MERCURY ELECTRODES IN NUCLEIC ACID ELECTROCHEMISTRY: SENSITIVE ANALYTICAL TOOLS AND PROBES OF DNA STRUCTURE. A REVIEW

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This review is devoted to applications of mercury electrodes in the electrochemical analysis of nucleic acids and in studies of DNA structure and interactions. At the mercury electrodes, nucleic acids yield faradaic signals due to redox processes involving adenine, cytosine and

guanine residues, and tensammetric signals due to adsorption/desorption of polynucleotide chains at the electrode surface. Some of these signals are highly sensitive to DNA structure, providing information about conformation changes of the DNA double helix, formation of DNA strand breaks as well as covalent or non-covalent DNA interactions with small molecules (including genotoxic agents, drugs, etc.). Measurements at mercury electrodes allow for determination of small quantities of unmodified or electrochemically labeled nucleic acids. DNA-modified mercury electrodes have been used as biodetectors for DNA damaging agents or as detection electrodes in DNA hybridization assays. Mercury film and solid amalgam electrodes possess similar features in the nucleic acid analysis to mercury drop electrodes. On the contrary, intrinsic (label-free) DNA electrochemical responses at other (non-mercury) solid electrodes cannot provide information about small changes of the DNA structure. A review with 188 references.

Keywords: Mercury electrodes; Nucleic acids; DNA structure; DNA interactions; DNA damage; DNA hybridization; Solid amalgam electrodes; Mercury film electrodes; Electrochemistry; Electrochemical sensors.

1. INTRODUCTION

In 1953 James Watson and Francis Crick uncovered the double-helical structure of DNA [1,2]. Their discovery had a significant impact on further progress in biological sciences. Watson's and Crick's model, based on pairing complementary bases (adenine with thymine or uracil, and cytosine with guanine), offered explanation of crucial processes which remained mystery at that time (e.g., how the genetic information is stored in the DNA molecules, how it is transferred from parental to progeny cells or how it is translated into the protein structure). Due to its newly established central role in molecular biology, DNA attracted the attention of a number of scientists who started to apply various analytical methods in the DNA research. Polarography appeared useful in studies of a variety of biologically active substances, including proteins[3,4], and some laboratories tried to apply it also in nucleic acid (NA) analysis. Probably the first polarographic experiments with DNA were performed by Berg[5], who, however, concluded that DNA is polarographically inactive. Soon after release of Berg's work, Paleček published a series of papers[6–8] on oscillographic polarography of DNA showing that DNA not only yields polarographic signals, but also that the measurements may provide information about the DNA structure, they can be used for determination of NA components and degradation products[8], etc. These observations became the basis for later applications of electrochemistry in studies of DNA structure, interactions and damage, trace determinations of RNAs and DNAs, etc. (reviewed in[9–17]). Nowadays NA electrochemistry, focused mainly on the development of sensors for DNA

hybridization or other important interactions (reviewed in[16–23]), represents one of the hottest topics of interdisciplinary research, in which themes of modern molecular biology meet with those of biochemistry, biophysics, physical chemistry and physics.

The early DNA electrochemical studies in the '50s as well as the polarographic investigations carried out during the '60s and '70s were connected with the dropping mercury electrode (DME) (reviewed in[11–13,15,16]). The polarographic techniques proved excellent for the detection of small structural changes in double-stranded DNA, albeit the DNA quantities required for the analysis were rather large. Later the hanging mercury drop electrode (HMDE) in connection with stripping voltammetric techniques was introduced in NA electrochemistry, which resulted in remarkable improvement of the sensitivity of the measurements (reviewed in[14–17]). At the end of the '70s, Brabec et al. showed[24] that DNA yields signals at carbon electrodes (CE) due to electrochemical oxidation of adenine and guanine residues. CE started to be used in NA electroanalysis as an alternative to the mercury electrodes (ME). In the second half of the '80s it was shown that NAs can easily be immobilized at the surfaces of HMDE[25] or CE[26] and that both electrode types are thus applicable in medium exchange electrochemical techniques. Electrodes modified with NAs (or proteins) make the basis for the development of electrochemical biodetectors (biosensors), a field of bioelectrochemistry that has enjoyed a remarkable boom over the past decade (reviewed in[16–23]).

At present, there is a strong bias towards using carbon and other kinds of solid (non-mercury) electrodes in NA electrochemistry and research oriented on the electrochemical biodetectors. The mercury electrodes are not very popular in this field. The reasons may be partly practical. Solid electrodes seem to better fulfil the requirements for simple, low cost, user-friendly biosensors applicable "in the field"; they can be more easily integrated into flow-through systems, etc. The choice of the electrode material may also be influenced by the electrochemical process of interest (e.g., DNA oxidation response or signals of redox markers at the CE) or by the technique of DNA immobilization at the surface (reviewed in[16,20,21]). On the other hand, other reasons for apparent marginalization of the ME may be rather irrational, such as fears of the "poisonous" metallic mercury (in some countries even having resulted in ban on using the mercury electrodes). It should be emphasized that, although the solid electrodes proved useful in many analytical applications, the mercury electrodes possess some features making them superior to other electrode types. In general, the mercury drop electrode has a perfectly smooth surface and is ideally renewable,

offering the best reproducibility of measurements[27–29]. Unlike solid electrodes, the HMDE can work in the compression/expansion mode and changes in the electrode surface area may be utilized in studies of the properties of adsorbed layers[30–32]. Extremely high hydrogen overvoltage at the ME allows for measurements at highly negative potentials. This is crucial for detection of cathodic and tensammetric NA electrochemical signals at potentials between –1.2 and –1.5 V. (Potentials are given against the saturated calomel electrode if not stated otherwise.) Some of these signals exhibit unique sensitivity to DNA structure, allowing indicator-free studies of minor changes in DNA conformation, DNA interactions with low-molecular-weight substances, or DNA damage (reviewed in[13,15,16,22]). Comparable sensitivity to subtle changes in DNA structure has not been attained with any of the so far applied mercury-free solid electrodes.

Electrochemical properties of the NAs, application of different electrochemical techniques in NA analysis and development of electrochemical biosensors for DNA hybridization, DNA damage and interactions with various biologically relevant substances have been thoroughly reviewed[9–23,33]. This review is devoted to utilization of the ME in DNA studies, accenting the advantages of the DME and HMDE in measurements of intrinsic DNA electrochemical signals. Recent investigations focused on substitution of the HMDE with a mercury film (MFE) and/or solid amalgam (SAE) electrodes are also reviewed.

2. POLAROGRAPHY AND VOLTAMMETRY OF NUCLEIC ACIDS AT MERCURY ELECTRODES

2.1. Redox Processes

Nucleic acids consist of residues of phosphoric acid, sugar component (ribose or 2-deoxyribose) and nitrogenous heterocyclic bases – adenine (A), guanine (G), cytosine (C) or thymine (T)[34]. Among them, only A, C and G undergo redox processes at the ME in aqueous media[6,7,35–37]. In NAs, A and C yield a single cathodic peak (usually denoted as peak III in differential pulse polarography or peak CA in voltammetric techniques) around –1.4 to –1.5 V, depending on conditions (Fig. 1, Table I) (reviewed in[11–13,15,16]). Guanine is reduced at even more negative potentials ($\leq$ –1.6 V) and its reduction signal is not detectable due to overlapping with the background discharge. Nevertheless, the product of this reaction, 7,8-dihydroguanine[36], can be oxidized back to G around –0.3 V, yielding analytically useful peak G in cyclic (Fig. 1, Table I) or anodic stripping voltammetric

modes[37–40]. Reduction of DNA bases requires protonation and adsorption at the electrode surface. The signals are thus dependent on pH and the presence of salts. Cations are necessary to neutralize the charge of polyanionic DNA and prevent strong repulsion between the phosphate groups and the negatively charged electrode surface. Some cations (NH_4^+, Cs^+ or Mg^{2+}) have remarkably positive effects on the formation of the DNA cathodic or anodic peaks[37].

