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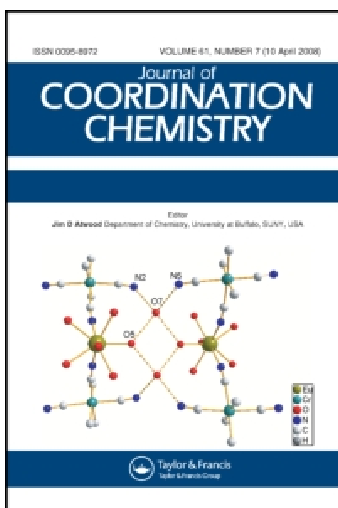

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REVIEW: INTERACTIONS OF METAL IONS WITH NUCLEIC ACIDS AND THEIR SUBUNITS. AN ELECTROCHEMICAL APPROACH

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REVIEW

INTERACTIONS OF METAL IONS WITH NUCLEIC ACIDS AND THEIR SUBUNITS. AN ELECTROCHEMICAL APPROACH

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Different electrochemical methods (differential pulse polarography, sweep voltammetry, alternating current voltammetry, chronocoulometry, cyclic voltammetry) can be used to investigate the interactions of metal ions such as osmium tetroxide, platinum complexes, Cu(II), Ni(II), Zn(II), Cd(II), Pb(II) and Eu(III) with DNA, t-RNA and their subunits. Modern electrochemical methods can detect very small perturbations in a double-helical structure as well as the behavior of single stranded (denatured) fragments of nucleic acids. The effectiveness of polarographic methods is comparable to enzymatic techniques which use specific nucleases. The polarographic data can also be used for the quantitative evaluation of the metal ion nucleic acid interactions (e.g., to calculate the association or stability constants and the number of binding sites). Some topics concerning these problems as well as general aspects of metal-nucleic acid interactions are discussed in this review.

KEYWORDS: metal complexes, DNA, RNA, voltammetric methods, polarography

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INTRODUCTION

Metal ions play an essential role in biological processes and the association of divalent metal ions with ribosomes is a well established fact. Metals are required in virtually all biological processes in which nucleic acids are engaged. During the past decade it was recognized that metal compounds may also be fatal for life; e.g. they can be important and dangerous carcinogens. Nickel, for example, in addition to chromium and cadmium, is considered to be a most effective carcinogen, and nickel sub-sulfide, Ni_3S_2, is the most potent metal carcinogen that has been tested on experimental animals.

Studies of a metal ion interaction with DNA are usually made on two levels: (1) addition of metal ions to natural DNA preparations and studies of the metal-DNA complexes obtained, and (2) a search for metals already present in DNA molecules extracted from various biological materials. The general features of metal ion effects on the structure of nucleic acids are already well established. There are two major binding sites for metal ions in a DNA molecule: phosphates and base donors. These two metal-DNA modes of interaction lead to distinctly different structural effects in the nucleic acid molecule. Metal interactions with phosphates stabilize the double helical structure while metal binding to the bases destabilizes the ordered DNA helical structure and induces a nucleic acid denaturation process a decrease of the melting point. Spectroscopic studies reveal a general picture of such an interaction which may not be relevant to the metal DNA interaction in a living cell. In the latter case the nucleic acid structure may be locally disturbed by the environment, e.g., by charged membranes or highly charged structural proteins. These local changes in the DNA structure may have a critical influence on the metal ion-nucleic acid interactions. Numerous studies of the electrochemical behavior of nucleic acids revealed that a charged electrode can serve as a simple and quite interesting model of the charged interfaces.

Polarographic techniques can serve not only as a source of electrically charged interfaces, but also as methods which can be used to register local variations in the DNA molecule or locally important interactions with metal ions. Polarography has proved to be quite useful in studies of the metal ion nucleic acid system, and, compared to other methods, provides unique information. This paper is a general review showing the potential of electrochemical techniques applied to metal-nucleic acid systems.

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Structures of DNAs and RNAs

Nucleotides are the building blocks of all nucleic acids. These structural units consist of three essential components: a nitrogen containing base (purine or pyrimidine), a pentose sugar and a phosphate group. Chemical structures of nine-membered double-ringed purines, adenine (A) and guanine (G), and six-membered single-ringed pyrimidines, cytosine (C), thymine (T) and uracil, (U) are shown in Fig. 1a.

Both DNA and RNA contain A, C and G; only DNA contains base T, whereas only RNA contains base U.[1,2]

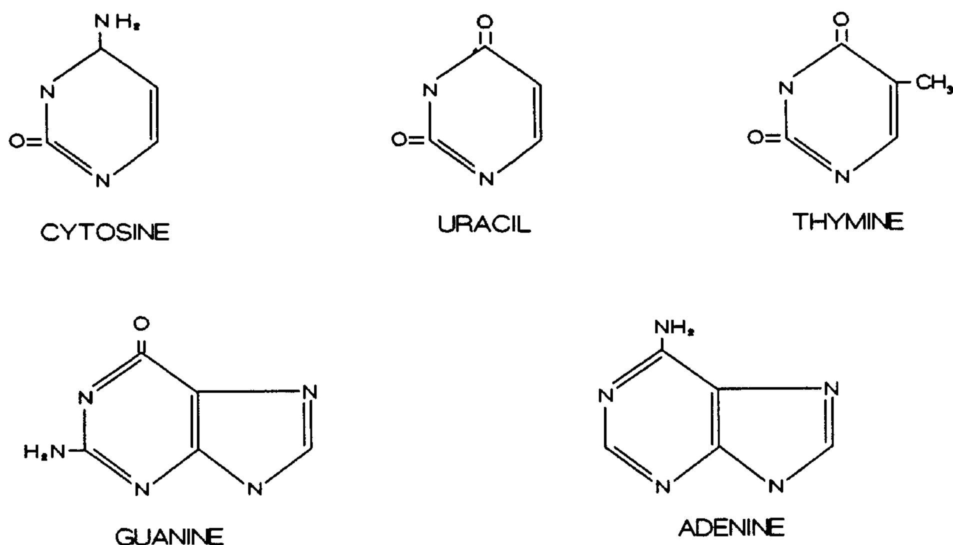
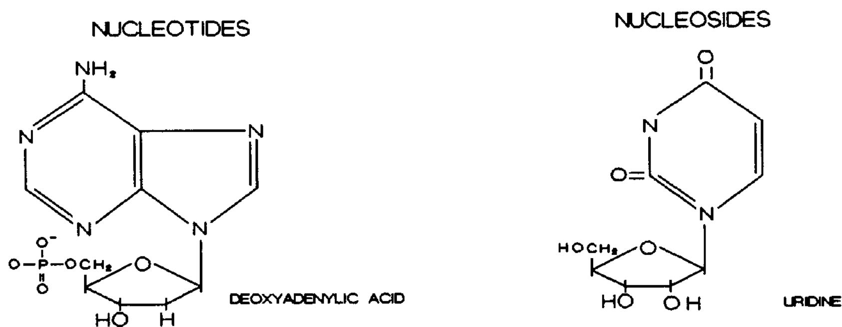


Figure 1a Chemical structures of nitrogenous bases in RNA and DNA.



RIBONUCLEOTIDES: adenylic acid, cytidylic acid guanylic acid, uridylic acid

RIBONUCLEOSIDES: adenosine, cytidine, guanosine uridine

DEOXYRIBONUCLEOTIDES: deoxyadenylic acid deoxycytidilic acid, deoxyguanylic acid deoxythymidylic acid

DEOXYRIBONUCLEOSIDES: deoxyadenosine, deoxycytidine,deoxyguanosine, deoxythymidine

Figure 1b The structures and names of nucleosides and nucleotides of RNA and DNA.

Pentose sugars found in nucleic acids give them their names. Ribonucleic acids (RNAs) contain ribose while deoxyribonucleic acids (DNAs) contain deoxyribose (Fig. 1b). According to the Watson and Crick model, a DNA molecule exists in the form of a double helix (Fig. 2). This model has the following major features:[2,3]

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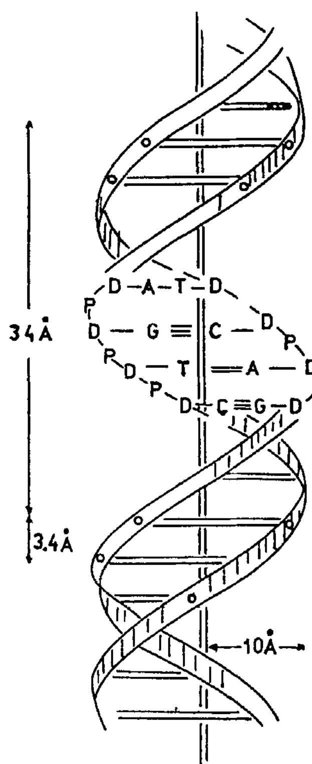

Figure 2 The helix structure of DNA.

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1. Two right-handed helical polynucleotide chains are coiled around the central axis.
2. Two chains are antiparallel, i.e., that viewed from one end of a helix, one chain is in the 5'–3' orientation while the second chain is in the 3'–5' orientation.
3. Bases of both chains are stacked on one another 0.34 nm (3.4 Å) apart.
4. Each turn of the helix is 3.4 nm long and contains 10 bases in each chain (B-form).
5. The nitrogenous bases of opposite chains are electrostatically attracted to one another as a result of the formation of hydrogen bonds: specifically only A-T and G-C pairs are allowed.
6. The double helix is 2.0 nm (20 Å) in diameter.

Under different conditions of isolation, purification and crystallization, several structural forms of DNA have been recognized. The A- and B-forms were discovered first. Watson and Crick's analysis was based on the B-form of DNA. The A-form is also a right-handed double helical configuration which, with its 11 base pairs in each turn, is slightly more compact than the B-form. The orientation of bases is slightly different in these two structures. The B-form seems to be a naturally occurring native structure of DNA, while the A-form can exist physiologically as a

result of the interaction of DNA with hydrophobic molecules or changing cellular conditions.[1] There is also a C-form, which resembles B-DNA with 9.33 pairs per turn.[2,3] The most recent form of DNA, discovered in 1979, is called Z-DNA. Similarly to A- and B-DNAs it consists of two antiparallel chains held together by Watson-Crick base pairs. Except for this, Z-DNA is quite different from B- or A-DNA. The left-handed helix of Z-DNA contains 12 base pairs per turn and resembles a zigzag conformation. There is increasing evidence suggesting that Z-DNA is present along with B-DNA under cellular conditions.[1-3] There is some speculation that Z-DNA may be important in the regulatory aspects of gene expression.

The second group of nucleic acids includes ribonucleic acids (RNA). Their structures are similar to those of DNA with several major exceptions: ribose replaces deoxyribose and uracil replaces thymine. Three classes of cellular RNA function during the expression of genetic information: ribosomal (rRNA), messenger (mRNA), and transfer (tRNA) RNA. Each class of RNA can be described in terms of its size, sedimentation behavior in a centrifugal field and genetic function.[1] The largest RNA is rRNA, which usually constitutes about 80% of all RNA in a cell. Transfer RNAs, the smallest RNAs, carry amino acids to the ribosome during translation. These molecules form a class of small globular polynucleotide chains of about 75 to 90 nucleotides. The t-RNA chain is folded in the form of a clover-leaf pattern which consists of loops and stems. It is well established that the native structure of tRNA is promoted in solution by small amounts of divalent cations, in particular Mg(II) ions.[1,3,5]

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Modes of Metal Ion Interactions with Nucleic Acids: Structural and Biological Aspects

Twenty-five elements are currently thought to be essential to warm-blooded animals. Ten can be classified as trace metal ions: Fe, Cu, Mn, Zn, Co, Mo, Cr, Sn, V, and Ni, and four as bulk metal ions: Na, K, Mg, and Ca. It is likely that other metal ions are also required at very low concentrations.

