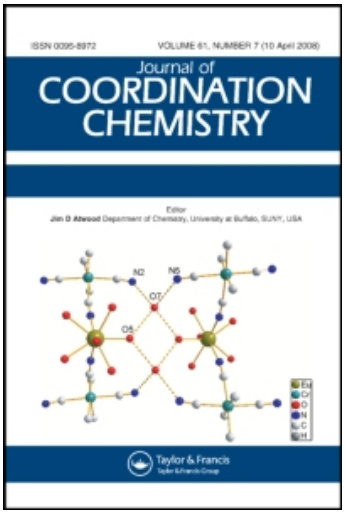

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Voltammetric Study of the Interaction of Pb(II) with the Double Stranded Polynucleotides Poly(dA-dT) · Poly(dA-dT) and Poly(dG-dC) · Poly(dG-dC)

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VOLTAMMETRIC STUDY OF THE INTERACTION OF Pb(II) WITH THE DOUBLE STRANDED POLYNUCLEOTIDES POLY(dA–dT)·POLY(dA–dT) AND POLY(dG–dC)·POLY(dG–dC)

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The interaction of Pb(II) ions with the double stranded polynucleotides poly(dA–dT)·poly(dA–dT) and poly(dG–dC)·poly(dG–dC) was studied with sweep voltammetry, cyclic voltammetry and alternating current voltammetry. The results have shown that the metal interacts with the phosphate groups in both polynucleotides and with base donors of the A–T polymer. The same result is observed for the Pb(II)-native DNA system.

Keywords: Lead(II), DNA, polynucleotides, electrochemistry

INTRODUCTION

Polynucleotides represent very useful models for understanding the structure and other properties of natural DNAs and RNAs. In the double-stranded polynucleotides we have the typical Watson–Crick double helical structures with properties similar to those of single- (ss) and double-stranded (ds) DNAs.[1,2] The polynucleotide models applied in this study can be especially useful for an understanding of the interactions of metal ions with DNA regions which are rich in A–T or G–C pairs.

During the polarographic process, ds polynucleotides display a reduction peak similar to that of native DNA.[1–3] Analysis of the voltammetric reduction peaks indicates variations in the ds polymers, which occur as a consequence of polynucleotide interaction with the charged electrode or other external effects which influence their helical structure.

Recent work[4–7] has shown that lead ions may interact with nucleic acids *via* phosphate groups, leading to stabilization of the helical structure or aggregation of nucleic acid. Interaction with nucleic bases is also possible[4,6,7] and this destabilizes the double-stranded structure. The latter interaction can be quite specific though the actual results do not reveal the details of the metal–nucleic acid interactions. It has also been shown that labilization of the double helical structure of nucleic acids may considerably influence its interaction with metal ions.[6,8–11]

EXPERIMENTAL

Poly(dA–dT)·poly(dA–dT) and poly(dG–dC)·poly(dG–dC) were obtained from Boehringer, Mannheim. Calf-thymus DNA (mol. weight 1.2×10^6, protein content

below 0.5%) was purchased from Worthington. Differential pulse polarographic (DPP) measurements[1] indicated that less than 1% of denatured DNA was present in the samples used. All other chemicals were of analytical grade. Concentrations of polynucleotides and DNA were determined spectrophotometrically assuming a molar absorbance for poly(dA–dT)·poly(dA–dT) of 6.6×10^3 at 262 nm, for poly(dG–dC)·poly(dG–dC) of 8.4×10^3 at 253 nm and for DNA of 6.6×10^3 at 260 nm.

The s.v. measurements were performed on a potentiostat generator (Type PG 30/1, Lódź, Poland). The three electrode system comprised a hanging mercury drop (HMDE, surface area 1.65 cm^2) as a working electrode, a platinum wire as an auxiliary electrode and a saturated calomel electrode as reference (scan rate of 10 $V \cdot s^{-1}$, adsorption potential $E_i = -0.2$ V, switching potential $E_s = -1.7$ V and accumulation time 180 s (without stirring)).

In c.v., the potential of the HMDE was changed linearly with time starting from $E_i = -0.2$ V and changing to potentials at which reduction of Pb(II) ions occurred (−0.8 V). The linear sweep was then reversed to oxidize the lead amalgam which was formed during the forward scan.