In single-stranded (ss) DNA, the base residues are exposed to the environment and can freely communicate with the electrode surface. Consequently, the ssDNA yields well-developed polarographic and voltammetric signals (Fig. 1, Table I). On the other hand, the base accessibility is limited in the double-stranded (ds) DNA. The intensity of polarographic or voltammetric signals of the dsDNA depends on localization of the respective electroactive site within the DNA double helix (Fig. 1) and on the DNA confor-

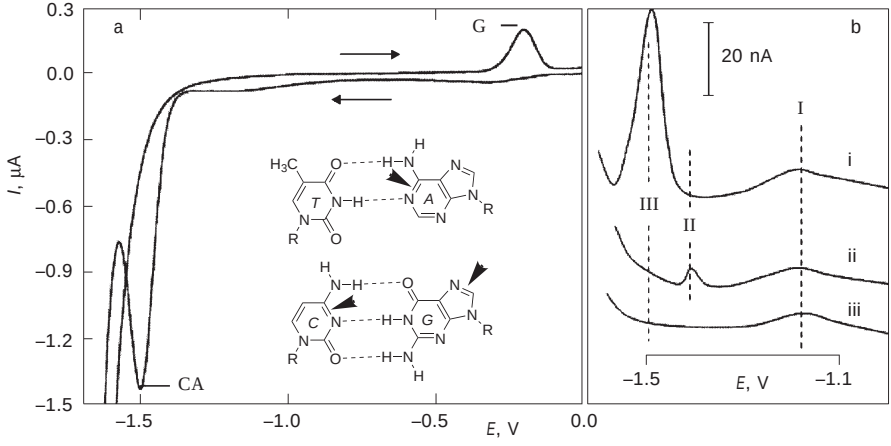

FIG. 1

a Cyclic voltammogram of single-stranded DNA at HMDE. The cathodic peak CA is due to irreversible reduction of cytosine (C) and adenine (A) residues. Guanine (G) is reduced at potentials ≤ –1.6 V and its reduction product yields the anodic peak G. Inset, scheme of Watson–Crick base pairs with electroactive sites undergoing redox processes at the HMDE (denoted by arrows). b Differential pulse polarograms of i, single-stranded (ss); ii, double-stranded linear (lin); iii, covalently closed circular (supercoiled, sc) DNA of a plasmid pAT153. The CV (a) was measured using the adsorptive transfer stripping (AdTS) technique (see Section 3) at DNA concentration of 30 μg ml^{-1}, accumulation time 60 s, initial potential –0.1 V, switching potential –1.85 V, scan rate 1 V s^{-1}. In the DPV (b), drop time was 1 s, pulse amplitude 50 mV and DNA concentrations 50 μg ml^{-1} (ssDNA) or 100 μg ml^{-1} (lin and scDNA). Both CV and DPP were measured in 0.3 M ammonium formate, 50 mM sodium phosphate, pH 6.9

mation. Reducible sites of A and C are involved in the Watson–Crick hydrogen bonding system, being hidden in the double-helix interior[12–15,33]. Intact dsDNA therefore yields only minor cathodic peaks. These signals are, in general, remarkably influenced by perturbations in the DNA structure (for more details, see Sections 4 and 5). Intensity of the anodic peak G is less dependent on the DNA structure. At full coverage of the HMDE, dsDNA yields peak G whose height is about 50% of the height of the anodic peak of ssDNA [17,37,39,40]. Such behavior is in agreement with the location of the G electroactive site close to the surface of dsDNA within the major groove of the double helix (Fig. 1). Oxidation response of G and A moieties at the CE, involving sites accessible via the major or minor groove of the DNA double helix, exhibit also a relatively low sensitivity to the DNA structure[16,22,41,42].

TABLE I
Polarographic and voltammetric signals of DNA at mercury electrodes

Peaks	Usually used technique	DNA constituent(s) responsible for the peak	Electrode process	Aproximative potential	Sensitivity to DNA structure
Peak G	CV, anodic SWV	guanine	reduction/ oxidation[b,c]	–0.3	+
Peak CA	LSV, SWV	adenine + cytosine	reduction[c]	–1.5	+++
Peak I	DPP	sugar-phosphate backbone	adsorption/ desorption	–1.1	–
Peak II	DPP	adenine + cytosine (dsDNA; transiently open, distorted/damaged regions)	reduction[c]	–1.4	+++
Peak III	DPP	adenine + cytosine (ssDNA)	reduction[c]	–1.5	+++
Peak 1	a.c. techniques LSV, SWV	sugar-phosphate backbone	adsorption/ desorption[d]	–1.2	–
Peak 2	a.c. techniques LSV, SWV	distorted ds regions; edges of base pairs	adsorption/ desorption[d]	–1.3	+++
Peak 3	a.c. techniques LSV, SWV	base residues in ssDNA regions	adsorption/ desorption[d]	–1.45	+++
Peak 3*	a.c. techniques	base residues in ss regions within scDNA	adsorption/ desorption[d]	–1.38	+++

[a] Potentials against saturated calomel electrode. [b] Guanine is reduced on the HMDE at potentials ≤ –1.6 V. The product of this reaction, 7,8-dihydroguanine, is anodically oxidized back to guanine. [c] In weakly acidic or neutral media in the presence of ammonium ions. [d] In weakly alkaline media.

2.2. Tensammetric Processes

It was shown for the first time by Miller[43,44] that DNA displays a distinct potential-dependent adsorption/desorption behavior at the ME. Both ss and dsDNA are adsorbed at the mercury surface in a wide potential region, displaying maximum adsorption around the potential of zero charge (pzc). At certain potentials, segments of the adsorbed DNA may be desorbed from the surface (and/or undergo reorientation), yielding tensammetric (capacitive) signals (reviewed in[13,15,16,18]). Such signals have been usually observed using a.c. polarographic or voltammetric techniques in weakly alkaline media where the pH-dependent faradaic processes (see Section 2.1) are suppressed. Depending on the medium composition and DNA structure, individual components of polynucleotide chains may be involved in the DNA adsorption and contribute to the tensammetric peaks. In low-salt media (such as 10 mM NaCl), the negative charges of phosphate groups are not screened by counterions and interaction of dsDNA with the electrode is mainly electrostatic[45,46]. DNA is attracted to the positively charged and strongly repelled from the negatively charged electrode. Changes of the surface charge polarity at the pzc lead to adsorption/desorption of the electrostatically adsorbed dsDNA and formation of a capacitive peak denoted as peak 0. In denatured DNA the electrostatic adsorption loses importance because of a strong hydrophobic interaction of freely accessible base residues with the mercury surface. At moderate ionic strengths (≥ 50–100 mM NaCl), cations from the medium efficiently screen the phosphate negative charges and both ds and ssDNA are adsorbed at the ME as electroneutral species[45–48]. Both DNA forms yield a tensammetric signal around –1.1 to –1.2 V (peak 1, Fig. 2, Table I). This peak (which is not significantly influenced by the DNA structure) is related to reorientation of polynucleotide segments adsorbed at the electrode surface via their sugar-phosphate backbone. Other tensammetric peaks observed at more negative potentials are dependent on DNA conformation via changes in accessibility of DNA base residues to the contact with the electrode surface (Fig. 2, Table I). These signals include peak 2 (due to distorted or damaged double-helical segments[13,15,22,49]), peak 3* (locally open regions in covalently closed circular DNA molecules[50]) and peak 3 (single-stranded DNA [13,15,45,46,50]). Relations between the DNA structure and its electrochemical responses are discussed in Section 4.