There are some metals, such as Cd or Pb, whose physiological functions are not known.[6,8] These metals, along with mercury, are major pollutants. The polluting elements lead to severe diseases such as nephritis (Cd), neuritis, encephalitis (Pb, Hg), or Alzheimer disease (Al).[6-8]

Metal ions play a significant role in metabolic reactions in all living organisms. The action of metal ions in most enzyme-catalyzed reactions, like DNA or RNA degradation by nucleases, is only partly understood. The fundamental role of magnesium in stabilization of the tertiary structure of t-RNA is well established.[5-9] Many metal complexes (e.g. platinum-amine complexes) act as chemotherapeutic agents, inhibiting synthesis of DNA which is often their target molecule.

The metal-nucleic acid coordination modes in the cases mentioned above differ widely due to the variety of binding sites available in nucleotides or nucleic acids as well as the variety of nucleic acid structures possible. The metal species (aqua-ion, partly complexed metal ion, etc.) interacting with nucleic acid ligands are also critical for the interaction mode.

The interactions between metal species and DNA may be summarized as:
— covalent inner-sphere binding
— covalent and non-covalent outer-sphere binding
— intercalation
— strand breakage

1. Inner- and Outer-Sphere Binding

A number of aquo-ions bind to DNA bases at different levels of covalency, mostly through phosphate moieties. The stabilizing and destabilizing effects of metal aquo-ions depend, among other factors, on the amount and the kind of metal ion in solution. In the series Mg(II), Co(II), Ni(II), Mn(II), Zn(II), Cd(II), and Cu(II), the affinity for base complexation relatively to phosphate binding increases from the left to the right.[10,11] The stabilizing effect of metal ions on the DNA helical structure results from neutralization of the phosphate negative charges by metal cations. The metal ion interactions with bases lead to destabilization of the DNA duplex.

Pb(II) and Cu(II) ions bind to phosphate groups only at very low metal concentrations (metal to DNA(P) ratio around 1:30).[12,13] Higher metal ion concentrations lead to interactions with bases which destabilize the nucleic acid helical structure. This markedly decreases the melting point (T_m) and promotes transition to the random coil state. On the other hand, copper also facilitates the renaturation process of DNA.[11,14] Behavior similar to Cu(II) was found for Hg(II) (it forms T-Hg-T links) and Ag(I) (binding to G-C rich regions).[2,15]

In the case of RNAs divalent metal ions, like Cu(II), Zn(II), and Pb(II) have more destructive effects on nucleic acid structure than in the case of DNAs. These metal ions catalyze the nonenzymatic cleavage of the phosphodiester bond.[16,17] Metal ions, like Mn(II), Cd(II), Ni(II), and Zn(II), bind to various sites, helical or loops like ones, of the t-RNA structure both in a cooperative and non-cooperative manner.[18–21]

The above examples relate to simple metal ion species (aquo-ions) not involved in coordination with ligands more complicated than water. Very often the metal species interacting with a nucleic acid is a more or less complicated complex molecule in which some metal binding sites are occupied by effective ligands. These ligands can influence considerably the metal-nucleic acid interactions. One of the best studied series of complexes is the platinum chloramines from $[PtCl_4]^{2-}$ to $[Pt(NH_3)_4]^{2+}$.[7,22] One of these species is cisplatin, *cis*- $[Pt(NH_3)_2Cl_2]$, (DDP), a widely used inorganic anticancer drug.[22,23] Cisplatin reacts with DNA in the cell nucleus, but the mechanism of its anticancer activity is not yet understood. One of the key issues in platinum drug pharmacology is to explain why *trans*-$[Pt(NH_3)_2Cl_2]$ is clinically almost completely inactive while *cis*-isomer is a very effective drug.[22,23] In both complexes chlorides are leaving groups, and the reaction with DNA is a two-step process. Initial binding leads to monofunctional adducts followed by closure to form bifunctional lesions. The structures of monofunctional intermediates are difficult to determine because of their transient nature. The preferred site of initial binding for both *cis*- and trans-DDP to DNA is the N_7 of guanine.[22] The monofunctional adducts of *cis*-DDP could close to form a chelate between the N_7 and O_6 positions of a single guanosine base.[2,7,22] This, however, has never been directly proven. The predominant bifunctional adducts formed by *cis*-DDP and DNA *in vitro* (and *in vivo*) are cross-links with dinucleotides containing two deoxyguanosines

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(>60%) or deoxyguanosine and deoxyadenosine (~22%). The binding sites are predominantly N_7 sites of purines.

The inert metal-amine complexes, like $[Co(NH_3)_6]^{3+}$, interact with nucleic acids *via* outer-sphere binding. They are very effective in stabilizing secondary and tertiary structures of DNA and t-RNA.[24–26] The inert nature of these complexes induces the outer sphere mechanism with bonding through coulombic attraction.[6] Hydrogen-bonding, either directly to phosphates or *via* water molecules, can also stabilize the helix structure. This kind of interaction has been observed in the initial binding of $[Ru(NH_3)_5H_2O]^{2+}$ to DNA.[27] This type of complexe also induces the B ⇒ Z transition in poly(dG-dC)·poly(dG-dC).[28]

The X-ray structure of $[Co(NH_3)_6]^{3+}$ bound to yeast phenylalanine to-RNA[29] has shown selective binding to purine-purine sequences especially guanines *via* hydrogen bonds to N_7 and O_6 atoms and to neighboring phosphates in three regions of t-RNA. No direct metal-nucleic acid coordination occurs, in contrast to $[Co(H_2O)_6]^{2+}$, which binds directly to N(7) of G(15) residue.[30] This complex induces the B ⇒ C transition.

2. *Intercalation*

The term 'intercalation' defines the binding of aromatic planar molecules. The classical interaction involves the insertion of a planar molecule between neighboring base pairs of DNA to which it is held by van der Waals forces.[31] This requires the extended helix and locally unwound DNA. The hydrodynamic parameters of DNA changes; the viscosity increases while the sedimentation coefficient decreases. The transition melting point also increases due to an intercalator-base pair stacking interaction which stabilizes the helical over the unwound form.

Many intercalators have biological activity. The platinum complexes with planar ligands, such as bipyridine, terpyridine, or o-phenantroline, bind to DNA or RNA by the intercalation mechanism. A similar pyridine derivative $[Pt(en)py_2]^{2+}$, in which pyridines are forced by nonbonded steric constraints out of the coordination plane, does not intercalate.[32]

Chelating ligands, like 1,10-phenanthroline or 2,2'-bipyridine (bipy), and their metal complexes are also intercalating agents. In this case coplanarity is not always possible as the geometry varies from octahedral $[M(phen)_3]$ to planar or tetrahedral $[M(phen)_2]$. These complexes have been studied extensively for their biological activity.[33] Zinc(II) complexes $[Zn(phen)Cl_2]$, $Zn(phen)_2]^{2+}$, and octahedral $[Zn(phen)_3]^{2+}$ were shown to unwind closed circular DNA by intercalation.[7]

Octahedral chelates, $[M(L\text{-}L)_3]$, are enantiomeric, and racemic forms have different affinities for DNA. The interaction is stereoselective as found, *e.g.*, for $[Ru(phen)_3]$ complexes.[34] Both optical isomers of $[Ru(phen)_3]$ bind to the left handed Z-DNA while only the Δ isomer interacts significantly with B-DNA.[7]

The other group of effective intercalators includes metalloporphyrins. A copper complex interacts with poly(dG-dC) and could be analyzed by the neighbor exclusion model of intercalation while complexes with Ni(II), Co(II) Zn(II), Fe(III) and Mn(III) react only with poly(dA-dT) as well as with DNA, presumably, electrostatically.

Many intercalators show antitumor activity both *in vitro* and *in vivo*.[35,36] Such activity has been found for the acridine orange — platinum (ethylenediamine)

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complex. In this complex, the acridine moiety modulates the affinity of the platinum complex to DNA. A synergistic combination of DNA recognition (intercalator) and DNA fixation (metal complex) could substantially broaden the types of active metal species.[37]

3. Strand Breakage

Metal ions are clearly useful in promoting cleavage of nucleic acids. In enzymatic systems they function by activating the phosphate ester bond for hydrolysis, either electrophilically *via* direct coordination or by delivery of coordinated nucleophiles. In synthetic systems the metal functions primarily as a redox system. The synthetic nucleases depend on metal ions for activity, but do not react catalytically in the true sense.

A good example of synthetic metallonuclease is methidiumpropyl — EDTA — Fe(II) (MPE-Fe(II)). This nuclease is composed of two functional regions, methidium, an aromatic intercalator which binds through the minor groove of DNA, and Fe(II)-EDTA, linked through a short hydrocarbon chain. MPE-Fe(II) cleaves DNA in two steps: i) the binding of the methidium group to DNA, delivering high local concentrations of reactive metal species to the DNA-ribose backbone and ii) the generation of reactive species at the metal center which is responsible for strand scission. In the presence of ferrous ion and oxygen, MPE-Fe(II) causes single-strand breaks in DNA.[38] A metal ion is essential to the activity of MPE, as is the case with metalloenzyme systems. However, to cleave DNA strands iron acts as a redox center, while in natural nucleasees metals acting as effective Lewis-acids, like Zn(II) Mg(II) or Ca(II), are required.

Copper-1(1,10 phenanthroline) complexes cleave DNA in an oxygen dependent reaction. The radical species are generated through one-election oxidation of a bound copper complex by hydrogen peroxide.[38] The Cu(II)-(phen) complex intercalates into the helix, and radicals produced in close vicinity of the DNA molecule are particularly damaging. The A-, B-, and Z-DNA react with this type of complex with different rates.[39]

Metalloporphyrins can cleave DNA through either chemical or photochemical activation. Porphyrins, four-coordinate structures, intercalate to G-C rich regions of double helical DNA, cleaving, after activation, the nucleic acid strands. This process strongly depends on the nature of the coordinated meal ion and the activator used.[39]

Electrochemical Behavior of Nucleic Acids

There is rich experimental evidence concerning the modes of interaction of nucleic acids with various kinds of membranes. An electric-field induced cell-to-cell fusion has been demonstrated as well.[40] The membrane electric fields are limited in length to the same orders of magnitude as those produced on electrodes. Similarly to the mercury electrode surface, the biological charged membranes are of hydrophobic character. The interaction of biomolecules, especially nucleic acids, with the surface of the mercury electrode may represent a model of nucleic acid interaction with various biological surfaces. It has been shown, for example, that DNA replication in procaryotic cells proceeds within a complex of membrane and DNA.[41] It has also been shown that double stranded polynucleotides adsorbed on a natural or a

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synthetic phospholipid membrane have their secondary structure significantly changed.[42] Different experiments suggest the opening of the double helix with the base adhering to the membrane.