The a.c. voltammetric measurements were carried out under potentiostatic control with a P.A.R. Analyzer, model 170, in a thermostatted Methrom cell with the three electrode system. The voltammograms were recorded with an adjusted phase difference of 90° with respect to the polarizing voltage applied (5 mV peak to peak). The a.c. frequency was 100 Hz.

The solutions were deoxygenated with a slow stream of analytical grade nitrogen and during the measurements nitrogen was passed over the solution surface. Acetate buffer containing 0.05 mol dm^{-3} sodium acetate (pH = 5.6) was used as a supporting electrolyte. The concentrations of poly(dA–dT)·poly(dA–dT), poly(dG–dC)·poly(dG–dC) and DNA were 30, 25 and 100 $\mu g/cm^3$, respectively. All polarographic measurements were performed at 25°C.

RESULTS

Alternating current voltammetry

Measurements of the time dependence of current at the adsorption potential $E_i = -0.2$ V by a.c. voltammetry may provide information about the adsorption rates of polynucleotides at the HMDE surface.[1,12,13] The dependence of the current (I_{oc}) on the square root of adsorption time (T_s) for both polynucleotides is shown in Figures 1 and 2. The adsorption rate of the poly(dG–dC)·poly(dG–dC) polymer does not much depend on the metal to phosphate ratio (P) (Fig. 1). There is a slight increase of I_{oc} with increasing P. The behaviour of the poly(dA–dT)·poly(dA–dT)–Pb(II) system is, however, different from that shown in Figure 1. The adsorption rate strongly depends on P values. For low P values (< 1), I_{oc} is less than for the metal-free polymer while for P = 10 one observes a slow attainment of electrode surface saturation as measured by the change of I_{oc} *vs* $t_s^{1/2}$ (Fig. 2). These results indicate that Pb(II) ions distinctly alter the hydrodynamic behaviour of poly(dA–dT)·poly(dA–dT), whereas their influence on the properties of poly(dG–dC)·poly(dG–dC) polymer is very small.

Sweep-voltammetry

This technique may provide useful information concerning hydrodynamic and interfacial properties of DNA.[6,13] The parameters characterizing the sv reduction peaks depend strongly on the structure of ds DNA or ds polynucleotide.[2,3] In

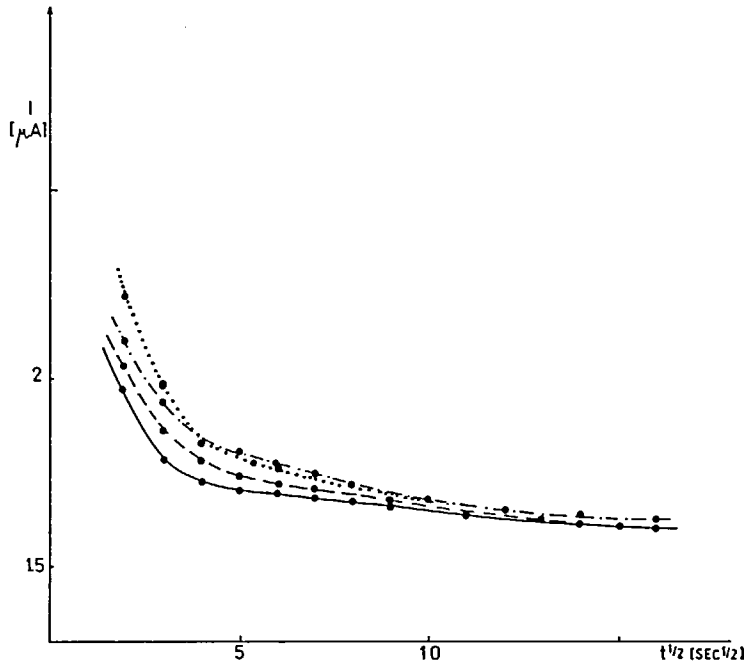

FIGURE 1 Influence of P value (metal to phosphate molar ratio) on the dependence of capacitive a.c. current (I_{oc}) on time; $E_i = -0.2$ V, $c_{poly(dG-dC)\,poly(dG-dC)} = 25\,\mu g/cm^{-3}$ in 0.5 mol dm^{-3} sodium acetate, pH = 5.6; P = 0 (———), P = 0.5 (– – –), P = 3 (— · —), P = 10 (····).