Adsorption/desorption behavior of nucleic acids is influenced not only by the NA conformation, but also by the type of its backbone. It has been shown that natural RNAs yield tensammetric signals at potentials differing from those of the DNA peaks[51–53]. Methods for trace determination of RNA

in the presence of excess of ds or ssDNA, based on measurements of an RNA-specific peak R, were proposed. Peptide nucleic acid (PNA), a synthetic DNA analogue, in which the entire sugar-phosphate backbone is replaced by an electroneutral pseudopeptide chain (reviewed in[54]), exhibits a distinct tensammetric behavior strongly differing from that of negatively

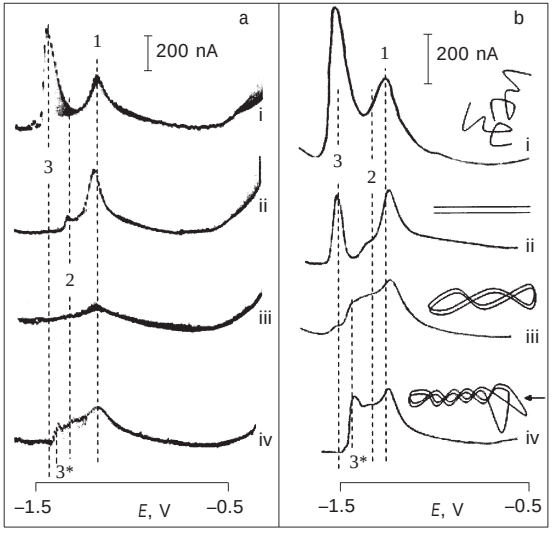

FIG. 2

Tensammetric signals of DNA at mercury electrodes respond to DNA structural transitions. a Alternating current (a.c.) polarograms, b sections of a.c. voltammograms measured at HMDE, of i, ssDNA; ii, linDNA; and iii, iv, scDNA of the plasmid pAT153. Peak 1, yielded by all of the DNA forms, is due to reorientation of DNA segments adsorbed at the electrode via the sugar-phosphate backbone. Peak 2 is specific for dsDNAs involving conformationally distorted double-helical segments. Peak 3 corresponds to DNA chains adsorbed via freely accessible base residues. In the a.c. polarography, peak 3 is yielded only by the ssDNA. At the HMDE, the latter peak is produced by the linDNA which undergoes partial denaturation at the electrode (see Section 4.2). The covalently closed circular molecules of scDNA cannot be denatured at the electrode surface and therefore do not produce peak 3 even at the HMDE. The scDNAs in iii and iv differ in the number of their negative superhelical turns (Section 4.3). Higher level of the negative DNA superhelicity in iv induces local opening of the DNA double helix. Unpaired bases within these covalently closed scDNA molecules (denoted by arrow in the scheme) yield peak 3*. The polarograms were recorded in 0.3 M NaCl, 30 mM $NaHCO_3$, pH 9.5, for DNA concentration of 100 μg ml^{-1}, drop time 10 s, scan rate 1 mV s^{-1}, peak-to-peak amplitude 10 mV, frequency 230 Hz. The phase-in component of a.c. current was recorded. Voltammetric measurements were performed in the AdTS mode, with DNA concentration 80 μg ml^{-1} (scDNA) or 50 μg ml^{-1} (den and linDNA), accumulation time 60 s, initial potential –0.1 V, scan rate 20 mV s^{-1}, other conditions as in a

charged polynucleotides[55,56]. PNA, in agreement with its electroneutrality, is less strongly adsorbed at the positively charged mercury surface than DNA of the same nucleotide sequence[55]. On the other hand, the PNA undergoes no adsorption/desorption processes manifested by formation of tensammetric peaks and remains strongly adsorbed at the negatively charged surface at potentials up to –1.8 V.

2.3. Electrochemical Techniques Applied in Nucleic Acid Analysis

Polarographic techniques with the dropping mercury electrode were the main methods applied in the first two decades after the discovery of DNA electroactivity (reviewed in[11–13,33]). The first measurements of native (ds) and denatured (ss) DNA and of apurinic acid (partly hydrolyzed DNA lacking purine bases) were made with the oscillographic polarography[6–8] (OP). Specific signals ("indentations") were attributed to electrode redox processes involving cytosine, adenine and guanine residues, and an influence of DNA structure on its polarographic response was established. Later differential pulse polarography[11–13,33,57–65] (DPP) proved an excellent technique for measurements of DNA redox response while a.c. polarography[43–46,66–69] was used in the studies of adsorption/desorption processes. Mainly DPP peak II and peak III achieved analytical importance. Peak III is specific for ssDNA and was employed in DNA denaturation experiments[12,33,59,70] and in detecting small amounts of ssDNA in dsDNA samples[57]. Peak II is yielded by ds (but not ss) DNA at a potential by ≈80 mV less negative, compared with the ssDNA peak III (Fig. 1b)[12,13,33,58]. The peak II displays a unique sensitivity to dsDNA structure, including conformational changes of the DNA double helix and DNA damage (see Sections 4 and 5).

Introduction of the hanging mercury drop electrode with voltammetric techniques extended the possibilities of nucleic acid electrochemistry (reviewed in[14–17]). Adsorptive stripping (AdS) voltammetry of NAs, based on accumulation of NAs at the electrode surface prior to the potential scan, resulted in remarkable increase in sensitivity[15,38,39,71]. Application of cyclic voltammetry (Fig. 1) enabled utilization of the anodic peak G for detection of small amounts of NAs[15,39,71]. Later on, modern electroanalytical techniques, including square-wave voltammetry[38,56] (SWV) and constant current chronopotentiometric stripping analysis[72–75] (CPSA), were applied in measurements of both anodic and cathodic NA response. Techniques allowing measurements at relatively high scan rates (such as LSV or SWV) are convenient mainly when potential-induced dsDNA structural changes at the HMDE surface (see Section 4) should be minimized[16]. Application of

both CPSA and SWV followed by numerical data processing (such as baseline correction) resulted in further improvement of the sensitivity of NA measurements at both HMDE [72,74] and CE [76,77]. Tensammetric responses of different forms of DNA, RNA, synthetic oligo- and polynucleotides were studied (and analytically utilized) using DPV [52,53,75], SWV [38,75,78], phase-sensitive a.c. voltammetry[49,50,78–80] and modern a.c. impedance techniques[55,81,82], including impedance spectroscopy[83,84]. Elimination voltammetry with linear scan[85,86] has recently been used to analyze electrode processes of both DNA and its monomeric components at the HMDE [87,88]. Voltammetric and chronoamperometric measurements at a compression dropping mercury electrode were used to study properties of adsorbed layers of ss and dsDNA [32].

2.4. Analysis of Monomeric NA Components at Mercury Electrodes

2.4.1. Stripping Voltammetric Determination of DNA Bases

DNA bases and purine nucleosides/nucleotides form sparingly soluble compounds with mercury ions[89]. These species can be generated at the ME surface upon application of certain potentials (at which the electrode mercury is anodically oxidized) in a solution of DNA components. Signals corresponding to the formation and dissolution of the base-Hg complexes were observed already in the early times of the NA electrochemistry as specific oscillopolarographic indentations[6,90]. Using the HMDE and cathodic stripping voltammetric techniques, very low concentrations (down to 1–10 nmol l^{-1}) of pyrimidine and purine DNA bases[91], their derivatives (including clinically used drugs such as 5-fluorouracil[92]) and purine nucleosides/nucleotides can be determined (reviewed in[16]). Recently a similar technique, based on accumulation of adenine or guanine complexes with copper ions at the HMDE followed by cathodic reduction of the copper, was proposed[93,94]. It has been shown that these principles can be utilized for a sensitive determination of acid-hydrolyzed DNA [93,95–98] (for practical applications, see Sections 6 and 7).

2.4.2. Two-Dimensional (2D) Condensation of NA Components

NA bases possess the ability of forming compact films (self-assembled monolayers) at the mercury surface. The potential-dependent 2D condensation of NA bases at the ME, manifested as capacitance “pits” on capacitance–

potential curves (measured at DME or HMDE by a.c. polarography/voltammetry, or by impedance techniques) was observed for the first time by Vetterl in the '60s [67–69,99]. It has been shown that the condensation ability is in general remarkably higher in bases and nucleosides/nucleotides naturally occurring in DNA and RNA than in other purine and pyrimidine derivatives. Formation of the "pits" depends on pH, ionic conditions and temperature[67,68,99–104]. It has been proposed that the 2D condensation is associated with the transition of the base residues from a flat-lying to perpendicular (relative to the electrode surface) orientation[103–106]. Similar effects have been recently observed on the surface of gold single-crystal electrodes and on mercury films[107,108].

