds-oligodeoxyribonucleotide. The addition of 38 μl of 8.17×10^{-7} M ds-oligo to the electrochemical cell decreases completely the oxidation current of zinc. The volume of ds-oligo used in the zinc ions complexation was determined through the inflection point (I.P.) of the titration curve and was employed to calculate the stoichiometry of the reaction. By this method, the stoichiometry rate was found to be one ds-oligodeoxyribonucleotide per 2 Zn^{2+} ions in agreement with the above ASV results.

The dissociation constant (K_d) of the Zn^{2+}-ds-oligo complex calculated by Eq. (3) was 1.62×10^{-9} M (Fig. 5) [48].

Five experimental evidences demonstrate that these two zinc ions interact with the two terminal phosphate groups (5′), stabilizing the double helix of the ds-oligo: (a) The concentration of zinc ions that had been used in the experiments was low (small Zn^{2+}:DNA-P rate), which favors the stabilization of double helix by Zn^{2+} ions [35]; (b) The destabilization of the double helix is negligible when the concentration of the monovalent cation (K^+) is ≥0.1 M (supporting electrolyte—0.1 M KNO_3) and the concentration of Zn^{2+} ions is $\leq10^{-4}$ M; (c) The absence of the peak around −1.3 V in the cyclic voltammograms obtained for the ds-oligo indicates that it is native. Consequently, Zn^{2+} ions cannot be linked by GC bridges because this phenomenon occurs only when DNA is denatured; (d) If zinc ions were linked by GC bridges, the obtained stoichiometry would be 10 Zn^{2+} ions per one ds-oligo molecule; (e) DNA regions that are rich in AT residues favor the interaction of zinc ions with phosphate groups [35].

Curve I (oxidation current) versus $v^{1/2}$ (square root of scan rate) show that the oxidation–reduction process of zinc is no longer controlled by diffusion in the presence of the 19 mer double-stranded oligo, which is in agreement with the ARC5IIC2 results [41,44,48].

4. Conclusions

The methodology developed in this work allowed determination of stoichiometry, dissociation constant and a possible mechanism of the interaction between Zn^{2+} ions and the 19 mer ds-oligodeoxyribonucleotide using a simple and rapid voltammetric procedure. The stoichiometry values obtained by anodic stripping voltammetry (2:1) and cyclic voltammetry (2:1) are identical. The low K_d values found for Zn^{2+}-ds-oligodeoxyribonucleotide complex using anodic stripping voltammetry ($K_d=4.71\times10^{-8}$ M) and cyclic voltammetry ($K_d=1.62\times10^{-9}$ M) are in agreement with the binding constants values determined for zinc–phosphate groups complexes under various conditions [49] and demonstrate the high affinity of ds-oligo to the Zn^{2+} ions, which are stabilizing its duplex by their electrostatic interaction with phosphate groups. These findings were key clues for the better understanding of Zn^{2+} functions in the DNA cleavage catalyzed by zinc nucleases [40,41]. Zinc ions are probably involved in the binding site to the DNA

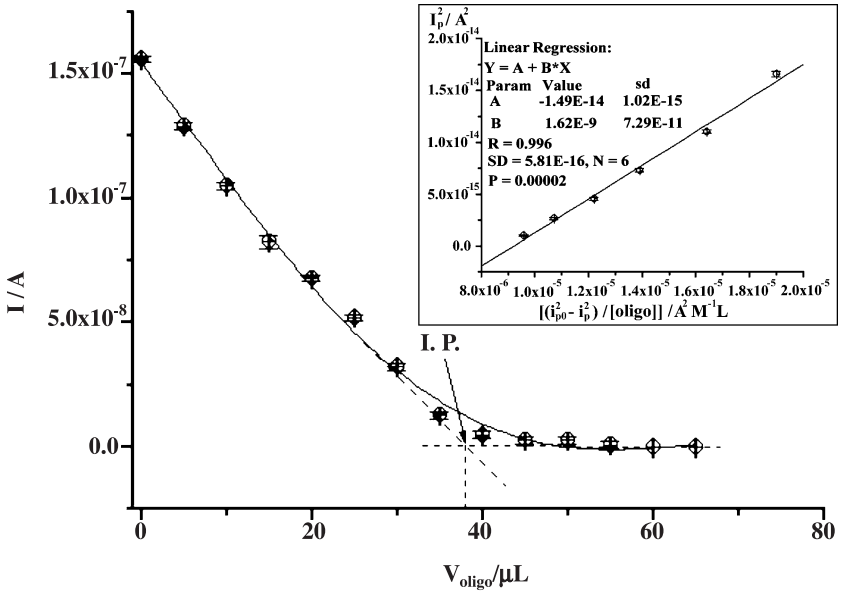

Fig. 5. Zinc ions binding to the 19 mer ds-oligodeoxyribonucleotide studied by cyclic voltammetry. The main figure shows the amperometric titration graph of 6.07×10^{-9} M Zn^{2+} ions in 10 ml of 0.1 M KNO_3 with the 19 mer ds-oligodeoxyribonucleotide. Successive additions of 5 μl of 8.13×10^{-7} M ds-oligo were made. I.P.=inflection point. (Inset) The plot i_p^2 vs. ($i_{p0}^2-i_p^2$/[oligo]) used to calculate the dissociation constant of Zn^{2+}-ds-oligo complex. *R*=correlation coefficient; SD=standard deviation; *P*=probability.

molecule and it can be suggested that they are responsible for a tight binding between DNA and the enzyme. Recently, a mechanism [41], with respect to the stereochemical course and zinc functions, for the 19 mer double-stranded oligodeoxyribonucleotide cleavage catalyzed by mung bean nuclease, was proposed using the voltammetric experimental data presented here and those obtained with Zn^{2+}–Mung bean nuclease system [50].

The results obtained here and recently reported works in the protein electrochemistry field [50–53] highlight the possibilities that voltammetric techniques offer for the quantification of metal-macromolecule interactions and understanding of how complex living systems function.

Acknowledgments

The authors wish to express their gratitude to Professors A.C. Barbosa and L. Morhy for providing certain facilities, to Dr. Genaro Ribeiro de Paiva for his help in the synthesis of the 19 mer ds-oligo and in the electrophoresis experiments, to Dr. Daniel Rigden for his help in revising the English text and to CNPq, CAPES, FAPDF for financial support.

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