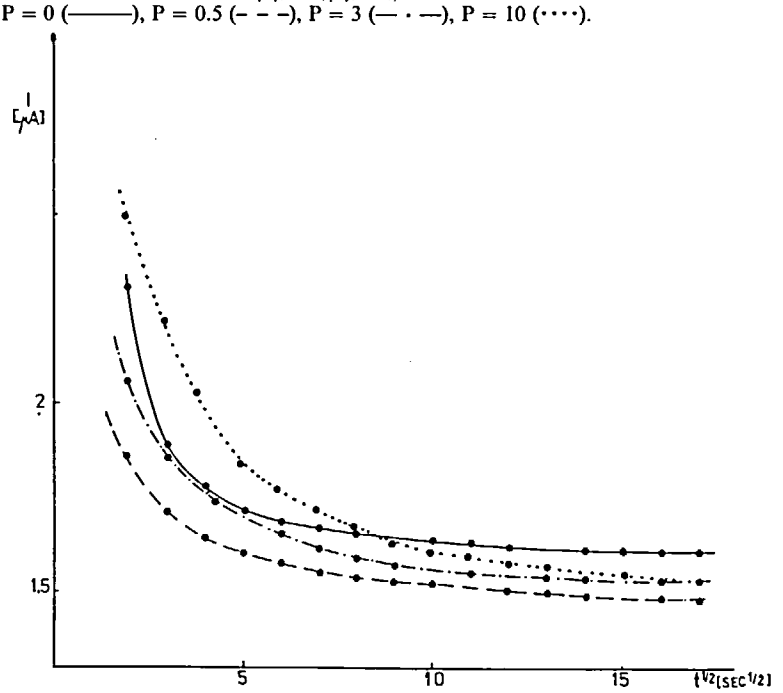

FIGURE 2 Influence of P value on the dependence of capacitive a.c. current (I_{oc}) on time; $c_{poly(dA-dT)\cdot poly(dA-dT)} = 30\,\mu g/cm^3$, other conditions as in Fig. 1.

poly(dG–dC)·poly(dG–dC) the cytosine residue undergoes a 2-electron reduction process[1] at about −1.584 V (Fig. 3a). In the presence of Pb(II) ions this reduction peak shifts slightly (to −1.604 V for P = 10) and current increases by less than 15% (Fig. 3a). In the case of poly(dA–dT)·poly(dA–dT), adenine is reduced on the electrode surface (overall 4-electron reduction[1]) at −1.576 V, Fig. 3b). The presence of Pb(II) ions shifts considerably the reduction peak of poly(dA–dT) towards more negative potentials (−1.628 V for P = 10, Fig. 3b). The increase of the limiting current of the reduction peak can be over 30% (P = 10), when compared to the metal-free polymer (Fig. 3b). Thus, as seen in the case of s.v. measurements, more drastic changes, caused by Pb(II), are observed in the poly(dA–dT)·poly(dA–dT) system, by comparison to the G–C polymer.

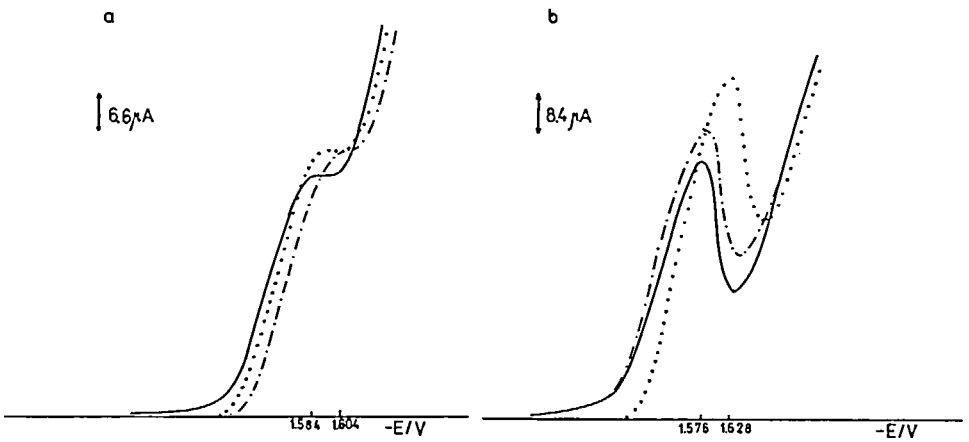

FIGURE 3 S.v. voltammograms of polynucleotides in the presence of Pb ions; a: poly(dG–dC)·poly(dG–dC); b: poly(dA–dT)·poly(dA–dT); P = 0 (———), P = 3 (— · —) and P = 10 (· · · ·); adsorption time 180 s, E_i = −0.2 V, scan rate 10 V s^{-1}, other conditions as in Figs 1 and 2, respectively.