3. DNA-MODIFIED ELECTRODES AND ADSORPTIVE TRANSFER STRIPPING ANALYSIS

Thanks to strong adsorption of NAs at the mercury (and also some kinds of carbon) electrode surface, it is possible to transfer an electrode with accumulated NA (i.e., a NA-modified electrode) from the NA solution into a blank background electrolyte where the measurement is then performed (Fig. 3)[25,26,109]. Electrochemical responses of DNAs, RNAs as well as synthetic polynucleotides and oligonucleotides obtained in this way are practically identical with the curves measured in the conventional AdS mode (with the NA in the background electrolyte solution). This procedure (called adsorptive transfer stripping, AdTS), proposed in the late '80s [25,109], offered quite new possibilities of electrochemical analysis of NA and other biopolymers. First, it became possible to analyze very small analyte volumes (using standard electrochemical equipment) because NAs can easily be accumulated on the electrode from several microliters of solution at an open current circuit. This is crucial for studies involving series of expensive and/or difficult-to-prepare samples, such as synthetic polynucleotides, oligonucleotides and DNA analogues[55,56], natural RNAs [51–53], supercoiled (sc) plasmid DNAs [50,110], chemically modified nucleic acids[111,112], etc. Second, composition of the sample solution can differ from the background electrolyte. In polarography and conventional voltammetry, analyte solution has to fit conditions, under which the given electrode reaction takes place. In AdTS, it is possible to study influence of the medium composition on DNA structure without such limitations because the medium is exchanged prior to the measurement. The same NA sample can thus be analyzed by different electrochemical methods which require different background electrolytes (e.g., measurements of DNA redox or tensammetric re-

sponses in neutral or weakly alkaline media, respectively). Moreover, a number of electroactive substances that interfere with the NA analysis in the conventional voltammetry can be removed during the medium exchange provided that these species are not firmly adsorbed on the electrode[25,26,52,109]. Reaction mixtures of DNA or RNA containing monomeric NA components[38,51,53,109,113], DNA damaging agents such as transition metal complexes[73,78,110], substances binding to DNA covalently[111] or non-covalently[49] can thus be readily analyzed via the AdTS procedure. Third, DNA-modified electrode can serve as a simple biodetector (biosensor) consisting of an electrochemical signal transducer (the electrode) and sensitive (recognition) DNA layer anchored on its surface (Fig. 3) (lit.[22,42,110,114–116]; reviewed in[16–23]). Such a device can be subjected to agents inducing changes in the DNA layer, which are subsequently electrochemically detected. For example, HMDE modified with scDNA functions as biodetector for agents causing DNA strand breaks (sb) (Section 5.1)[22,78,110]. Processes leading to DNA damage at the electrode surface can be modulated by the electrode potential[114,115,117].

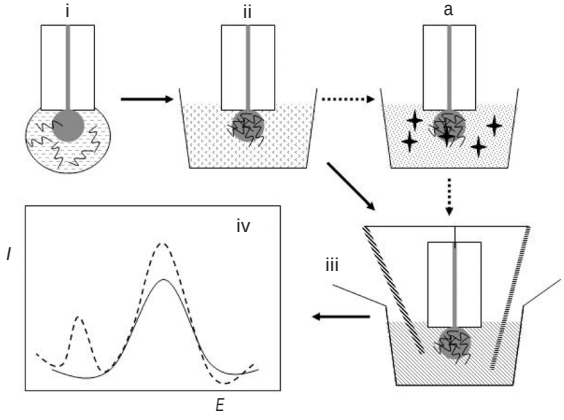

FIG. 3

Scheme of adsorptive transfer stripping (AdTS) voltammetry of NAs at the HMDE. i, The electrode is immersed into a 3–5 μl drop of DNA solution; ii, the NA-modified electrode is washed; iii, transferred into blank background electrolyte solution, followed by iv, voltammetric measurement. In contrast to the conventional voltammetry (with DNA in the background electrolyte), the composition of the analyzed sample must not be the same as that of the background electrolyte solution. Strongly adsorbed layer of NA resists the medium exchange while weakly adsorbing substances can be washed out and do not interfere with the NA analysis. a, The NA-modified electrode can be exposed to various substances interacting with the immobilized DNA, thus serving as a simple electrochemical DNA biodetector. Due to interactions with agents in solution, structure of the anchored DNA may be altered, resulting in changes in the observed electrochemical response (dashed curve in iv)

4. DNA STRUCTURE IN SOLUTION AND ON THE MERCURY SURFACE

Low accessibility of the A and C electroactive sites in dsDNA is the cause of a strong influence of DNA structure on its cathodic response at the ME (Fig. 1). Similarly, DNA tensammetric signals are structure-sensitive due to alterations of the accessibility of hydrophobic base residues upon the DNA structural changes. In general, ssDNA yields well-developed peaks, while intact (native) dsDNA appears electrochemically "silent" under certain conditions (reviewed in[12,13,15,16,22]). Changes of the dsDNA conformation may result in partial or full exposure of some base residues to the environment and subsequently in formation of specific DNA signals. In polarographic modes working with small potential excursions during the DME lifetime (such as DPP), the measured signals well reflect the DNA structure in solution (reviewed in[12,13]). Changes of the DNA conformation due to the contact with the charged electrode surface at potentials of the structure-sensitive DNA signals at the DME can be neglected. On the other hand, significant structural changes of dsDNA may take place at the HMDE (working with the same surface during the whole potential scan)[13,66,110,118,119]. These effects, involving slow potential-dependent DNA unwinding at the electrode surface, should be taken into account when voltammetric methods are applied in studies of the DNA structure. The DNA surface denaturation can be analytically exploited in some cases (e.g., detection of DNA damage, Section 5)[110].

4.1. Studies of DNA Structure by Polarographic Methods

DPP measurements of DNA provide specific cathodic signals yielded by either ssDNA (peak III) or dsDNA (peak II)[11–13,33,57,58,63,70] (Fig. 1b). A similar behavior was observed for ds or ssRNA which produce analogous peaks, albeit at somewhat different potentials from DNA[65]. In agreement with free accessibility of the C and A residues in ssDNA, the latter yields a well developed peak III at relatively low concentrations (micrograms per milliliter)[57]. This signal was utilized in DNA denaturation studies as well as in methods for detection of small amounts of ssDNA in dsDNA. The DPP peak II of dsDNA is observed at the potential by about 80 mV less negative than the peak III of ssDNA and is usually by two orders of magnitude smaller (for intact calf thymus dsDNA at room temperature). However, the height of peak II responds sensitively to perturbations in the dsDNA structure. Results of DPP measurements performed by Paleček in the '60s provided an early evidence of premelting conformational changes of the DNA double

helix (reviewed in[12,13]). The peak II remarkably increases with temperature in the region far below the DNA "melting" point. Under such conditions, the DNA remains double-helical and no effects can be detected by methods usually used in DNA denaturation studies (such as absorption spectrophotometry). It has been proposed that transient openings of the DNA double helix (of random nucleotide sequence), resulting in short-term exposure of individual base pairs to the environment, are responsible for the peak II formation[12,13]. At higher temperatures (but below the DNA denaturation point), these perturbations become more frequent. At low temperatures, they take place mainly around the ends of dsDNA molecules (where the transient base pair openings are more likely). Accordingly, covalently closed circular (ccc) DNAs possessing no strand ends produce no peak II (Fig 1b)[64]. The height of the DPP peak II increases as a result of formation of DNA sb (due to treatment with DNase I, ionizing radiation or ultrasound[58,120]), i.e. with the number of the ends of polynucleotide chains. UV irradiation[60] or chemical modification of DNA [10,121–123], resulting in damage to the DNA bases and subsequent distortions of the DNA double helix also lead to an increase in the peak II height. The DPP measurements can be used to differentiate between various lesions induced by different DNA damaging agents. When a DNA lesion involves unpaired bases, peak III is observed[10,121,123]. On the other hand, non-denaturational distortions of the DNA double helix without disruption of the Watson–Crick base pairs contribute to changes of the peak II intensity[10,121].