Recently, significant progress has been made in the study of the properties and structure of nucleic acids using voltammetric methods involving mercury electrodes. Adsorption of biomacromolecules (*e.g.* nucleic acids) is quite different from that of small molecules. The determination of the adsorbed biopolymer conformation is one of the most important questions in adsorption studies.

DNA molecules are very long polymers. The bacterial chromosome of *E. Coli* is formed by a single molecule of double helical DNA which consists of 3.4 million base pairs. Its contour length, 1.2 mm, corresponds to a macroscopic dimension while its width, 20 Å, is on the atomic scale. Even at pH around three each phosphate group in DNA carries a single negative charge. There are also ionizable groups present in the nucleic bases, including ring nitrogens. DNA, is in fact, an anionic polyelectrolyte at pH above four. The negative charges of the phosphate groups play an important role in stabilization of nucleic acid conformation. Double-stranded DNA is sensitive to changes in ionic strength below $0.1–0.2\ \mathrm{mol\ dm^{-3}}$ while in a single-stranded DNA the charge repulsion becomes significant at ionic strength $1.0\ \mathrm{mol\ dm^{-3}}$. The double helical structure of DNA yields a rigid cylinder as a model for the shape of the molecule and behaves in solution as a polymer of limited flexibility. The rod configuration is observed when the molecular weight is below 3×10^5. Single-stranded DNA is flexible in contrast to the stiffness of double helical DNA.

The process of the reduction or oxidation of the biopolymer at an electrode is not easy to understand. Nucleic acids contain a number of electroactive sites, whose reducibility or oxidizability may be affected by their position in the ordered structure and by the adsorbtivity of the given segment of the biomolecule. In the study of structures and properties of natural DNAs and RNAs synthetic polynucleotides are very useful models.

In nucleic acids the elctroactive groups are only bases.[43] Adenine (A) and cytosine (C) are reduced at mercury electrodes while adenine and guanine (G) are oxidized at graphite electrodes. The reduction sites of A and C form part of the hydrogen-bond system involved in the helical structure, while the oxidation sites in A and G are far away from this hydrogen-bond system. The reduction of A and C takes place only when both of these bases are protonated at N_1 (A) and N_3 (C), which happens at pH less than 7.[44,45] The reduction schemes of protonated adenine and cytosine are given in Scheme 1.[44,45]

In direct current (DC) polarography, native calf thymus DNA and double-stranded DNAs show no reduction waves at room temperature even at high concentrations. Denatured DNA and other single-stranded polynucleotides containing adenine and/or cytosine are polarographically reducible while polynucleotides with only non-reducible bases are inactive in the DC method.[46]

Due to its low sensitivity DC polarography is not very suitable for DNA analysis. Much better results were obtained when differential pulse polarography (DPP) was used. The behaviour of native and denatured DNAs during the DPP reduction is shown in Fig 3. Both the double- and single-stranded DNA produce peak I at -1.1 V while peak II at -1.375 V and peak III at -1.450 V are characteristic of the native and denatured DNA molecules, respectively. The first peak (I) does not depend distinctly on the DNA conformation and from the analytical point of view

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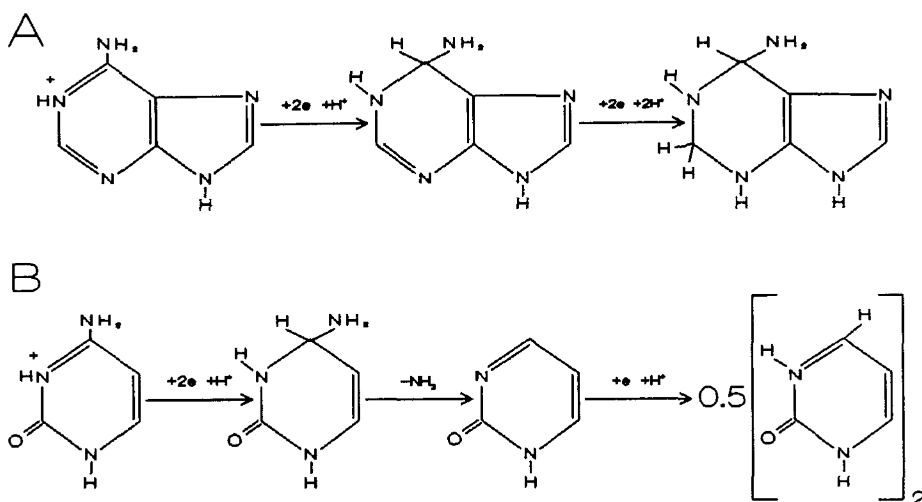

Scheme 1 Scheme of electrochemical reduction of protonated adenine (A) and cytosine (B).

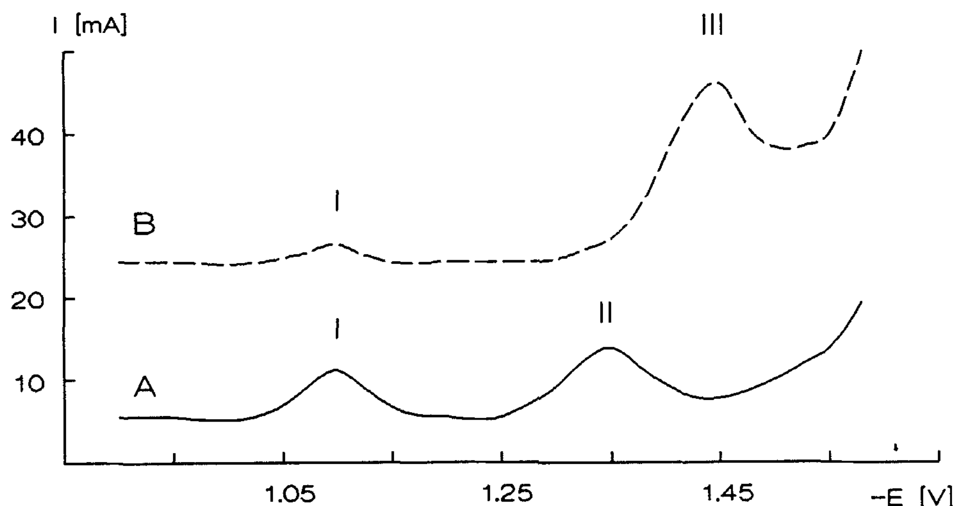

Figure 3 The DPP reduction of native (double stranded) DNA (A), and denatured (single stranded) DNA (B), respectively. (in 0.2M ammonium formate, pH = 7.0, SCE as a reference electrode).

is not very interesting. This peak is non-Faradaic (capacitive).[46,47] Peaks II and III which represent the reduction of A and C bases are of great analytical significance. It has been shown that all reducible single-stranded polynucleotides produce well developed peaks similar to peak III of the denatured DNA.[46,48,49]

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Most double-stranded polynucleotides produce peaks very close to peak II of the native double helical DNA.[48,49] To explain the DP polarogram of native DNA and the origin of peak II it was proposed that small fragments of native DNA differ from the classical B-form. Partially destabilized (unwounded) fragments allow bases to be more accessible to the electrode process.[46,49] The sources of these local changes in the DNA conformation are different, *e.g.*: anomalies in the primary structure of the double helical DNA, bending and kinking of DNA, single-stranded interruptions, rare or damaged bases, mismatched bases, etc. The DPP signal of the native DNA grows markedly with increasingly temperature in the temperature range below denaturation.[49] This phenomenon is called the premelting state. This premelting state depends on the nucleotide sequence.

Peak III of single-stranded polynucleotides is used to evaluate nucleic acid denaturation.[45,49,50] Changes in the height of this peak monitor the course of denaturation or renaturation of DNA or double-stranded RNA, and it can be used to detect even traces of single-stranded material in the native DNA, RNA and other double helical polynucleotides.[49,51]

Chemical modification involving a small amount of nucleotide residues in the double helical DNA is sensitively reflected by changes in peaks II and/or III. Formation of single-strand breaks (ssb) and double strand breaks (dsb) results in an increase in peak II without changes in its reduction potential. Modification of the guanine residue by some carcinogens (*e.g.*, N-acetoxy-N-2-acetyloaminofluorene)[52] creates locally disorganized regions in the double helix and causes an increase in peak II while shifting its potential towards more negative values.

Formation of relatively short, single-stranded regions, *e.g.*, due to splitting of the opposite strand in the DNA double helix by exonuclease III, results in the appearance of peak III.[46]

DPP is currently used in studies of DNA damaged by various agents and the results suggest that this technique possesses great sensitivity and a high degree of selectivity for detection of different types of structural defects in the double helix of DNA. In the DPP measurements with DME the change in potential during the lifetime of a mercury drop electrode is small. Thus, there are no major variations of the structure of adsorbed nucleic acid in comparison to that of DNA in bulk solution. On the contrary, measurements with a hanging mercury drop electrode (HMDE) yield information about conformational changes on the surface due to prolonged DNA interaction with the electrode charged to a certain potential.[46,53,54]

In linear sweep voltammetry (LSV), HMDE is kept for a certain time interval at the initial potential E_i. During this waiting time DNA is adosrbed at the electrode and may undergo changes in its secondary structure due to its prolonged interaction with the electrode surface. The height of the voltammetric peak is proportional to the amount of single-stranded (ss) DNA reduced at the electrode surface, and serves as an indication of the extent of surface denaturation of double-stranded DNA.[53,54] Opening of the double helix due to the interaction of DNA with the electrode surface was demonstrated in 1972.[55] At pH ~ 7, the opening process is relatively slow (about 100 s) and is limited to potentials close to -1.2 V *vs* SCE. This potential region is called region U. The opening of a double helix has been explained by strains in the DNA molecule derived from the strong repulsion between the negatively charged phosphates and the electrode surface to which the polynucleotide chain is anchored *via* hydrophobic bases. In a wide range of more positive potentials, centered at -0.6 V (region T), faster changes occur, which are limited

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to several percent of the molecule in the vicinity of certain anomalies in the DNA primary structures, *e.g.*, single-strand breaks. Changes in ionic strength influences both potentials in the region U and intensities in the region T.[46,55,56] It should be noted that contact of the double-stranded DNA with the mercury pool electrode for about 70 min begins the process of opening the double helical structure of DNA.[57]

The voltammetric peak of denatured DNA adsorbed on an electrode corresponds to a DNA structure with maximum accessible bases, and it is possible to estimate quantitatively the fraction of DNA labilized in solution by such processes as γ-irradiation, methylation, etc.[58,59] In the case of chemical methylation of native DNA, three to four base pairs are labilized when one base is methylated.[60] The γ-irradiation destabilizes 3 to 8% of the base pairs per kilorad (10 Gy).[58,59] LSV is found to be a very sensitive method and can be used in conformational analysis.