TABLE I
Cyclic voltammetry data for the Pb(II)–acetate buffer system*

V [mV/s]	I_c [μA]	$-E_c$ [V]	I_a [μA]	$-E_a$ [V]	ΔE [V]	I_c/I_a
5	5.33	0.430	18.45	0.375	0.055	0.3
10	6.97	0.420	18.04	0.370	0.050	0.4
20	8.20	0.420	15.58	0.365	0.055	0.5
50	12.71	0.435	18.04	0.365	0.070	0.7
100	19.68	0.445	24.60	0.365	0.080	0.8
200	23.37	0.450	24.47	0.360	0.090	0.9

*0.05 M acetate buffer; pH = 5.6; adsorption time, 180s: E_i (initial potential) = −0.2 V; E_s (reverse potential) = −0.8 V; I_c = current of cathodic peak; E_c = potential of cathodic peak; I_a = current of anodic peak; E_a = potential of anodic peak; $\Delta E = E_c - E_a$; $c_{Pb} = 6.6 \times 10^{-5}$ mol dm^{-3}.

Cyclic-voltammetry

This technique can be used to evaluate more precisely the complexation of Pb(II) ions with polynucleotides. In c.v. measurements the potential of the HMDE changes

TABLE II
Cyclic voltammetry data for the Pb(II)-poly(dG-dC)·poly(dG-dC) system*

	P = 0.5						P = 3						P = 10					
V [mV/s]	I_c [μA]	$-E_c$ [V]	I_a [μA]	$-E_a$ [V]	ΔE [V]	I_c/I_a	I_c [μA]	$-E_c$ [V]	I_a [μA]	$-E_a$ [V]	ΔE [V]	I_c/I_a	I_c [μA]	$-E_c$ [V]	I_a [μA]	$-E_a$ [V]	ΔE [V]	I_c/I_a
5	0.22	0.430	0.88	0.400	0.030	0.3	1.42	0.430	6.18	0.395	0.035	0.2	6.4	0.435	24.4	0.375	0.060	0.3
10	0.34	0.435	0.80	0.405	0.030	0.4	1.98	0.430	5.54	0.395	0.035	0.4	7.6	0.440	20.0	0.380	0.060	0.4
20	0.44	0.425	0.80	0.390	0.035	0.5	2.77	0.425	2.66	0.390	0.035	0.5	10.0	0.430	19.6	0.370	0.060	0.5
50	0.70	0.425	0.92	0.390	0.035	0.8	4.19	0.420	6.02	0.375	0.045	0.7	15.6	0.435	22.0	0.370	0.065	07.
100	1.00	0.420	1.02	0.385	0.035	1.0	6.14	0.435	6.93	0.385	0.050	0.9	20.8	0.450	26.0	0.350	0.100	0.8
200	1.56	0.425	1.56	0.385	0.040	1.0	8.32	0.435	8.51	0.385	0.050	1.0	29.2	0.460	32.8	0.360	0.100	0.9

* 0.05 M acetate buffer; pH = 5.6; adsorption time, 180 s; E_i (initial potential) = −0.2 V; E_s (reverse potential) = −0.8 V; $c_{poly(dG-dC)\cdot poly(dG-dC)} = 7.6 \times 10^{-5}$ M (25 μg/cm³); P = 0.5, *e.g.*, $c_{Pb} = 3.8 \times 10^{-5}$ M; P = 3, *e.g.*, $c_{Pb} = 22.8 \times 10^{-5}$ M; P = 10, *e.g.*, $c_{Pb} = 7.6 \times 10^{-5}$ M; I_c = current of cathodic peak; E_c = potential of cathodic peak; I_a = current of anodic peak; E_a = potential of anodic peak; $\Delta E = E_c - E_a$.