Measurements of tensammetric DNA signals (a.c. polarographic peaks 2 and 3, Fig. 2) provided qualitatively similar information about the DNA structure and its changes as the DPP measurements (reviewed in[10,13,16]). Alternating current polarography and a.c. impedance measurements at the static mercury drop electrode (SMDE) have been applied in studies of superhelicity-induced structural transitions of ccc plasmid DNA molecules[50] (Section 4.3).

4.2. Changes of dsDNA Structure at the HMDE Surface

When a.c. voltammetric curves of linear dsDNA are measured at the HMDE and the potential is scanned from positive to negative values, the ssDNA-specific peak 3 is observed in addition to the peak 1 and peak 2 (Fig. 2b)[13,15,16,81,110,124]. Similar behavior can be observed when the faradaic peak CA is measured. On the other hand, when the potential is scanned in the opposite direction (from the negative to the positive values), no a.c. voltammetric peak 3 is observed and the voltammogram is qualitatively

identical to the dsDNA a.c. polarogram (Fig. 2a)[16,81,124]. At the DME, analogous phenomena could be observed only upon large potential excursions during the drop lifetime (in normal pulse polarography[13,15,16,81,110,124]). Although there were attempts to explain these phenomena by formation of unusual dsDNA structures (such as ladder DNA conformation[125], "π-state" DNA [9] or aggregates of dsDNA molecules at the negatively charged mercury surface[9,124]), potential-induced unwinding (surface denaturation) of the DNA double helix at the electrode surface is the most probable explanation[13,66,110,118,119]. Heights of the ssDNA-specific peaks (peak 3, peak CA) slowly increase due to the exposure of dsDNA at the HMDE surface to potentials around –1.2 V (potential "region U", in neutral media between about –1.0 and –1.3 V). The same treatment has no effect on the response of ssDNA. The region U roughly corresponds to potentials of the DNA peak 1. It has been proposed (lit.[13,118] and references therein) that the potential-induced surface denaturation of dsDNA is caused by repulsion of phosphate groups by the negatively charged electrode surface. The DNA remains adsorbed by randomly unpaired hydrophobic bases (e.g., at molecule ends or around single strand interruptions) resulting in strains in the DNA molecules and in the DNA unwinding. When the potential is scanned from the positive to the negative values, the region U is crossed before potentials of the ssDNA-specific peaks are reached. The model of the DNA surface denaturation has been supported by the behavior of dsDNA containing interstrand crosslinks and of cccDNAs. Introduction of the interstrand crosslinks on dsDNA treatment with bifunctional platinum complexes results in inhibition of the DNA unwinding within the region U [126]. The cccDNA does not contain any strand ends and its denaturation is topologically restricted[127–129]. Accordingly, cccDNAs yield no voltammetric peak at potentials corresponding to the ssDNA peak 3 regardless of the scan direction[50,110].

At potentials less negative or more negative than those of region U, no significant structural changes (comparable to those occurring in the region U) of dsDNA take place[13,16]. DNA structure and arrangement of adsorbed DNA molecules at the HMDE surface tends to be fixed[26,49,109,130]. Such a conclusion has been strongly supported by the observed irreversibility of the potential-induced DNA surface denaturation[118] as well as by the results of medium-exchange experiments, in which DNA was adsorbed on the HMDE surface under conditions inducing DNA structural changes[49,109,130]. When DNA was adsorbed at higher temperatures and the measurements were performed at room temperature, changes of the DNA voltammetric response typical of the premelting transitions were observed[130]. Adsorption

of dsDNA in the presence of intercalative drugs (such as chloroquine, $[Co(phen)_3]^{3+}$ or 9-aminoacridine) followed by removal of the intercalators results in formation of a specific dsDNA structure (tentatively denoted as intDNA [49]) on the electrode surface, yielding a characteristic large a.c. voltammetric peak 2 and unusually small peak 3 [49]. Binding of intercalators to dsDNA in solution results in distinct conformational changes, namely untwisting of the DNA double helix and lengthening of the DNA molecules[34,127]. It has been proposed that after intercalator removal the intDNA involves untwisted double helical regions adsorbed on the surface and superhelical loops stretching to the solution. The intDNA is resistant to unwinding in the region U unless single-strand breaks (ssb) are introduced into the surface-confined DNA [49]. Native DNA immobilized on the HMDE surface resists even incubation in 0.2 M NaOH (the medium inducing complete DNA denaturation in solution) (Fig. 4). The potential-induced DNA unwinding is enhanced in strongly alkaline (as well as in acidic[13]) media suggesting that the irreversible DNA surface denaturation is facilitated by reversible destabilization of the DNA duplex in NaOH solution. In contrast to the resistance to alkaline denaturation, dsDNA on the electrode surface exhibits slow increase in the relative height of peak 3 on incubation in 5 M

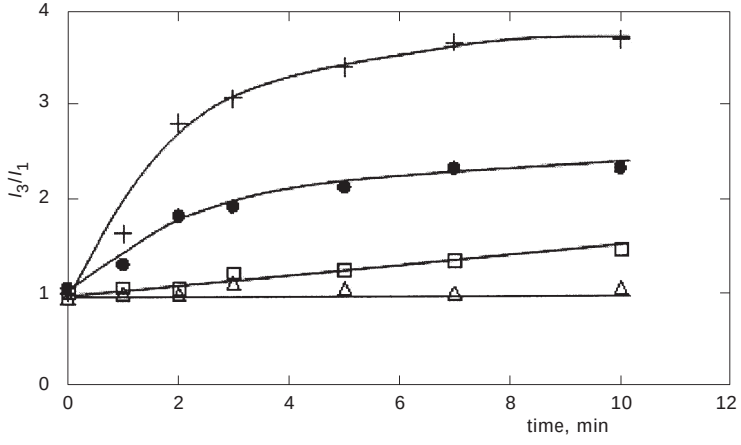

FIG. 4

Effects of denaturing agents on a.c. voltammetric responses of ds linDNA adsorbed at the HMDE surface. The linDNA-modified electrode was incubated: in 0.2 M NaOH at room temperature (either at open current circuit (△), or at –1.2 V (●)); in 5 M $NaClO_4$ at 65 °C (□); in 1% formaldehyde, 5 M $NaClO_4$ at 65 °C (+). After time intervals given in the graph, the electrode was washed, transferred into voltammetric cell and voltammograms were recorded. The heights I_1 and I_3 of a.c. voltammetric peak 1 and peak 3, respectively, were measured (under conditions as in Fig. 2b) and the ratios I_3/I_1 plotted

$NaClO_4$ at 65 °C (Fig. 4). Such behavior suggests that thermal denaturation of surface-confined dsDNA is partially irreversible due to thermal motions of the unpaired complementary DNA strands at the surface. In the presence of 1% aqueous formaldehyde, an agent modifying unpaired base residues thus preventing DNA renaturation, the irreversible thermal DNA denaturation at the HMDE is further enhanced (Fig. 4).

4.3. Behavior of Supercoiled, Relaxed Covalently Closed Circular, Open Circular and Linear DNA Molecules on the Mercury Electrodes

Double-stranded DNAs (of identical nucleotide sequences) may adopt different structures and/or topological states. For example, small circular DNA molecules, called plasmids, occur in bacterial or some eukaryotic cells[34]. When both strands of these DNAs are covalently closed, their molecules may exist in relaxed, negatively or positively supercoiled (superhelical) forms[34,127–129]. Relaxed cccDNA spread in a plane possesses the same number of double helix turns (the linking number, Lk) as the same DNA in its linear (lin) or open circular (oc) form (lin and ocDNAs containing free strand ends are inherently relaxed). Lk of cccDNA, however, may be higher or lower than that of the relaxed DNA and cannot be changed without interruption of DNA strands. DNA molecules with linking deficit or excess are negatively or positively supercoiled, respectively. Such DNAs tend to compensate their linking deficit or excess by adopting specific configuration, in which the double helix winds around itself, forming superhelical turns[127,129]. DNA molecules differing in the Lk value (or in the superhelix density, σ) are called topoisomers. Depending on σ, specific structure transitions may occur in the scDNA. Negative DNA superhelicity induces local DNA openings (Fig. 2) and stabilizes non-B structures adopted by segments of certain nucleotide sequences, such as cruciforms, Z-DNA or intramolecular triplexes[127,129,131]. Various forms of circular and linear plasmid DNA molecules have been studied by AdTS voltammetry at the HMDE [50,110].