Adsorption of DNA at a mercury-electrolyte interface can be studied by measuring changes in the differential capacity of the electrical double layer of the electrode. This can be done by alternating current (AC) polarography. In this technique, variations in the differential capacity are detected at adsorption potentials not far from the zero-charge potential of the mercury electrode (*i.e.*, −0.48 V *vs* a reference saturated calomel electrode, SCE, in 0.2 M ammonium formate). With the help of HMDE potentiostatically controlled at the adsorption potential, the time course of DNA adsorption can be followed after superimposition of AC voltage (amplitude 5–10 mV) and measuring the decrease of the alternating current flowing through the interface. Adsorption of DNA at the electrode surface proceeds irreversibly and depends on the ionic strength, the form of the macromolecule, the medium, pH and the molecular weight of DNA.[46,61]

The AC voltammetric technique can be developed to probe the macromolecular damage caused by mutagens carcinogens,[46,62] and other substances. The method is based on diminuation of the AC signal with time corresponding to changes of the capacity of the electric double layer at HMDE, Diffusion controlled adsorption of DNA molecules from the bulk of the solution causes progressive diminuation of the capacity of the electric double layer. The time corresponding to saturation of the electrode surface depends on the diffusion coefficient of the DNA species which is modified by the action of alkylating mutagens. The time of saturation decreases with the decrease of the percentage of the methylated bases of DNA.[62] Highly methylated DNA (11.5% of methylated bases) causes rapid saturation of the electrode surface with an adsorption time 30–40 times lower than that obtained for native DNA. The sensitivity of detection of the AC method is one methylated base per 200 intact bases. If circular DNA is used instead of calf thymus DNA, the sensitivity can be improved by one order of magnitude or more.[62] The AC test requires no more than 10 min.

Electrochemical Approach to Metal-Nucleic Acid Interactions

It has been mentioned in the preceding section that various polarographic techniques can be applied to different kinds of investigations of the state of the nucleic acid molecule such as its conformations and changes in double helical structure which occur on the electrode surface.[63]

The significance of modern electrochemical techniques in the study of genetic material lies in the high sensitivity of these methods for small perturbations in the double helical structure. Polarographic methods ignore thousands of base pairs

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regularly arranged in the double helix and recognize the presence of a small number of bases which form anomalies in the native structure of nucleic acid. There are only a few techniques which can be applied for similar purposes (*e.g.*, enzymatic techniques using certain specific nucleases).

Our knowledge concerning the interaction of nucleic acids with different electrically charged interfaces in a cell *in vivo* is still limited. Thus, electrochemical methods may serve as extra physiological models of interactions of nucleic acid with charged surfaces. In addition all measurements are performed in aqueous solutions with physiological salts (Na^+, K^+) at neutral pH as a function of temperature.

Thus, the results obtained from modern electrochemical methods for the nucleic acid — metal ion system can offer us:

1. valuable information on the properties and structure of a nucleic acid in solution and on the charged surface;
2. information on the influence of different substances, including metal ions, on the structure of these biomolecules;
3. the mode of action for a metal ion, whose half-potential (or peak potential) is a function of electron density and biophysical properties;
4. information about the amount of metal ion bound to a nucleic acid and the structure and stability of the complexes formed;
5. a dynamic model for the interaction between metal ion and nucleic acid and information about consequences of such an interaction in the presence of charged surfaces;
6. the possibility to study very low concentrations of metal ions and DNA.

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It should also be mentioned that, there is a relatively new, extremely sensitive spectroscopic method, related to electrochemical methods called surface enhanced Raman scattering (SERS) spectroscopy. This method was first applied to the study of adsorbed nucleic acids in 1979.[64]

This technique allows one to observe the vibrational spectra of biomolecules adsorbed at a positively charged silver surface. SERS spectroscopy is a very sensitive method of detection for moieties of an adsorbed biopolymer situated close to a charged surface.

Native DNA exhibits 30 to 40 normal Raman scattering bands in the spectral range 200–1800 cm^{-1}.[65] The more intense bands are caused by vibrations of the bases (A,G,C and T). The local destabilization of the adsorbed DNA induced by interfacial forces originates from the charged silver surface.[53,66] Thermal denaturation of calf-thymus DNA has also been studied by the SERS technique.[65,67] The results indicate that the bands assigned to nucleic bases are sensitive to the thermal transition from the helical double-stranded to the disordered single-stranded structure. In this process the DNA strands become open, and the corresponding bases easily reorientate to interact directly with the surface.

The SERS results obtained for methylated and γ-irradiated DNA adsorbed at charged interfaces agree with the results of previous studies performed with voltammetric techniques.[59,66,68]

1. Osmium-Textroxide Nucleic Acid Systems

Osmium tetroxide has been found to be a very suitable electroactive marker for structural analysis of nucleic acids by electrochemical methods. It is polarographi-

cally reducible in its free state. In the presence of DNA osmium tetroxide binds covalently to the 5–6 double bond of pyrimidine residues in single-stranded fragments of nucleic acids. The DNA bound osmium forms an electroactive probe detectable by means of polarographic techniques.[47,69,70] The osmium bound to denatured DNA produces several DPP peaks. Some can be used to evaluate the amount of denatured DNA over a wide concentration range.[47,69,70]

Native DNA was assumed until recently to be non-reactive with osmium tetroxide.[71] It has been shown, however, that OsO_4 can react with labilized fragments of double-stranded DNA although to a limited extent.[47,69] The amount of osmium bound to DNA increases in the presence of structural defects induced in DNA by various factors such as ionizing and UV radiation.[47,69,70]

2. Interaction of Platinum Complexes with DNA

A number of platinum complexes having anticancer activity are polarographically active.[72–74] Cis-dichlorodiaminoplatimun(II) (*cis*DDP) exhibits in DPP a reduction peak around -1.5 V, whose height is proportional to the concentration of *cis*DDP in the concentration range 10^{-6} to 10^{-2} mol dm^{-3}. The determination of unbound platinum drug is possible in a wide range of concentrations.[72] The binding of an active platinum drug results in minor conformational changes of DNA, with the double helical structure conserved.[75] The interaction of antitumour platinum complexes with native DNA at low levels of binding (0.001–0.02 metal per phosphate, r_b) does not result in the formation of single-stranded DNA. The results obtained for the influence of platinum compounds on the stability of DNA during the thermal denaturation demonstrate that *cis*DDP and *cis*-dichloro-*bis*-isopropylamino-platinum (II) cause the melting point to decrease considerably with increasing r_b, while other active compounds affect T_m only slightly.

The interaction of inactive antitumour platinum complexes induces more severe alterations which have the character of denaturation of longer regions of DNA.[75] It has also been demonstrated that active antitumour compounds of tetravalent platinum bind to DNA without prior reduction. This binding can induce conformational alterations of DNA similar to those caused by *cis*-DDP.[73,74] The binding of antitumour active dichloro-Pt(IV) complexes to DNA is weaker than the binding of their Pt(II) analogs.[75]

Recent study of the *cis*DDP interaction with DNA suggests that the major binding mode of this complex is intrastrand coordination to two consecutive guanine bases.[76]

The effect of platinum interaction with DNA adsorbed on a charged mercury electrode differs from that found in the bulk of the solution.[77,78] Modification of the DNA structure by bifunctional platinum compounds (*cis*- and *trans*-DDP) results in a substantial lowering of the extent of the interfacial conformational rearrangement, the modification by *trans*-DDP being more effective. Monofunctional *dien*-Pt(II) influences the unwinding of DNA on the mercury electrode only negligibly. It has been concluded that interstrand cross-links induced in DNA by platinum compounds are responsible for the antitumour activity of these drugs.[77,78]

The SERS technique has been used to follow the interfacial behavior of DNA modified by platinum complexes.[65,79] The presence or absence of specific SERS bands yields information concerning the stereochemistry of Pt binding to a nucleic acid. It has been suggested that *cis*-DDP coordinates to DNA in the interior of the

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doule-helical structure, probably between adjacent guanines[79] as no Pt-N(7) stretch at 514 cm^{-1} could be observed. On the other hand, the characteristic symmetric Pt-N vibration at this frequency is observed for the [Pt(dien)Cl]-DNA system indicating a preferential binding of monofunctional complex outside the double-helical structure.[65,79]

3. Interaction of Copper(II) Ions with DNA

The interaction of cupric ions with nucleic acids is rather well established[11,15,35,80,] although some controversies concerning the exact model still exist.[81,82] It is generally accepted, that Cu(II) ions may induce the renaturation or denaturation of DNA and that the concentration of metal ions and ionic strength are critical for these processes. Two kinds of Cu(II) coordination to DNA are proposed: denaturing and non-denaturing.[81] Polarographic studies[13,14,63,80] have shown that local labilization of the double-helical structure influence significantly the mode of metal interaction with nucleic acid. It has been suggested that defects caused by electrochemical reduction in the DNA structure induce the unwinding process of the double helix, which favors metal interactions with nucleic bases. Cu(II) ions are much more effective in coordination with nucleic acids than many other metal ions. The effect of cupric ions on DPP reduction is seen immediately after addition of metal salt to DNA solutions.[14,64,80] Strong involvement of cupric ions in the binding of nucleic bases is particularly resident when DNA structure is labilized by polarographic reduction.[80] The DPP experiment has also shown the renaturing ability of the Cu(II) ions.[63]

Cu(II) interaction with calf-thymus DNA was studied by means of DPP and sweep voltammetry.[14] Most of the complexes formed at high ionic strength (0.2 M) and low metal concentration are nondenaturing. Their formation has a minor effect on unwinding of DNA. Excess Cu(II) leads, however, to distinct denaturation of the DNA structure, through formation of very strong bonds with nucleic acid bases (preferentially guanosines). Metal ions have little effect on denaturation induced by polarographic reduction on a mercury electrode.[14] Cupric ions have no detectable renaturing ability towards thermally denatured DNA at 0.2 M ionic strength while this ability is clearly seen when ionic strength is low (0.05 M).[14,63,80] Additional information could be obtained from the reduction wave of the metal ion measured in the presence of DNA. The behavior of the metal reduction DPP peak suggested, among other things, that even at low metal concentrations Cu(II) binds directly to nucleic bases.[80] Very effective involvement of Cu(II) ions in direct interaction with nucleic bases supports the conclusion that the major species existing in Cu(II)-DNA solutions, especially after DNA unwinding caused by DPP, is the denatured Cu(II)-DNA complex.[63,80]

4. Interaction of Ni(II), Zn(II) and Cd(II) Ions with DNA, RNA and Nucleic Acid Subunits

The application of polarographic techniques to the study of the Ni(II) interaction with nucleic acids is discussed in detail in the literature.[83,84,86] Although electrochemistry of Ni(II) complexes is a rather complicated process, the results obtained for Ni(II) complexes with nucleic acids and their subunits allow one to draw several very valuable conclusions. The interaction of Ni(II) with phosphate groups leads to

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a decrease of the limiting current of the metal reduction waves while Ni(II)-base coordination causes splitting of the DPP reduction waves (peaks). Thus, a distinction between complexation of bases and phosphates is an easy task. This allows one to evaluate the stability constants of Ni(II) complexes with bases, nucleosides and nucleoside phosphates (Table 1). The values of stability constants agree with those obtained by other techniques.[87,88] The relatively high stability found for metal nucleoside-triphosphate systems (Table 1) may indicate the formation of the macrochelate species involving base and phosphate coordination to the same metal ion.[88] Thus, in the case of nucleic acid subunits, both the base and phosphate serve as donors for Ni(II) binding.