TABLE III
Cyclic voltammetry data for the Pb(II)–poly(dA–dT)·poly(dA–dT) system*

	P = 0.5						P = 3						P = 10					
V [mV/s]	I_c [μA]	$-E_c$ [V]	I_a [μA]	$-E_a$ [V]	ΔE [V]	I_c/I_a	I_c [μA]	$-E_c$ [V]	I_a [μA]	$-E_a$ [V]	ΔE [V]	I_c/I_a	I_c [μA]	$-E_c$ [V]	I_a [μA]	$-E_a$ [V]	ΔE [V]	I_c/I_a
5	0.23	0.430	0.80	0.400	0.030	0.3	0.79	0.430	7.33	0.390	0.040	0.1	5.6	0.440	18.8	0.380	0.060	0.3
10	0.28	0.420	0.70	0.395	0.025	0.4	2.57	0.425	7.33	0.380	0.045	0.3	8.0	0.440	16.8	0.380	0.060	0.5
20	0.42	0.425	0.74	0.395	0.030	0.6	3.56	0.425	7.13	0.380	0.045	0.5	10.8	0.440	19.2	0.375	0.065	0.6
50	0.66	0.430	0.86	0.395	0.035	0.8	4.75	0.425	7.92	0.375	0.050	0.6	16.4 9.6	0.435 0.505	23.6	0.365	0.07 0.140	
100	0.96	0.430	1.06	0.395	0.035	0.9	7.92	0.440	9.31	0.385	0.055	0.8	22.4 10.24	0.440 0.515	28.0	0.360	0.080 0.155	
200	1.4	0.420	1.38	0.385	0.035	1.0	10.9	0.440	11.88	0.385	0.055	0.9	18.4 9.2	0.455 0.540	21.2	0.360	0.095 0.180	

* 0.05M acetate buffer; pH = 5.6; adsorption time, 180 s; E_i (initial potential) = −0.2 V; E_s (reverse potential) = −0.8 V; $c_{poly(dA-dT)\cdot poly(dA-dT)} = 9.1 \times 10^{-5}$ M (30 μg/cm³); P = 0.5, *e.g.*, $c_{Pb} = 4.6 \times 10^{-5}$ M; P = 3, *e.g.*, $c_{Pb} = 27.3 \times 10^{-5}$ M; P = 10, *e.g.*, $c_{Pb} = 91 \times 10^{-5}$ M; I_c = current of cathodic peak; E_c = potential of cathodic peak; I_a = current of anodic peak; E_a = potential of anodic peak; $\Delta E = E_c - E_a$.

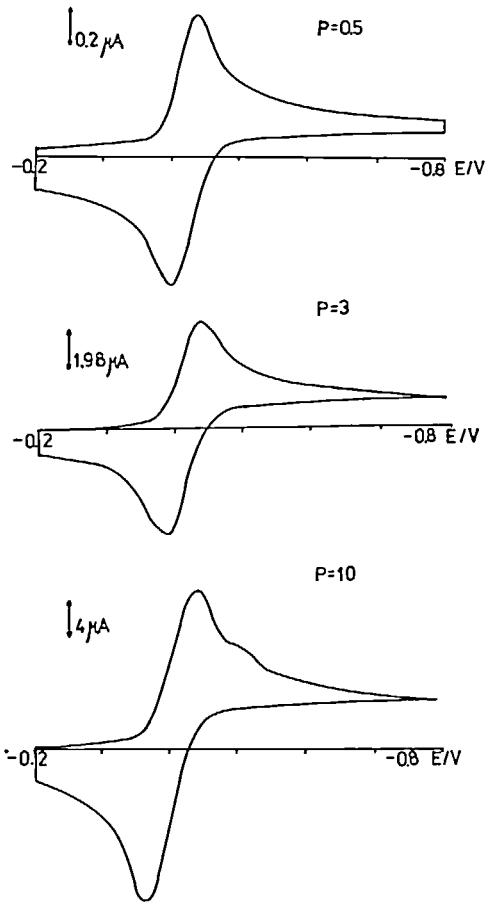

FIGURE 4 Cyclic voltammograms of Pb(II) ions in the presence of poly(dA–dT)·poly(dA–dT); adsorption time 180 s, scan rate 100 mV s^{-1}, $E_i = -0.2$ V, $E_s = -0.8$ V and other conditions as in Fig. 2.