The cccDNAs are not susceptible to the potential-induced surface denaturation within the region U and do not yield the a.c. voltammetric peak 3 [22,50,110]. Such behavior is in agreement with the absence of free strand ends in the cccDNA molecules associated with topological hindrance for the irreversible DNA unwinding[127,128]. Introduction of single strand interruptions or linearization of the cccDNA molecules (both in solution or at the electrode surface) manifests itself by formation of peak 3 [78,110,114,115,117]. Qualitative differences in the a.c. voltammetric responses of cccDNAs and

DNA molecules possessing free ends has been utilized in development of methods for detection of DNA damage (Section 5; reviewed in[22]).

Opening of the scDNA double helix induced by high negative superhelicity[127,129,131], associated with exposure of some base residues to the environment, results in formation of a specific tensammetric signal[50]. Potential of this peak, denoted as peak 3*, is remarkably less negative than that of ssDNA peak 3. Peak 3* is observed at both the HMDE and DME (SMDE) (Fig. 2), indicating that the superhelicity-induced DNA openings occur already in solution. Dependence of the height of the peak 3* on the negative σ shows a distinct transition and fits analogous dependences of the cathodic DNA peak CA intensity (suggesting increased accessibility of DNA bases for their electrochemical reduction) and of the reactivity of the DNA bases towards chemical probes[50]. Besides this helix opening transition, the a.c. voltammetric measurements indicated another structural transition at lower $-\sigma$ values associated with changes in the DNA adsorbability at the HMDE[50].

5. DETECTION OF DNA DAMAGE AND DNA INTERACTIONS WITH SMALL MOLECULES

DNA in cells can be damaged by a variety of agents which may affect its ability to maintain, replicate and express genetic information[34,132–134]. Interactions of DNA with such species may result in modification of the base residues and/or interruptions of the DNA sugar-phosphate backbone (formation of sb). Damage to DNA often leads to mutations and to serious diseases including cancer or hereditary disorders. Analysis of the level of DNA damage, as well as detection of genotoxic agents in the environment, water, food, etc., is therefore closely related to human health protection. Methods used for detection of DNA damage should be sensitive enough to find one lesion per 10^4 to 10^6 undamaged entities. The currently used techniques are usually based on DNA hydrolysis followed by HPLC detection of damaged nucleotides[135,136]. Another possible approach is to analyze DNA without hydrolysis, utilizing changes of the DNA features induced upon its damage. Electrochemical analysis of DNA at the ME appears convenient for this purpose (reviewed in[22]).

5.1. Detection of DNA Strand Breaks

As discussed above (Section 4), polarographic and voltammetric signals of DNA are sensitive to the presence of free ends of the polynucleotide chains.

Early DPP measurements made with DNA treated with deoxyribonuclease I or exposed to ionizing radiation revealed low dose-dependent effects on the peak II intensity, suggesting that the latter signal responds to formation of DNA ssb [13,58,60]. Voltammetric techniques in connection with the HMDE provide a similar information about DNA breakage by ultrasound[80] or γ-rays[137]. Later ccc (sc) plasmid DNAs have been employed in studies of DNA damage with the HMDE [22,71,73,78,110,114,115,117]. The scDNA is more resistant to irreversible DNA denaturation than oc or linDNAs because its chains cannot be separated from each other. Methods for the detection of ssb formation in scDNA, involving DNA denaturation in solution followed by AdTS cyclic[71] or a.c. voltammetry[110] at HMDE, have been proposed. The cccDNA does not undergo even unwinding at the HMDE within the region U [110,138], resulting in the absence of tensammetric peak 3 on curves of the intact scDNA and restricted reducibility of the scDNA C and A residues[73,138]. Appearance of the peak 3 (or of faradaic peak CA [73]) therefore indicates disruptions of the DNA sugar-phosphate backbone[22,50,78,110,114,115,117] (Fig. 5). Detection of ssb using scDNA and a.c. voltammetry at HMDE is highly sensitive (one strand scission among 2×10^5 intact phosphodiester bonds)[110]. HMDE modified with the scDNA represents a simple biodetector of DNA-cleaving species (Fig. 5) applicable in analysis of real samples, including natural or industrial waters, food, etc.[78]. It has been shown that DNA at

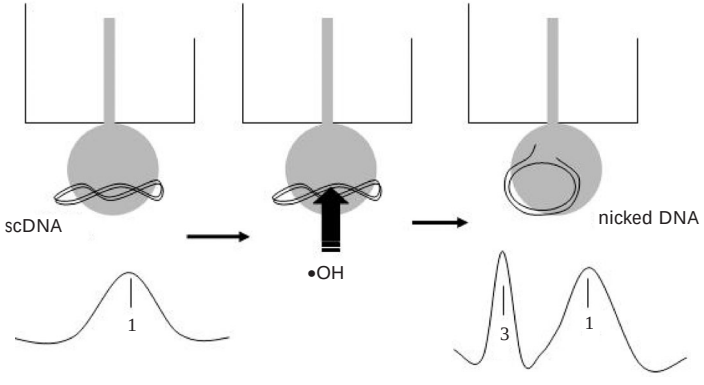

FIG. 5
Scheme of detection of DNA damaging agents using the scDNA-modified mercury electrode. The scDNA is adsorbed at the HMDE surface, thus forming a biodetector (biosensor). The biodetector is immersed into an analyzed sample. In the presence of species cleaving the DNA sugar-phosphate backbone, strand breaks are generated in the anchored DNA. The nicked ocDNA yields a.c. voltammetric peak 3 which is not produced by the intact scDNA. Instead of the HMDE, MFE or SAE can be used

the HMDE can be cleaved by various agents, such as chemical nucleases[78,110,114,115], reactive oxygen species[114,115] or enzymes[117]. The influence of the electrode potential on damage to the surface-confined DNA was investigated[114,115]. In the presence of transition metal ions or their complexes (iron/EDTA or copper/1,10-phenanthroline), formation of DNA-damaging oxygen radicals can be modulated via electrochemical processes. Measurements of DNA oxidation response at the CE do not offer sensitive detection of the DNA sb because of lack of differences between the signals of sc, oc and linDNAs [22,41,42].

5.2. Damage to DNA Bases

DNA bases can undergo chemical or photochemical reactions[34] that may result in changes of their electrochemical features. Due to covalent modification of the imidazole ring of G (with dimethyl sulfate, diethyl pyrocarbonate, an anticancer drug thiotepa, etc.), the electroactive site responsible for the G redox processes at the HMDE is lost, and the peak G diminishes[38]. An analogous principle has often been employed in connection with oxidation response of the G residues at CE (lit.[139–141]; reviewed in[22]). Measurements of the decrease in initially large signal are, however, inherently poorly sensitive (to obtain a decisive response, a portion of the damaged electroactive moieties has to exceed the standard relative deviation of the measurement, which is usually reported as several per cent). Better results can be obtained when modification of the base moieties results in appearance of new electrochemical signals. For example, an adduct of A residues with chloroacetaldehyde yielded a cathodic peak at the HMDE at a less negative potential, compared to the signal of the unmodified polyadenylic acid[142]. One of the most abundant products of natural DNA damage, 8-oxoguanine, yields a specific oxidation peak at CE [143,144]. When a molecule binding to the DNA is electroactive, the modified DNA may yield signals provided by the introduced moiety. For example, adducts of DNA with the anticancer drug mitomycin C yields signals at the HMDE due to the mitomycin C quinone group[145–148]. NAs modified with complexes of osmium tetroxide with nitrogenous ligands (Os,L), reacting primarily with thymine residues, produce several signals at the ME related to the osmium electrochemistry, including a large peak involving catalytic hydrogen evolution[111,112,149–152]. Some Os,L have been used as chemical probes of DNA structure[50,129,152], and the extent of DNA modification was determined electrochemically at the ME in some studies[50]. These substances can also be used as electroactive DNA markers in DNA hybridization assays[153–156] (see Section 6). Damage or modification of