The addition of Ni(II) ions to DNA containing solutions decreases the limiting current of the DNA reduction peak.[63,80] This suggests a stabilization of the double-helical structure of DNA resulting from a Ni(II) interaction with the nucleic acid phosphate chain. Such interactions neutralize the negative charge of phosphates and enhance the hydrogen bonds between the bases while decreasing the repulsive interactions of the negatively charged electrode surface and the phosphates. The latter interactions are the main cause of destabilization of the double-helical structure on a charged electrode. This effect of Ni(II) ions does not depend on the exposition time or the metal to DNA phosphate molar ratio (P).[63] The local breaks in the DNA double-helical structure caused by consecutive polarographic reductions (*vide supra*) changes however, the DPP pattern of the Ni(II)-DNA polarograms. The breaks induce DNA unwinding[63,80] which facilitates the direct metal-base interaction. The DPP polarograms exhibit two metal reduction peaks: one, attributed to free or phopshate bound metal ions, and the other at more positive potential, (−0.9 V) corresponding to base bound Ni(II),[80] Fig. 4a,b. The height of the peak at −0.9 V (Fig. 4a) increases with an increase of the number of destabilized regions of DNA and reaches a maximum for denatured DNA (Fig. 4b). The observed limiting current of the DNA reduction wave is proportional to the member of destabilized fragments of DNA; it is possible to use polarographic methods to evaluate quantitatively the fraction of unwound DNA.[59,63,88] For example, the amount of labilized DNA bases in Ni(II)-DNA solutions reaches 0.3% for P=0.5 while it is equal to 3.5% when P=5.[63,88,89]

The opening of the helical structure of native DNA adsorbed on the electrode is especially effective at HMDE.[47,53] Thus, the application of sweep voltammetry can

Table 1 Stability constants for equimolar complexes of Ni(II) for different nucleic bases, nucleosides, nucleotides and phosphates in 0.2 M $NaClO_4$ measured at pH = 6.0.

Ligand	Log K_1	References
Inosine	3.42±0.1	86
ITP	4.27±0.2	86
Uracil	3.12±0.1	86
Uridine	3.89±0.1	86
ATP	4.30±0.15	86
Phosphate	3.26±0.1	86
Cytosine	3.06	83
Cytidine	3.55	83
2'-Deoxycytidine	3.94	83
2'-Deoxycytidine-5' -Monophosphate	3.90	83

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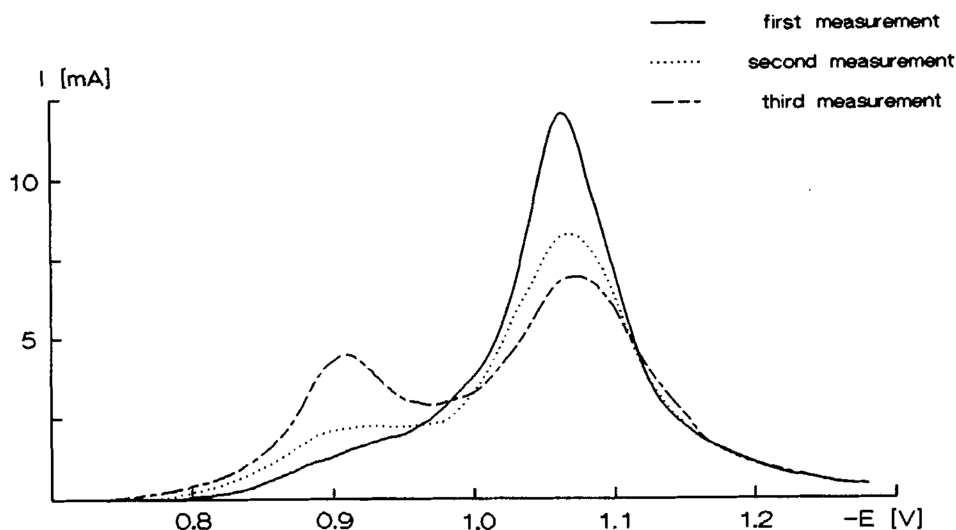

Figure 4a The DPP reduction peaks of Ni(II) obtained for the Ni(II)- native DNA system, measured three times in about 2h intervals (in 0.05M sodium acetate buffer, pH = 5.6, SCE as a reference electrode).

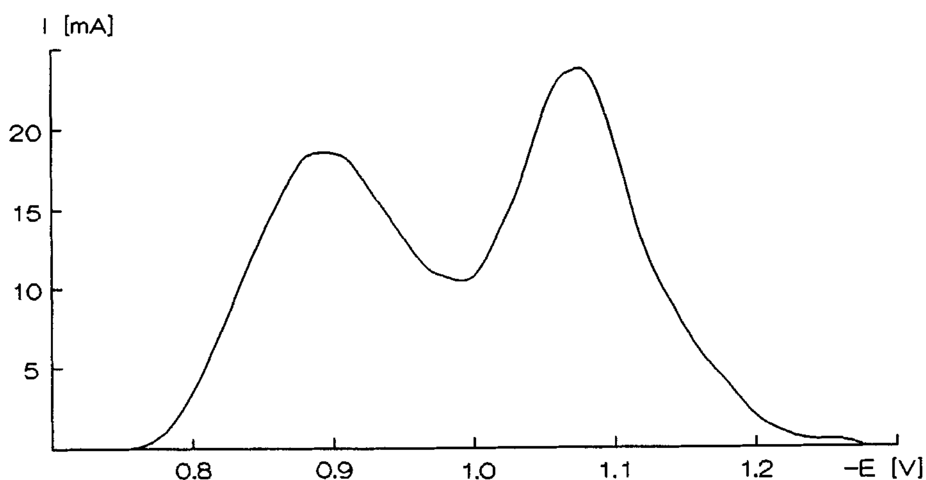

Figure 4b The DPP reduction peaks of Ni(II) for the Ni(II)-denatured DNA system, (in 0.05M sodium acetate buffer, pH = 5.6, SCE as a reference electrode).

supply additional evidence indicating that local labilization of the double-helical structure may distinctly influence the interaction modes between metal ions and nucleic acids. For the Ni(II)-native DNA system the low metal ion ratios, (P=0.5 and P=1), do not influence the DNA structure while an excess of metal (P=5) induces considerable denaturation of DNA adsorbed on HMDE.[63,90] This behavior of the Ni(II)-DNA system on a hanging electrode indicates that at low metal

concentration the major interaction sites of Ni(II) are phosphates (stabilizing effect),[10,11,35] while an excess of Ni(II) will compete in binding to base donors. Thus, the cooperative effect of metal ion coordination to bases and an electric field lead to considerable labilization of the double-helical structure. This conclusion was clearly supported by the study of the Ni(II)-denatured DNA system.[63,90]

The molecular structure of t-RNA is very specific due to the fact that it contains both open (loops) and double-helical fragments which are very well defined. The DPP method has been used to evaluate the interaction of Ni(II) and Zn(II) with t-RNA.[86] Free Ni(II) ions are reduced at −1.0 V. The addition of an excess of t-RNA (low ionic strength 0.005M, no Mg(II) ions) shifts the DPP reduction peak to −1.025 V. This peak is observed until the metal to t-RNA ratio reaches 3:1. At higher metal ratios the second reduction peak appears at −0.826 V and its height increases steadily as Ni(II) is added to the t-RNA solutions, Fig. 5. When Mg(II) ions are added, only one reduction wave at −0.776 V is observed. These results suggest that Ni(II) ions bind to at least two kinds of sites. The first binding site(s) is common for Ni(II) and Mg(II) and both ions compete in their interactions with nucleic acid.[91,92] Analysis of all polarographic data[86] indicates that Ni(II) ions begin their interaction with t-RNA *via* strong-binding sites of Mg(II) ions (phosphate pockets). The Scatchard method[19,85] allows one to establish more precisely two distinguishable phosphate binding sites with different association constants (Table 2). The strong-binding sites contain six Ni(II) ions while for the less specific and weaker interaction with phosphates 25 metal ions are involved.[86] Electrochemical

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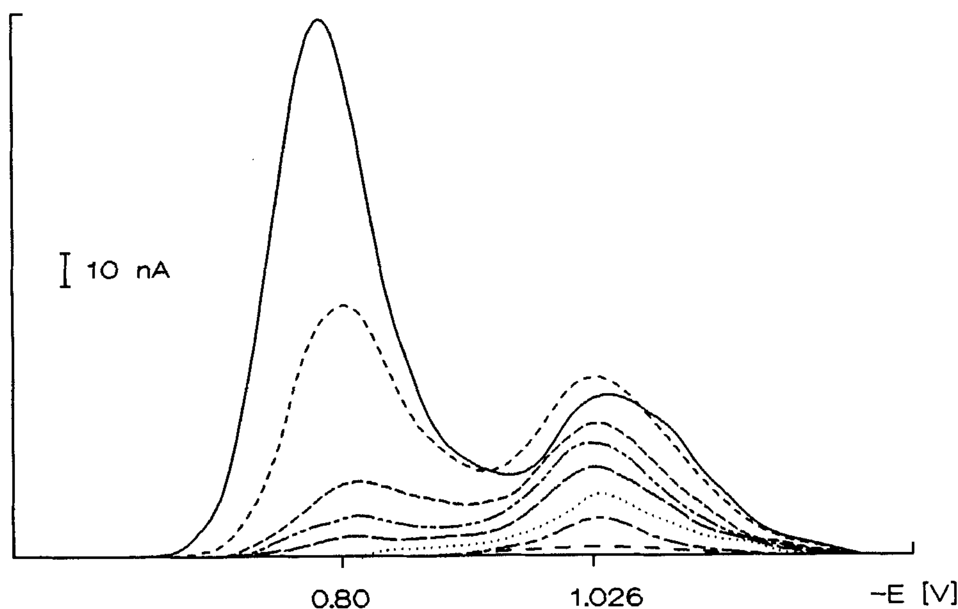

Figure 5 The DPP reduction peaks of Ni(II) in the presence of $tRNA^{Phe}$ (100 μg/ml): c_{Ni} = 2.5 × 10^{-6} (– – – –); 5 × 10^{-6} (—–·); 7.5 × 10^{-6} (·····); 1 × 10^{-5} (— —); 1.50 × 10^{-5} (—·–·—); 1.75 × 10^{-5} (–·–·–); 2.0 × 10^{-5} (-·-·-); 2.25 × 10^{-5} (——) mol dm^{-3}, (in 0.005 M $NaClO_4$, pH = 6.5, SCE as a reference electrode).

Table 2 Association constants K_1 and K_2 and number of available binding sites N1 and N2 for Ni(II), Zn(II), Cd(II), Mn(II), and Mg(II) t-RNA systems.

System	N1	N2	N1 + N2	$K_1[M^{-1}]$	$(\log K_1)$	$K_2[M^{-1}]$	$(\log K_2]$	ref.
Ni-tRNA	6	25	31	7.07×10^5	5.85	7.18×10^3	3.86	86
Ni-tRNA	6	25	31	1.70×10^6	6.23	6.9×10^3	3.84	86
Zn-tRNA	3	39	42	3.80×10^5	5.57	1.00×10^4	4.00	86
Cd-tRNA^{Tyr}								
(a)	6.5	37	43.5	5.60×10^5	5.75	3.00×10^4	4.48	19
(b)	3.5	39	42.5	2.00×10^6	6.30	2.00×10^4	4.30	19
Mn-tRNA^{Phe}	4	38	42	6.50×10^5	5.81	3.00×10^4	4.48	19
Mg-tRNA^{Phe}								
(c)	4–6	19±5	24	9.00×10^4	4.95	6.00×10^3	3.78	104

[a] Polarographic method, at 0.005 M, tris buffer, pH 8, 8°C. [b] As above at 0.02 M tris buffer, pH 8, 10°C. [c] Melting curves, fluorescence technique, 0.01 M sodium cacodylate and 0.022 M NaCl, pH = 6.0, 10°C.

behavior of Ni(II) ions in the presence of t-RNA clearly indicates the involvement of base nitrogens in the reduction mechanism of metal ion, *i.e.*, the direct coordination of some metal ions to the base donor system.