linearly with time, starting at −0.2 V, towards more negative potentials where reduction of metal ions occurs (around −0.38 V). After traversing the potential region where reduction of Pb(II) takes place, the direction of sweep is reversed (at E = −0.8 V) and oxidation of the lead amalgam formed during the forward scan can be detected. The results of the c.v. measurements for both polynucleotides with metal ions are collected in Tables I to III and presented in Figures 4 and 5. The current density is proportional to the square root of potential sweep rate. This indicates diffusion controlled reduction of Pb(II) ions.[14]

According to the results obtained for the Pb(II)-acetate system (Table I) the cyclic voltammograms for this metal ion consist of single cathodic (E_c) and anodic (E_a) peaks for different concentrations of Pb(II). The reaction is electrochemically irreversible ($E_c - E_a = 0.080$ V and $I_1/I_2 = 0.8$, I_1 and I_2 being the intensities of the corresponding peak currents, Table I).

The behaviour of Pb(II) in the presence of poly(dG–dC)·poly(dG–dC) polymer is similar to that observed in the Pb-acetate system (Table II, Fig. 5). Only a very slight shift of E_a towards more negative potentials is observed (Table II). The cyclic

voltammograms for lead(II) ions in the presence of poly(dA–dT)·poly(dA–dT) consist of single cathodic and anodic peaks for P = 0.5 and 3 (Table III, Fig. 5). In these cases the process is electrochemically irreversible. Increase of metal concentration to P = 10 leads to the observation of two c.v. peaks corresponding to cathodic reduction (Fig. 5, Table III) with reduction potentials at −0.440 and −0.515 V, but only one anodic peak is observed at −0.360 V. This result may indicate the formation of two different complexes of Pb(II) with poly(dA–dT)·poly(dA–dT). One is similar to that observed in Pb-acetate or Pb-poly(dG–dC)·poly(dG–dC) systems and the other, giving a more irreversible electrochemical reaction ($E_c - E_a$ = 0.155 V), exists only in Pb-poly(dA–dT)·poly(dA–dT) solutions.

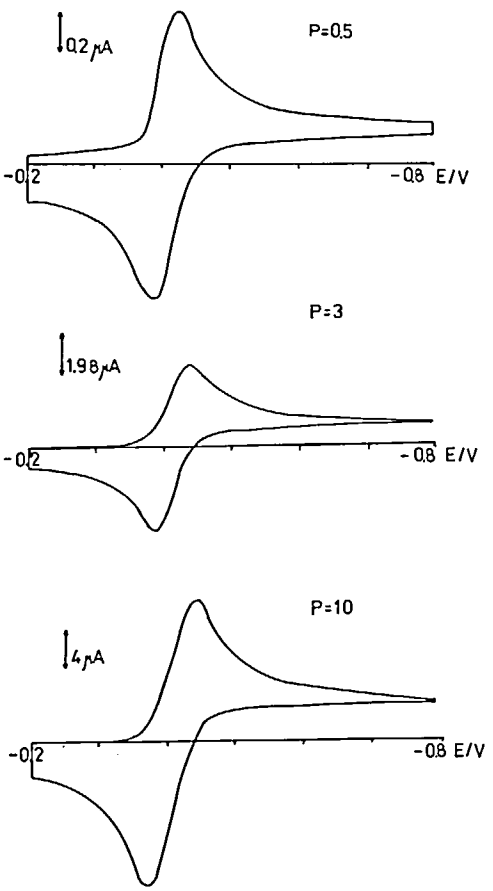

FIGURE 5 Cyclic voltammograms of Pb(II) ions in the presence of poly(dG–dC)·poly(dG–dC), conditions as in Figs 1 and 4.