DNA bases is often accompanied by distortions of the DNA double helix at the damaged sites. These structural perturbations can be detected electrochemically at the ME. For example, UV-irradiated dsDNA (involving mainly pyrimidine dimers)[60] or dsDNA treated with platinum complexes (forming different types of covalent adducts mainly with guanine residues)[10,121,123,126] were analyzed by electrochemical methods. Depending on the nature of the formed lesions, changes of the DPP peak II and/or peak III were observed (see Section 4.1). In some cases, lesions involving damaged bases can be converted into sb (e.g., by specific enzymes[21,157]) and detected indirectly using the technique described in Section 5.1

5.3. Substances Interacting with DNA Non-Covalently

A number of potentially genotoxic agents bind DNA non-covalently[34]. In some cases these interactions precede covalent attack on the DNA molecule. The non-covalent binding may involve electrostatic attraction between cationic moieties and the DNA phosphate groups, binding of some substances within the grooves of the DNA double helix, and intercalation of planar aromatic systems between DNA base pairs involving stacking interactions[34]. Such interactions may affect electrochemical behavior of the DNA-binding substance, of the DNA or of both. Association of toxic transition metals (Cd, Pb) with DNA was studied using CV, a.c. voltammetry and chronocoulometry at the HMDE [158,159]. Distinct interactions of the $[Co(NH_3)_6]^{3+}$ complex on DNA and RNA were studied by AdTS DPV and utilized in a microanalytical method for determination of RNA in the presence of DNA [52]. Polyuridylic acid adsorbed on HMDE surface accumulates Hg^{2+} ions[160]. Electroreduction of Ni^{2+} ions at the ME is catalyzed upon the metal binding to denatured and native DNA [161]. Copper is stabilized in its monovalent (Cu^{+}) state when coordinated by purine or cytosine bases and nucleosides. At the HMDE (and also at CE), this phenomenon results in splitting of Cu^{2+} reduction into two one-electron steps[162,163]. Cu^{+} stabilization upon binding to DNA bases has been proposed to be involved in the electrode potential-dependent damage to HMDE surface-confined DNA in the presence of copper and oxygen[115]. DNA interactions with metalloporphyrins containing ions of Cu, Ni, Cd or Zn, were investigated using CV at HMDE [164]. Complexes of DNA with various intercalators and minor groove binders, including chloroquine, $[Co(phen)_3]$, $[Ru(bipy)_3]$, doxorubicin, 9-aminoacridine[49], Acridine Orange[165] and actinomycin D [166] were analyzed by AdTS a.c. voltammetry. Alterations of the DNA structure upon binding of the intercalative drugs to dsDNA can be detected via changes of

the intensities of a.c. voltammetric peak 2 and peak 3 [49]. A natural bis-intercalator, antibiotic echinomycin exhibits differential CV response at the ss and dsDNA-modified HMDE, suggesting selective interactions with the DNA double helix[167].

6. APPLICATION OF MERCURY ELECTRODES IN DETECTION OF DNA HYBRIDIZATION

Two polynucleotide chains of complementary sequences can reform the double helix[168]. This process, called NA renaturation or hybridization, is being utilized for detection of nucleotide sequences. DNA hybridization is of great practical importance in contemporary medical diagnostics, forensic medicine, detection of pathogenic microorganisms including biological warfare, etc. (reviewed in[16–21]). In the DNA hybridization assays, one of the complementary strands – the hybridization probe – is specifically designed and synthesized as a relatively short oligonucleotide (tens of nucleotides). The probe is usually immobilized on a surface and subjected to the complementary NA strand in solution (target DNA or RNA). The target DNA (tDNA) is captured on the surface by forming duplex (hybrid) with the anchored probe and can be detected by various techniques. At present, the most frequently used methods involve radioactive or fluorescent DNA labeling. Optical detection is employed in most of the currently available DNA biosensors or chips (arrays)[169]. The optical analyzers are, however, relatively expensive which motivates the efforts to replace optical detection by other principles.

It has been shown that DNA hybridization assay can involve electrochemical detection of the hybrid formation (reviewed in[16–21]). The capture probe can be immobilized on the surface of an electrode, forming the recognition layer of an electrochemical DNA hybridization biodetector. A number of techniques of the detection of DNA hybrid at the electrode surface have been proposed, including utilization of intrinsic electroactivity of the tDNA [170], application of covalently bound electrochemically active[171–173] or enzymatic[174] tags, non-covalent redox indicators[175,176], employment of electrocatalytic enhancement of the DNA signals[170] or of DNA-mediated electron transfer[171], an electrochemical variant of the "molecular beacon" technology[173], etc. The capture probe immobilization has been attained in various ways, depending on the electrode material. For example, thiol-modified probes are usually used in connection with gold electrodes[171,173]. Other techniques involve covalent binding of probe oligonucleotides end-labeled with reactive groups to chemically modified carbon

or indium-tin oxide electrodes[20]. At the CE, the capture probes or tDNAs can be adsorbed without any chemical modification[176–178].

So far, there have been no reports about successful DNA hybridization on the surface of ME. The reason is probably a firm adsorption of hydrophobic DNA base residues on the mercury surface. Due to strong interaction with the surface, the bases of the immobilized strand are not available for forming the duplex with the complementary one in solution[21,177]. However, the ME have been successfully employed in DNA hybridization assays carried out in a "double-surface" mode.

6.1. Electrochemical Double-Surface DNA Hybridization Assay

In the double-surface method, the hybridization and detection steps are performed on two different surfaces[95,96,153–156] (Fig. 6). This technique was introduced to overcome some difficulties which limit performance of the single-surface techniques (i.e. those with the capture probe immobilized at the transducer electrode). Basically, the single-surface methods usually work well with model short tDNAs (tens of nucleotides), but often suffer from the loss of sensitivity and/or specificity when real tDNA samples (hundreds to thousands base pairs long DNAs) are analyzed. These difficul-

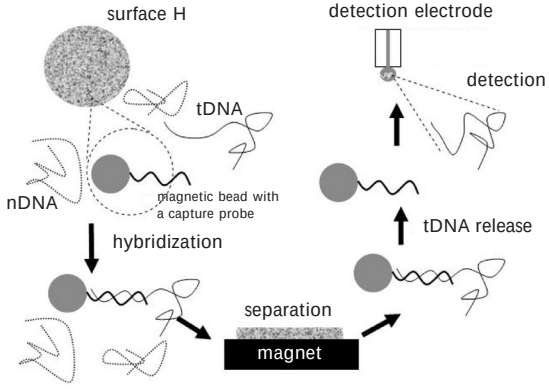

FIG. 6
Double-surface electrochemical technique of detection of DNA hybridization. The DNA hybridization takes place at surface H while detection of the hybridization event is performed at the detection electrode. Commercially available magnetic beads have been used as the surface H. Target DNA (tDNA) is captured on the beads via hybridization with complementary probe strands immobilized on the bead surface. Non-specific DNA (nDNA, dotted) is removed by repeated magnetoseparation and washing of the beads. Then the tDNA can be released from the surface H and electrochemically detected

ties originate mainly from non-specific interactions of non-complementary overhangs of the tDNA molecules and/or of other non-specific NAs at the electrode surface. Introduction of a separate surface for the hybridization step (surface H) in the double-surface technique makes it possible to optimize its properties and the procedure (including efficient washing and removal of non-specifically adsorbed species) without limitations related to the features of the transducer electrode. Commercially available magnetic beads coated with the capture probes fit well the requirements for the surface H and enable highly efficient and specific enrichment of the tDNA [93,95,96,153–156,179–182]. Moreover, the magnetic particles are applicable in microfluidic systems[183]. After hybridization and separation, the tDNA can be detached from the beads and analyzed by e.g., AdTS voltammetry (Fig. 6). Detection electrodes and suitable electrochemical techniques can be chosen with respect to the intrinsic features of the given tDNA and/or to the method of DNA labeling. Strong adsorption of the target (or probe) DNA on the electrode surface does not obstruct the double-surface assay[96,153,156,180]. The HMDE (or related electrodes such as mercury film or amalgam ones, Section 7) have been employed in the double-surface electrochemical DNA hybridization assays in connection with the following techniques.