In the case of the DNA-Zn(II) system, metal ions are preferentially bound to the phosphate sugar backbone, although some involvement of base coordination is also suggested.[10,11,15] Zinc is found to decrease the melting point at a high metal to DNA molar ratio and induces the renaturation process. The latter process suggests the formation of metal bridges between complementary bases of opposite strands in the unwound state. The preferential sites for Zn(II) ions are assumed to be G-C pairs with chelation involving N(7) of guanine and the phosphate group.[93] The DPP and sweep voltammetry (SV) studies of the Zn(II) and Cd(II) interactions with DNA have shown that even low concentrations of Zn(II) ions stimulate the unwinding process on a mercury electrode.[20,93] It is also possible that at low ionic strength metal ions increase the DNA adsorption on the electrode by ordering its structure. This conclusion is strongly supported by the SV results.[20,94]

As mentioned above, the use of DPP allows one to evaluate the concentration of unbound metal ion. Thus, it is possible to apply the Scatchard method in estimation of the association constants and the number of metal ions bound to different binding sites of the biopolymer. The data obtained for Zh(II)-t-RNA,[86] Cd(II), and Mn(II)-t-RNA[19] are summarized in Table 2. In all cases two types of binding sites are found. The stronger, pocket-like site is similar to that found for Mg(II) ions while the other sites are less specific, weaker, and more populated. These interactions are mostly of electrostatic nature, and depend strongly on the ionic strength, the presence of competitive ions (*i.e.*, Mg(II)) and the charges involved.[19,86] Mg(II) ions, which are essential for t-RNA biochemistry, are competitive with Ni(II), Zn(II), and Cd(II), especially when in excess, as the respective association constants obtained for these metal ions are relatively close to each other (Table 2). The influence of Cd(II) ions on DNA structure is less specific than that of Zn(II).[20,94] Although some stabilizing effect of Cd(II) on the DNA double-helical structure is observed, the renaturation process is considerably less than that in the presence of Zn(II) ions.[94] These conclusions are supported by spectroscopic data.[95,96]

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5. Pb(II) Interactions with DNA

Interaction modes of Pb(II) ions with nucleic acids are much more complicated than those discussed above. A small amount of Pb(II) (P=0.5) has some stabilizing effect on the double-helix of DNA while an increase of metal ion concentration (P=1) induces strong destabilization of the nucleic acid structure.[13,89] The latter interactions also cause unusual two-phase transition observed in the melting profiles.[13] This fact suggests some specificity in the Pb(II)-DNA interactions. To explore more quantitatively this system, a series of voltammetric methods, sweep voltammetry, alternating current voltammetry, cyclic voltammetry, and chronocoulometry, have been applied.[21] The Faradaic and capacitive currents were used to examine the hydrodynamic properties of the DNA and Pb(II)-DNA systems, the complexation of Pb(II) to DNA in solution, and the interfacial behavior of these systems, Fig. 6. The measurements of time dependence of capacitive current at the adsorption potential $E_s = -0.2$ V by AC voltammetry provide information about the adsorption rates of DNA at the HMDE surface. The effect of Pb(II) ions shows that adsorption mechanisms strongly depend on the metal to phosphate ratio (P). Comparison with Pb(II)-free, native DNA shows that in the presence of small amounts of Pb(II) (P = 0.5) the diffusion rate of DNA-bound Pb(II) decreases. At P=1 a nonlinear decrease of capacitive component against time of adsorption indicates interfacial rearrangements, while for P>1, the rapid saturation of the electrode surface suggests that complexation of metal ions induces a large decrease of hydrodynamic volume of DNA (Fig. 6). These conclusions are supported by chromatography on hydroxyapatite and viscosity measurements.[89]

The sweep voltammetric response of DNA can be used to measure the

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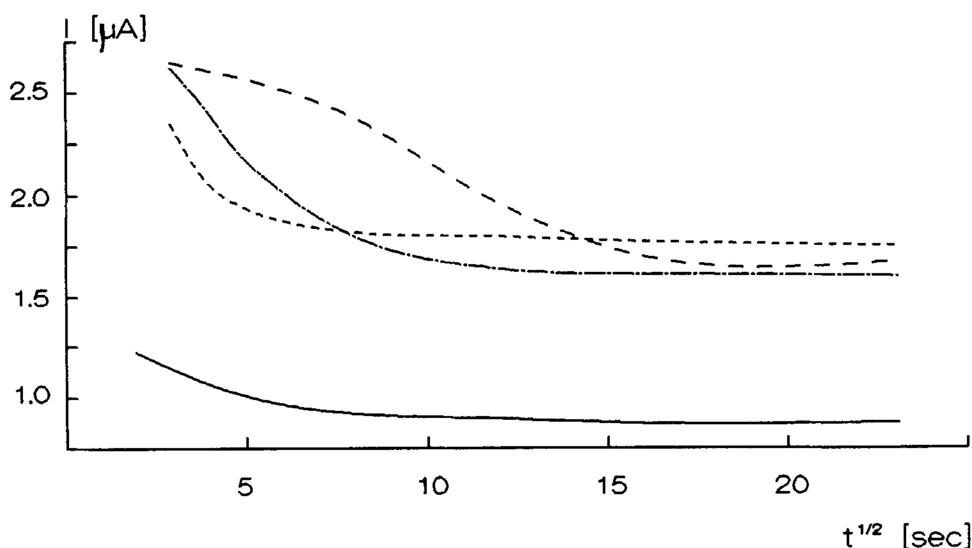

Figure 6 Effect of molecular ratios of Pb(II) / DNA (P): O (·····); 0.5 (–·–·–); 1 (– – –); 3 (——); on the time dependence of the capacitive A.C. current. Adsorption potential $E_S = -0.2$V (v.s.SCE), sodium acatate buffer, pH = 5.6.

accessibility of adenine and cytosine moieties to an electrode process in the presence and absence of metal ions. The results that Pb(II) interferes considerably with the reduction of adenine and cytosine of the adsorbed DNA.[21] For P>1, the Faradic response of Pb(II)- DNA drops to a negligible value. This indicates the compacting process for native DNA, which hinders the accessibility of nucleic base residues to electrochemical reduction.[21,89] For P<2 in the presence of DNA the cyclic voltammograms of Pb(II) show single cathodic and anodic peaks, E_c = −0.480 V, E_a = −0.423 V which can be attributed to labile complexation[21,97], Fig. 7A. For P>2 the situation is more complicated. Cyclic voltammograms display complex shapes, Fig. 7a,b,c and d. Two peaks could be distinguished at E_c = −0.500 V and E_c = −0.530 V. The more positive redox peak at E_c = − 0.500 V is assigned to a diffusion-controlled electrode process similarly to that observed for low P values. The second peak at E_c = − 0.530 V is assigned to strongly adsorbed Pb(II) at the electrode surface.[21] This adsorption of metal ions mediated by a film of condensed DNA has also been examined with a chronocoulometric method. This technique makes possible the calculation of the amount of adsorbed Pb(II) in moles per cm^2 of an electrode surface. The calculations show that at metal concentration up to

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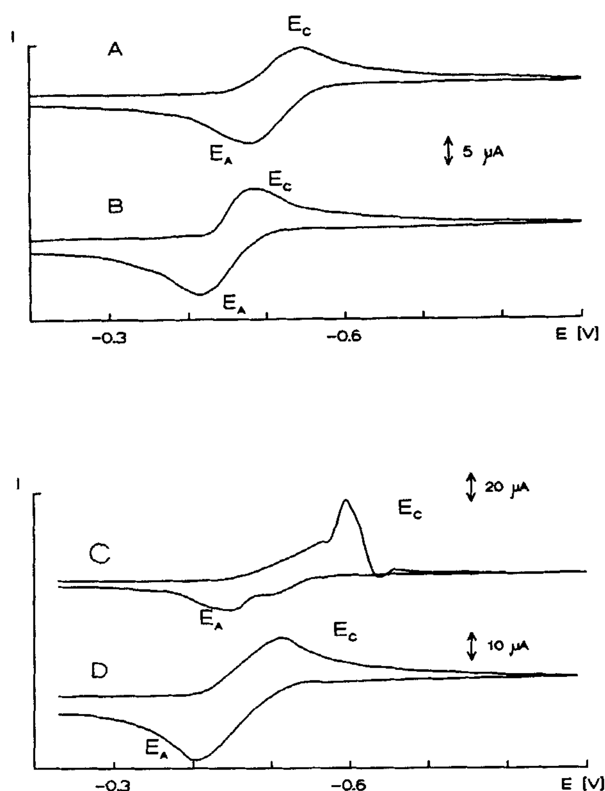

Figure 7 Effect of molecular ratios of Pb(II) / DNA (P) on the cyclic voltammograms of Pb(II). a: P = 1; c_{Pb} = 0.33 mM b: c_{Pb} = 0.33 mM, c: P = 3; c_{Pb} = 0.99 mM; d. c_{Pb} = 0.99 mM. (in sodium acetate buffer, pH = 5.6, SCE as a reference electrode).

6×10^{-4} mol dm^{-3} no variation of the surface excess Γ, (Γ is the amount of adsorbed Pb^{2+} in moles per cm^2), can be detected, regardless of whether DNA is adsorbed or not,[21] Fig. 8. On the other hand, for P>2 an enhanced adsorption of Pb(II) ions at the electrode surface is observed when compared to the Pb(II)-electrolyte system. This result confirms strong adsorption of Pb(II) ions induced by DNA as already observed in CV data. The chronocoulometric titration of DNA with Pb(II) also permits the binding isotherm for the complex with cooperative coordination of Pb(II) to DNA to be obtained. The Scatchard representation confirms this binding mode, Fig. 9.