DISCUSSION

Partial opening of DNA double-helical structure due to its interaction with a charged electrode was demonstrated in 1974.[15] At neutral pH the process takes place at a potential of about −1.2 V (*vs* saturated calomel electrode, region U).[2,15] The process

is relatively slow (tens of seconds) and involves an appreciable part of the DNA molecule. At more positive potentials (region T) one can observe faster changes, which are limited, however, to about several percent of the helix (in the vicinity of defects in the DNA helical structure).[1,15] In principle, the results obtained for synthetic polynucleotides agree with those obtained for native DNA.[2,3,15] It is accepted that the synthetic polynucleotides have less defects in their helical structure than does native DNA extracted from biological material.[16,17] The distinct heights of the voltammetric peaks, which do not depend on the initial potential in the T region (these may, however, increase considerably in the U region[3]), indicate that both polynucleotides undergo some denaturation on the charged electrode surface (*e.g.*, Fig. 3, see also ref. 2). The results also prove that the interaction of each polynucleotide with Pb ions is different. As was shown earlier,[6,7] the major interactions of helical nucleic acids with Pb(II) involve phosphate groups as a primary target for the approaching metal ion. This interaction may increase the stability of the double helix especially at low ionic strength. It does not, however, greatly influence the hydrodynamic properties of the polynucleotide. Thus, according to the a.c. measurements, the major interaction of lead ions with poly(dG–dC)·poly(dG–dC) for all P values studied involves phosphate oxygen atoms. The other voltammetric results agree with such an assumption. There is a slight change in the peak height of the s.v. signal, indicating that metal ions do not facilitate the surface denaturation process to any great extent, *i.e.*, interaction with base donors involved in the formation of the double-helical structure is negligible. The poly(dG–dC)·poly(dG–dC) ligand does not effect the c.v. peaks of Pb(II) ion as compared to the metal–acetate system due to the fact that in both cases the interaction of metal ions is limited mostly to oxygen donors. Considerably different results were obtained for the Pb(II)–poly(dA–dT)·poly(dA–dT) system (*vide supra*). Distinct variations of current intensity (Fig. 2) in a.c. polarograms indicate that A–T pairs may be involved directly in the interaction with metal ions *via* the base donors. The latter interaction destabilizes the double helical structure of the polynucleotide. The variations of current intensity in a.c. measurements clearly indicate that metal ions in excess (P = 10) clearly change the hydrodynamic behaviour of poly(dA–dT)·poly(dA–dT) (Fig. 2). More direct evidence for the different modes of Pb(II) ion binding with the A–T polymer is provided by c.v. measurements in which for P = 10 one can observe two reduction peaks. One at −0.440 V (Fig. 4) is similar to that observed for the two other systems mentioned above. The more irreversible reduction peak at −0.515 V corresponds to a different coordination mode for Pb(II), most likely involving a complex with a direct metal–base interaction. The same kinds of results are obtained for the Pb(II)–DNA system (Fig. 6). Thus, the model is a good mimic of native DNA and helps to explain the real situation in the Pb(II)–DNA system. The direct interaction with bases leading to destabilization of helical structure is also seen in the s.v. voltammograms in which the height of the reduction peak of the polymeric ligand increases distinctly in the presence of Pb(II) ions (Fig. 3b). These results taken together indicate that Pb(II) may interact with nucleic acids in two different ways, *i.e.*, *via* the phosphate chain in the G–C rich region and with base donors in the A–T rich region. This conclusion allows a reinterpretation of earlier spectroscopic studies of the melting profiles of DNA in the presence of lead ions.[4] The interaction of metal ions with A–T rich regions of DNA may stimulate their unwinding and could be the main reason for the decrease of melting temperature, T_m, of the remaining A–T pairs in the DNA structure. Interaction of Pb ions with A–T rich regions was also suggested by other studies.[18,19]

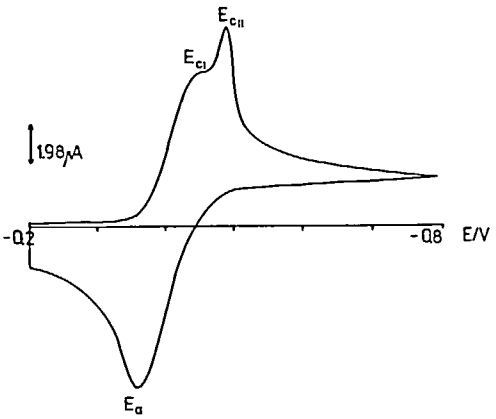

FIGURE 6 Cyclic voltammograms of Pb(II) ions in the presence of DNA for F = 3 (c_{Pb} = 99×10^{-5} mol dm^{-3}, c_{DNA} = 100 μg/cm^{-3}. $E_{cI} = -0.445$ V, $E_{cII} = -0.490$ V, $E_a = -0.360$ V, other conditions as in Fig. 4.

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