6.1.1. Label-Free Detection of Acid-Hydrolyzed Target DNA

This method takes advantages of ultrasensitive stripping voltammetric detection of purine DNA bases involving sparingly soluble complexes with mercury or copper ions (Section 2.4.1)[93,95,96]. In this technique, tDNA detached from the surface H is incubated in acidic medium. Due to the partial DNA hydrolysis, purine bases are released from the N-glycosidic bonds and can be determined in the hydrolysate. Cathodic stripping voltammetry of the acid-hydrolyzed DNA at the HMDE or silver SAE (AgSAE) (involving sparingly soluble compounds of the purines with the electrode mercury)[96,97] as well as measurements of copper–purine complexes at HMDE, AgSAE or CuSAE [93] provide high sensitivities of the assay. A 1000-base pair DNA fragment could be detected in attomole quantities[96]. This technique uses no DNA labeling, utilizing exclusively intrinsic electrochemical properties of the tDNA components.

6.1.2. Detection of Osmium-Labeled Target or Probe DNAs

As mentioned in Section 5.2, DNA covalently modified with Os,L yields well defined signals at the ME and CE. While measurements of reversible oxidation/reduction signals of DNA modified with OsO_4, 2,2′-bipyridine (Os,bipy) at the CE allow differentiation between free Os,bipy and the DNA-Os,bipy adducts[111,153], measurement of the catalytic signal produced by these species at the ME provide a remarkably better sensitivity[112,154,155]. Using AdTS DPV at the HMDE in acid medium, detection limits below 1 ng of DNA–Os,bipy per milliliter can easily be attained[112]. Os,bipy labeling of either tDNAs or signaling probes has been utilized in the double-surface electrochemical DNA hybridization detection. It has been shown that long tDNAs can be Os,bipy labeled outside the sequence recognized by the hybridization probe immobilized at the surface H [153,155]. In another approach, the (non-labeled) tDNA captured on the beads were hybridized with a Os,bipy-labeled signaling probe[154]. Os,L-labeling of DNA is especially convenient when the hybridizing sequence does not contain pyrimidine bases. After hybridization on the magnetic beads, the captured molecules are released from the surface H and the osmium tags are subsequently detected by AdTS voltammetry at either CE or ME [153–155]. Another approach involves enzyme-linked immunoassay of the Os,bipy-labeled tDNA on the magnetic particles followed by detection at the CE [156].

7. MERCURY FILM AND SOLID AMALGAM ELECTRODES IN NUCLEIC ACID ANALYSIS

As mentioned in Section 1, other electrode types than the mercury ones are preferred in the contemporary nucleic acid electrochemical analysis and biodetector development. However, in some applications the ME are clearly superior. There are thus attempts to develop new electrode types which would combine the unique features of the HMDE with some properties of solid electrodes (non-toxic material, mechanical resistance, applicability in simple and cheap sensor devices, etc.). Unlabeled as well as chemically modified NAs and their components have been recently analyzed at the MFE or SAE.

Cathodic responses of electroreducible nucleosides, and denatured and degraded calf thymus DNA at relatively high concentrations (hundreds of $\mu g\ ml^{-1}$) were observed at a mercury film plated on a silver electrode[184]. The results obtained with a mercury-coated glassy carbon electrode (MF/GCE) suggest that the MF/GCE is more suitable for practical usage[42,75,185]. Both

redox and tensammetric responses of DNA, RNA, synthetic polynucleotides and PNA can be detected with the MF/GCE [75]. Calf thymus ssDNA or yeast tRNA yield well-defined CPSA peak G at concentrations of 100 and 50 ng ml^{-1}, respectively. The MF/GCE modified with scDNA was used as a sensor for DNA damage and for substances inducing DNA sb [42]. Os,bipy-modified DNA has also been analyzed at the latter electrode[185]. Nucleic acid bases form compact layers on the MF/GCE surface, similar to those previously studied at the HMDE (Section 2.4.2)[107,108]. Interactions of ss and dsDNA with a bis-intercalator echinomycin were studied at the MF/GCE using impedance or CPSA measurements[83]. A MFE covered with DNA-modified nitrocellulose membrane was used in studies of sorption of toxic heavy metals (Cd, Pb)[186] or immunoassays of DNA interactions with platinum complexes[187,188].

Purine bases can be detected at AgSAE or CuSAE by the stripping voltammetric techniques (involving sparingly soluble Hg or Cu complexes) with sensitivities similar to those attained with the HMDE [93,97,98]. Both AgSAE and CuSAE were utilized for detection of acid-hydrolyzed DNA, including applications in the double-surface DNA hybridization assay[93]. The AgSAE was applied in various variants, including AgSAE coated with a mercury meniscus (m-AgSAE) or a mercury film (f-AgSAE) and the AgSAE with polished surface (p-AgSAE). The liquid mercury-free p-AgSAE is applicable in detection of A and G bases in the presence of copper[98]. Tensammetric and redox responses of sc, lin and ssDNA at the m-AgSAE [116] or f-AgSAE (R. Fadrna and M. Fojta, unpublished data) exhibited differences analogous to those observed at the HMDE (Sections 4 and 5). Both m-AgSAE and f-AgSAE can thus be used as probes of DNA damage.

8. CONCLUSIONS

During the more than 45-year history of the NA electrochemical analysis, various electrode types and materials were applied. A broad spectrum of experimental data suggests that different types of analysis require application of different electrode materials, their modifications and combination with various electrochemical techniques. In general, solid electrodes (such as carbon, gold, indium-tin oxide or chemically modified variants of some of them) are suitable for the measurements of DNA oxidation response, of signals of electroactive DNA labels or non-covalent redox indicators. Most of the contemporary investigations involving solid (non-mercury) electrodes are targeted on the development of electrochemical DNA hybridization detectors. On the other hand, intrinsic DNA oxidation signals measured at

the CE inherently exhibit only low sensitivity to DNA structure (primarily due to accessibility of the A and G oxidation sites within the DNA double helix). For these reasons, ME offering conformation-sensitive cathodic and tensammetric DNA signals are better suited when small alterations in the DNA double helix should be detected. Measurements at the ME allow for discrimination between various DNA structure entities, including strand interruptions, free ssDNA stretches, intramolecular locally open regions, distortions of dsDNA including those induced by covalently or non-covalently bound drugs, etc. ME are highly sensitive probes of DNA sb. Signals at the ME of osmium-modified DNA involving catalytic hydrogen evolution allow easy detection of picogram quantities of DNA. There is an apparent conflict between the exclusivity of the ME in some types of NA analysis and their limited usage in the up-to-date research in bioelectrochemistry and development of electrochemical DNA biodetectors. Recent studies suggest that the way from this blind alley might lead through applications of the solid amalgam and/or mercury film electrodes.

9. SYMBOLS AND ABBREVIATIONS

A	adenine
G	guanine
C	cytosine
T	thymine
NA	nucleic acid
ss	single-stranded
ds	double-stranded
sc	supercoiled
oc	open circular
ccc	covalently closed circular
lin	linear
sb	strand break
ssb	single strand break
Os,L	osmium tetroxide complex with a nitrogenous ligand
Os,bipy	osmium tetroxide, 2,2′-bipyridine
PNA	peptide nucleic acid
tDNA	target DNA
a.c.	alternating current
CV	cyclic voltammetry
DPP	differential pulse polarography
DPV	differential pulse voltammetry
CSV	cathodic stripping voltammetry
SWV	square wave voltammetry
LSV	linear sweep voltammetry
CPSA	constant current chronopotentiometric stripping analysis

AdS	adsorptive stripping
AdTS	adsorptive transfer stripping
pzc	potential of zero charge
ME	mercury electrode
DME	dropping mercury electrode
HMDE	hanging mercury drop electrode
SMDE	static mercury drop electrode
MFE	mercury film electrode
MF/GCE	mercury film-coated glassy carbon electrode
SAE	solid amalgam electrode
CE	carbon electrode
region U	region of potentials in which DNA adsorbed at the mercury electrode is slowly unwound (denatured)

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