It should be noted here that spectroscopic methods (*UV, CD*) cannot detect any significant secondary modification of the DNA B-structure for P<3, whereas for P>10 Pb(II) ions favor the C-form of nucleic structure.[13]

Pb(II) ions do not affect significantly the SERS spectra of DNA.[98] Up to P=3 no denaturation of nucleic acid is observed. However, detailed examination of SERS spectra shows some variation of the band intensities depending on P value, indicating some interfacial rearrangements.[98] The direct interaction of lead ions with nucleic acid causes drastic changes in the structure of the DNA molecule as described above. A similar effect was observed for the system containing polyamines[99] or trivalent metal ions.[100,101] Under similar experimental conditions there was no evidence of structural collapse with such metal ions as Mn(II), Zn(II), Mg(II), Cd(II), Cu(II) and Ni(II). The collapse of DNA tertiary structure induced by Pb(II) ions may play an important role in the higher organization of DNA. Indeed, most DNA in living organisms is present in a compact form.[2] Thus, comparison of

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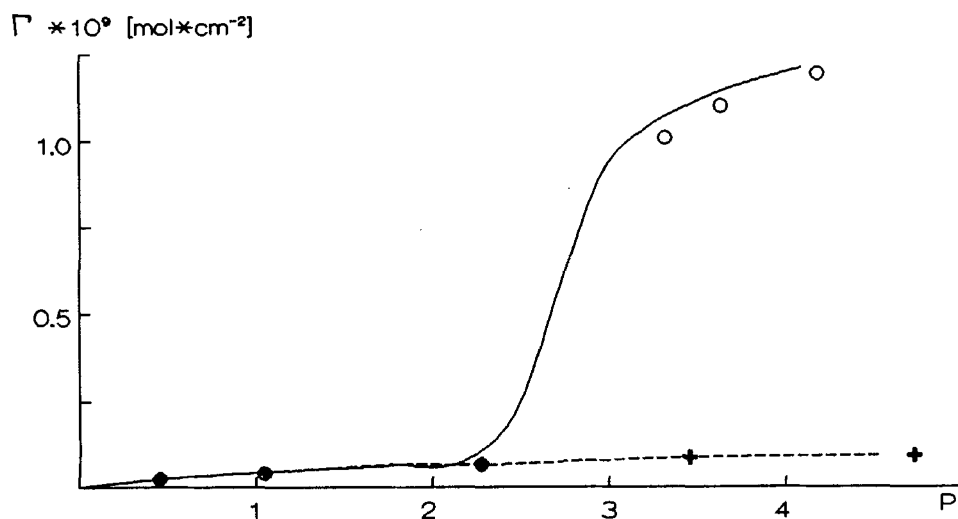

Figure 8 Effect of molecular ratios of Pb(II) / DNA (P) on surface excess of Pb(II), Γ. Pb(II)-buffer (- - -) and Pb(II)-DNA (——), (in 0.05M sodium acetate buffer, pH = 5.6, SCE as a reference electrode).

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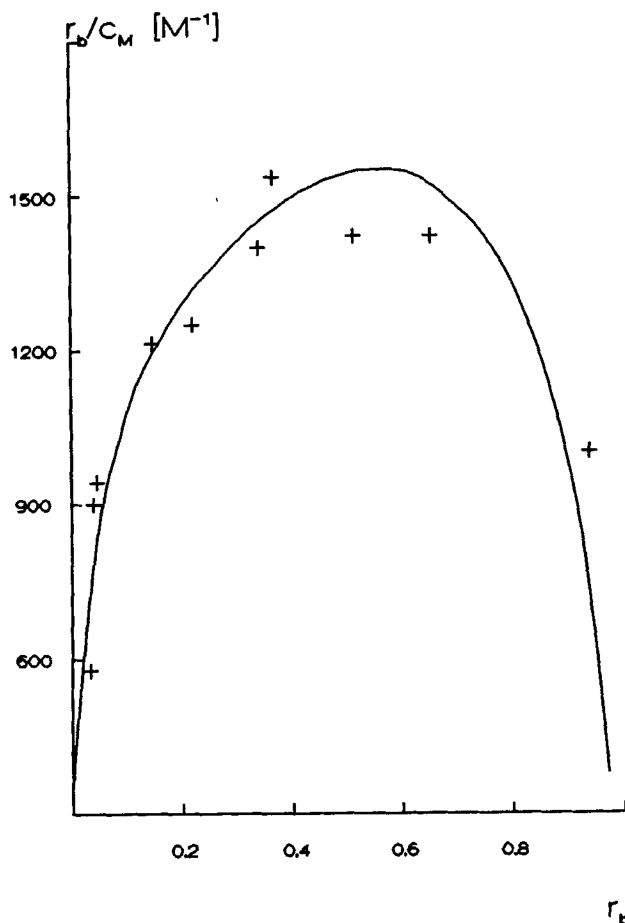

Figure 9 Scatchard plot for Pb(II) / DNA system; r_b – ratio of bound Pb(II) per mononucleotide residue.

this specific effect of lead to essential divalent ions, such as Mg(II), raises the question of whether Pb(II) interferes with the compaction mechanism of DNA.[21]

The interaction of Pb(II) with double-stranded polynucleotides, poly(dA-dT)·poly(dA-dT), and poly(dG-dC)·poly(dG-dC) has been studied with sweep and cyclic voltammetry and alternating current voltammetry.[102] Both polynucleotides are useful models for understanding the structure, and other properties of natural DNAs and RNAs. The polarographic reduction process of model double-helical polynucleotides is very similar to that of native DNA.[48,49] Analysis of the voltammetric reduction peaks indicates variations in double-stranded polymers, which occur as a consequence of polynucleotide interaction with the charged electrode or of other external effects on the helical structure. The voltammetric results show that the interactions of the polynucleotides mentioned above with Pb(II) ions are different for each polymer.[102] According to the AC measurements, the major interaction of lead ions with poly(dG-dC)·poly(dG-dC) involves phos-

phate oxygen atoms. Other voltammetric results agree with this assumption, indicating that metal ions do not facilitate the surface denaturation process to any great extent. This (dG-dC) polymer does not affect the CV peaks of Pb(II) ions in contrast to the metal-acetate system as both ligands coordinate to the metal ion *via* oxygen donors.[102] Considerably different results were obtained for the Pb(II)-poly(dA-dT)·poly(dA-dT) system. Distinct variation of current intensity in the AC polarograms indicates that A-T pairs may be involved directly in the interaction with metal ions *via* the base donors. This binding mode destabilizes the double-helical structure of the polynucleotide. Variations of current density in the AC voltammetry clearly indicate that metal ions, which are in excess, change the hydrodynamic behavior of the (dA-dT) polymer. More direct evidence for different modes of Pb(II) binding to this polymer is provided by the CV measurements in which one can observe two metal reduction peaks when metal ions are in excess. One, at −0.440 V, is similar to that observed for the system mentioned above. A more irreversible reduction peak at −0.515 V corresponds to a different metal complexation involving, most likely, a complex with direct binding, to a base donor system.[102] The same conclusion has been drawn for the Pb(II)-DNA system.[21,89,98] Thus, the model polynucleotides may mimic native DNA and help to explain the binding situation in the Pb(II)-DNA system: metal ions can interact with DNA in two different ways, *via* phosphates in the G-C rich regions and with based donors in the A-T rich regions. This latter interaction may stimulate the DNA unwinding and could be the main reason for the decrease of the melting point in this system.[13,102] Interaction of Pb(II) ions with A-T rich regions has also been suggested by other studies.[95,100]

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6. *Interaction of Eu(III) Ions with DNA*

Nucleic acids were thought to be conformationally related to the right-handed double helical A- or B-DNA. Recent X-ray studies, however, have shown for the $(dC \cdot dG)_n$ alternating copolymer[2] that a left-handed double helical configuration of DNA is also possible. It is termed Z-DNA.[1,2] Poly(dG-dC)·poly(dG-dC) undergoes the B–Z transformation in solutions with rather high ionic strength. The DPP measurements can detect the B–Z transition.[103] It was found that Eu(III) ions induce the B–Z transition in the poly(dG-dC)·poly(dG-dC) system effectively. Polarographic methods allowed examination of both Eu(III) ion complexation and the B–Z transition simultaneously.[103] The B–Z transition in this system was also followed by spectroscopic measurements. It has been calculated from the DPP data that one Eu(III) ion per 10–20 nucleotides is able to induce a B–Z conformational change.

CONCLUSIONS

The significance of modern electrochemical techniques in the study of metal ion interactions with nucleic acids follows among other things, from the fact that they can detect very small perturbations in the double-helical structure as well as from the behavior of single-stranded (denatured) fragments of DNA.

The effectiveness of polarographic methods is comparable to enzymatic techniques which use specific nucleases.

The sensitivity of electrochemical measurements also offers the possibility to follow the behavior of metal ions or nucleic acids in the metal-nucleic acid systems under the circumstances similar to natural conditions. In many cases polarographic data can also be used to evaluate quantitatively the metal ion-nucleic acid interactions, *e.g.*, to calculate the association constants or the number of binding sites, and to specify the nature of metal ion binding to a given coordination site in the biopolymer.

A mercury electrode immersed in nucleic acid containing solutions may serve as a suitable model for the study of interactions of the biopolymer with charged surfaces. As discussed above, these interactions may have critical influence on the binding of metal ions to DNA.

The range of potentials attainable at the mercury electrode, the intensity of the electric field, and the extremely short distances between a molecule and the surface of the electrode (tens of hundreds of Å) are similar to those occurring in living cells. These briefly mentioned advantages of the use of electrochemical methods in studying nucleic acids indicate their important impact on understanding of the basic biological processes.

It is worth mentioning that recent developments in microelectrodes may allow investigation of single molecular events involving DNA molecules.[105,106]

However, it is obvious that correlation between electrochemical results and the data obtained by means of other methods is necessary.

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References

1. W.S. Klug, M.C. Cummings, *Concepts of Genetics*, (Ch. E. Merrill Publ. Comp., Columbus, 1983).
2. W. Saenger, *Principles of Nucleic Acid Structure*, (Springer-Verlag, New York, 1984).
3. D. Watson and F.H.C. Crick, *Nature* **171**, 737 (1953).
4. D.M. Lilley, *Chem. Soc. Rev.* **18**, 53 (1989).
5. J. Ciesiolka, W.J. Krzyzosiak, *Post. Biochem.* **30**, 71 (1984).
6. Hay R.W., *Bio-Inorganic Chemistry* (J. Wiley and Sons, New York, 1984).
7. N. Farrell *Transition Metal Complexes as Drugs and Chemoterapeutic Agents*, (Kluwer Academic Publ. Dordrecht, 1989) vo,. 11.
8. Sposito G. *Metal Ions in Biological Systems* (ed. H. Sigel, Marcell Dekker, New York, 1984), vol. 20 p. 1.
9. S.R Holbrook, J.L. Sussman, R.W. Warrant, G.M. Church and S.H. Kim, *Nucl. Acid Res.* **4**, 2811 (1977).
10. G.L. Eichhorn, *Nature* **194**, 474 (1962).
11. G.L. Eichhorn and Y.A. Shin, *J. Am. Chem. Soc.* **90**, 7323 (1968).
12. M. Langlais, H.A. Tajmir-Riahi and R. Savoie, *Biopolymers* **30**, 743 (1990).
13. K. Gasiorowski, J. Swiatek and H. Kozlowski *Inorg. Chim. Acta* **106**, L1 (1985).
14. T. Pawlowski, B. Gulanowski, J. Swiatek, K. Gasiorowski and H. Kozlowski, *Acta Biochem. Pol.* **36**, 75 (1989).
15. I. Sissoeff, J. Grisvard and E. Guille, *Prog. Biophys. Molec. Biol* **31**, 165 (1976).
16. R.S. Brown, B.E. Hingerthy, J.C. Dewan and A. Klug, *Nature* **303**, 543 (1983).
17. G.L. Eichhorn, E. Tarien and J.J. Butzow, *Biochemistry* **10**, 2014 (1971).
18. A.A. Schreier and P.R. Schimmel, *J. Mol. Biol.* **86**, 601 (1974).
19. L. Kaba, J-M Sequaris, P. Valenta and H.W. Nurnberg *Toxicol. Environm. Chem.* **10**, 103 (1985).
20. J. Swiatek, T. Pawlowski, K. Gasiorowski and H. Kozlowski, *Inorg. Chim. Acta* **138**, 79 (1987).
21. J-M. Sequaris and J. Swiatek, *Bioelectrochem. Bioenerg.* **26**, 15 (1991).
22. C.A. Lepre and S.J. Lippard, *Nucl. Acid Molecular Biol.* (F. Eckstein and D.M. Lilley, eds., Springer Verlag, Berlin 1990) vol. 4, p. 9.
23. B. Rosenberg, Cancer **55**, 2303 (1985).
24. F. Ascoli, M. Branca, C. Mancini and B. Pispisa, *J. Chem. Soc. Faraday Trans* 1213 (1972).

25. R.L. Karpel, N.S. Miller, A.M. Lesk and J.R. Fresco, *J. Mol. Biol.* **97**, 519 (1975).
26. J. Widom and R.L. Baldwin, *J. Mol. Biol.* **144**, 431 (1980).
27. M.J. Clarke, B. Jensen, K.A. Marx and R. Kruger, *Inorg. Chim. Acta* **124**, 13 (1986).
28. J.M. Neumann, S. Tran-Dinh, J.P. Girault, J.C. Chottard, T. Huynh-Dinh and J. Igolen, *Eur. J. Biochem.* **141**, 465 (1984).
29. R.A. Byrd, M.F. Summers, G. Zon, C. Spellmeyer and L.G. Marzilli, *J. Am. Chem. Soc.* **108**, 504 (1986).
30. J.P. Caradonna, S.J. Lippard, M.J. Gait and M. Singh, *J. Am. Chem. Soc.*, **104**, 5793 (1982).
31. M.J. Clarke and H. Taube, *J. Am. Chem. Soc.* **97**, 1397 (1975).
32. K.W. Jennette, S.J. Lippard, G.A. Vassiliades, and W.R. Bauer, *Proc. Natl. Acad. Sci. USA* **71**, 3839, (1974).
33. M.J. Clarke, *Inorg. Chem.* **16**, 738 (1977).
34. M.D. Reily and L.G. Marzilli *J. Am. Chem. Soc.* **108**, 6785 (1986).
35. G.L. Eichhorn *Inorganic Biochemistry* (Elsevier, Amsterdam, 1973), vol. 2
36. S.J.N. Burgmayer and E.I. Steifel *J. Chem. Ed.* **943**, 1 (1985).
37. R. Faggiani, B. Lippert, C.J.L. Lock and R.A. Speranzini, *Inorg. Chem.* **21**, 3216 (1982).
38. L.A. Basile and J.K. Barton, *Metal Ions in Biological Systems* (H. Sigel and A. Sigel eds., Marcel Dekker, New York, 1989), vol. 25, p. 31
39. A.M.J. Fichtinger-Schepman, R.A. Baan, A. Luiten-Schnite, M. Van Dijk and P.M.M. Lohman, *Chem. Biol. Int.* **55**, 275 (1985).
40. S.B. Zimmerman, J. Vienken, *J. Memb. Biol.* **67**, 165 (1982).
41. J.M. Marmon, H.W. Taber, *J. Bacteriol.* **129**, 789 (1977).
42. V.G. Budker, A.A. Godovikov, L.P. Naumova, I.A. Slepneva, *Nucl. Acid Res.* **8**, 2499 (1980).
43. E. Palecek, *Topics in Bioelectrochemistry and Bioenergetics* (G. Milazzo ed., Wiley and Sons, London, 1983), vol. 5. p. 65
44. P.J. Elving, *Topics in Bioelectrochemistry and Bioenergetics* (G. Milazzo ed., Wiley and Sons, London, 1976), vol. 1, p. 179
45. G. Dryhurst, *Electrochemistry of Biological Molecules* (Academic Press, New York, 1977)
46. E. Palecek, *Proc. Conf. Electroanal. Hyg. Environm. Clin. Pharm. Chem.* (W.F. Smyth, ed., Elsevier, London, 1980).
47. E. Palecek, *Bioelectrochem. Bioenerg.* **15**, 275 (1986).
48. F. Jelen and E. Palecek, *Gen. Physiol. Biophys.* **4**, 219 (1985)
49. E. Palecek, *Methods in Enzymology* (L. Grossman and K. Moldave eds., Academic Press, New York, 1971), vol. 21, p. 3
50. E. Palecek and J. Doskocil, *Anal. Biochem.* **60**, 518 (1974).
51. E. Palecek and B.D. Frary, *Arch. Biochem. Biophys.* **115**, 431 (1966).
52. R.P.P. Fuchs, *Nature* **257**, 151 (1975).
53. H.W. Nurnberg, *Bioelectrochemistry* (G. Milazzo and M. Blank eds., Plenum Publ. Corp., New York, 1983), vol. 1
54. E. Palecek, S. Kwee, *Coll. Czech. Chem. Commun.* **44**, 448 (1977).
55. P. Valenta, H.W. Nurnberg, P. Klahre, *Bioelectrochem. Bioenerg.* **1**, 487 (1974).
56. V. Brabec, *Biophys. Chem.* **11**, 1 (1980).
57. V. Brabec and E. Palecek, *Biophys. Chem.* **4**, 79 (1976).
58. J-M. Sequaris, *Electrochemistry, Sensors Analysis* (M.R. Smyth and J.G. Vos eds., Elsevier Sci. Publ., Amsterdam, 1986), p. 191
59. J-M. Sequaris, P. Valenta and H.W. Nurnberg, *Int. J. Radiat. Biol.* **42**, 407 (1982).
60. J-M. Sequaris, H.W. Nurnberg and P. Valenta, *Toxicol. Environm. Chem.* **10**, 83 (1985).
61. B. Malfoy and J. Reynaud *J. Electroanal. Chem.* **67**, 359 (1976).
62. J-M. Sequaris and P. Valenta *J. Electroanal. Chem.* **227**, 11 (1987).
63. H. Kozlowski, T. Pawlowski and J. Swiatek, *Asian J. Chem.* **1**, 93 1989.
64. E. Koglin, J-M. Sequaris, P. Valenta, *Proc., 14th European Congr. Metal Spectr.* 1979, Frankfurt/M.
65. E. Koglin and J-M. Sequaris, *Topics in Current Chemistry* (Springer Verlag, Berlin, 1986), vol. 134, p. 1
66. J-M. Sequaris, *Application de la Voltammetrie a Etude des Acides Deoxyribonucleiques Natifs et Modifies* (These Doctorate d'Etat, Univ. Orleans, 1982).
67. J-M. Sequaris, E. Koglin, P. Valenta, H.W. Nurnberg, *Ber. Bunseng. Phys. Chem.* **85**, 512 (1981).
68. J-M. Sequaris, J. Fritz, H. Lewinsky and E. Koglin, *J. Coll. Int. Sci.* **105**, 417 (1985).
69. E. Lukasova, F. Jelen and E. Palecek, *Gen. Physiol. Biophys.* **1**, 53 (1982).

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70. E. Lukasova, M. Vojtiskova, F. Jelen, T. Sticzay and E. Palecek *Gen. Physiol. Biophys.* **3**, 175 (1984).
71. L.G. Marzilli, *Prog. Inorg. Chem.* **23**, 255 (1977).
72. O. Vrana and V. Brabec, *Anal. Biochem.* **142**, 16 (1984).
73. O. Vrana, V. Brabec and V. Kleinwachter, *Experientia* **40**, 446 (1984).
74. V. Brabec, O. Vrana, V. Kleinwachter, *Studia Biophys.* **114**, 199 (1986).
75. O. Vrana, V. Brabec and V. Kleinwachter, *Anti-Cancer Drug. Design.* **1**, 95 (1986).
76. O. Vrana, V. Brabec, *Studia Biophys.* **114**, 209 (1986).
77. V. Brabec, V. Kleinwachter, J.L. Butour and N.P. Johnson, *Biophys. Chem.* **35**, 129 (1990).
78. D. Kasparova, O. Vrana, V. Kleinwachter and V. Brabec, *Biophys. Chem.* **28**, 191 (1987).
79. J-M. Sequaris, E. Koglin, B. Malfoy, *FEBS Letters* **175**, 95 (1984).
80. T. Pawlowski, J. Swiatek, K. Gasiorowski and H. Kozlowski, *Inorg. Chim. Acta* **136**, 185 (1987).
81. W. Forster, E. Bauer, H. Schutz, H. Berg, L. Akimenko, L.E. Minchenkova, Yu.M. Evdokimov and Ya.M. Varshawsky, *Biopolymers.* **18**, 625 (1979).
82. V.A. Sorokin, Y.P. Blagoi, V.A. Valeev, S.V. Kornilova, G.O. Gladchenko, J.D. Reva and V.J. Sokhan, *J. Inorg. Biochem.* **30**, 87 (1987).
83. H. Elzanowska, J.H. Van de Sande, *Bioelectrochem. Bioenerg.* **19**, 425 (1988).
84. H. Elzanowska, J.H. Van de Sande, *Bioelectrochem. Bioenerg.* **19**, 441 (1988).
85. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660, (1949).
86. J. Swiatek and T. Pawlowski, *J. Inorg. Biochem.* **44**, 163 (1991).
87. H. Sigel, S.S. Massoud and R. Tribolet, J. Am. Chem. Soc. **110**, 6857 (1988).
88. H. Sigel, *Eur. J. Biochem.* **165**, 65 (1987).
89. J. Swiatek and B. Gulanowski, *Acta Biochim. Pol.* **37**, 7 (1990).
90. T. Pawlowski, J. Swiatek and H. Kozlowski, Proc. II Symp. Inorg. Biochem. Mol. Biophys. Karpacz, 276, (1989).
91. M.M. Teeter, G.J. Quigley and A. Rich, *Nucleic Acid-Metal Interactions* (H. Spiro, ed., New York, 1980) p. 146
92. P.R. Schimmel and A. Redfield, *Ann. Rev. Biophys. Bioenerg.* **9**, 181 (1980).
93. Ch. Zimmer, G. Luck and H. Triebel, *Biopolymers.* **13**, 45 (1974).
94. J. Swiatek, T. Pawlowski and H. Kozlowski, *Stud. Biophys.* **122**, 195 (1987).
95. H.A. Tajmir-Riahi, M. Langlais and R. Savoie, *Nucl. Acid Res.* **16**, 751 (1988).
96. S. Alex and P. Dupuis, *Inorg. Chim. Acta* **157**, 271 (1989).
97. J-M. Sequaris and M. Esteban, *Electroanal.* **2**, 35 (1990).
98. J. Swiatek, E. Koglin and J-M. Sequaris, *Proc. II Symp. Inorg. Biochem. and Mol. Biophys.* Karpacz, 1989, 335
99. R.W. Wilson and V.A. Bloomfield, *Biochemistry* **18**, 2192 (1979).
100. J. Widom and R.L. Baldwin, *Biopolymers* **22**, 1621 (1983).
101. P.G. Harrison and M.A. Healy, *Inorg. Chim. Acta* **80**, 279 (1983).
102. J Swiatek, *J. Coord. Chem.* **22**, 121 (1990).
103. J-M. Sequaris, M.L. Kaba, and P. Valenta, *Bioelectrochem. Bioenerg.* **13**, 225 (1984).
104. R. Roemer and R. Hach, *Eur. J. Biochem.* **55**, 271, (1975).
105. J.L. Li and M. Fleischman, *J. Electroanal. Chem.* **316**, 255, (1991).
106. M. Fleischmann, S. Pons and J.L. Daschbach, *Microelectrodes: Theory and Applications, A.S.I. Series E, 197* (M. I. Montenegro, M. Arlete Queiros and J.L. Daschbach ed., 1991) *p.* 393.

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