Cyclic Voltammetry of DNA at a Mercury Electrode: An Anodic Peak Specific for Guanine

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Abstract. Synthetic homopolyribonucleotides poly(A), poly(U), poly(C), and poly(G), poly(A, G, U), apurinic acid and native and denatured DNA from calf thymus were analyzed by means of cyclic voltammetry (CV) using a hanging mercury drop electrode. It was shown that guanine containing polynucleotides, i.e. poly(G), poly(A, G, U) and DNA yield an anodic peak of guanine in the vicinity of a potential of −0.3 V (against a saturated calomel electrode). The guanine peak appeared only at a sufficiently negative switching potential (about −2 V). The appearance of the guanine peak was conditioned by a reduction of guanine residues in the region of the switching potential and reoxidation of the reduction product in the vicinity of −0.3 V. Native and thermally denatured DNAs were investigated under the conditions of both complete and incomplete coverage of the electrode in various background electrolytes. Both DNA forms yielded anodic CV peaks of guanine with the peak of denatured DNA being always higher than that of native DNA. Irradiation of native DNA with relatively small doses of gamma radiation (5—120 Gy) resulted in an increase of the anodic peak. A comparison of changes induced by gamma radiation in the anodic (guanine) and cathodic (reduction of adenine and cytosine) peaks showed a steeper increase of the cathodic peak as compared to that of the anodic one. It has been concluded that in the given dose range the DNA double-helical structure is mainly damaged in the adenine-thymine rich regions.

Key words: DNA guanine residues — DNA radiation damage — Cyclic voltammetry — Mercury electrode

Introduction

More than 20 years ago (Paleček 1958, 1960a, b, 1961) nucleic acids and their components were found to be polarographically active. Adenine and cytosine are reducible at the mercury electrode, whereas guanine yields a specific anodic signal

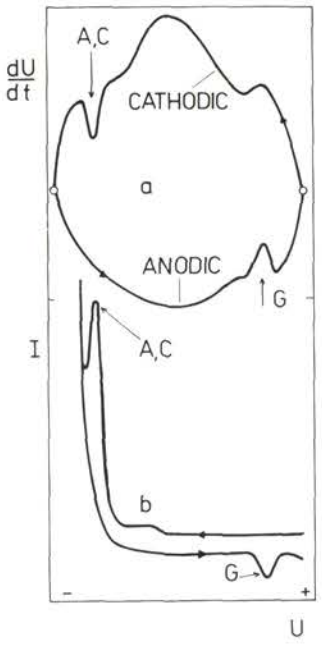

Fig. 1. Schematic representation of (*a*) oscillopolarographic and (*b*) cyclic voltammetric curves of denatured DNA. For the anodic signal G, guanine residues in the polynucleotide chain are responsible; this signal is observed only if the electrode was previously charged to sufficiently negative potentials (around −2 V) at which guanine is reduced. Due to scanning of the potential in the anodic direction the reduction product is oxidized and the anodic CV peak (or oscillopolarographic indentation) appears. A, C, cathodic signal due to reduction of adenine and cytosine residues.

(Fig. 1), which has been discovered by means of oscillographic polarography at controlled alternating current (Kůta and Paleček 1983). The reducibility of adenine and cytosine residues in the polynucleotide chain has been extensively investigated, especially with the use of differential pulse polarography (Paleček 1983). Using this approach, several important pieces of knowledge were obtained on the structure and properties of DNA (Paleček 1976). The disadvantage of this kind of polarographic analysis includes reduction of adenine and cytosine residues at about the same potential; consequently, their signals cannot be separated. Anodic indentation of guanine on curves $dU/dt = f(U)$ (Fig. 1) is not overlapped by a signal of another base, but it can only be detected by means of oscillographic polarography with alternating current, i.e. by a method with numerous disadvantages as compared with other electrochemical methods (Kůta and Paleček 1983). A study of guanosine by means of cyclic voltammetry (CV) has shown (Janík 1969) that its peak can be obtained only at scan rates higher than 3.5 $V.s^{-1}$. The

equipment necessary for rapid CV has not been a standard component of polarographic (voltammetric) analyzers and this finding (Janík 1969) has thus probably been the reason why this effect has not so far been studied more in detail by means of CV. Quite recently only rapid CV has been used in a study of guanosine (Trnková et al. 1980) and this method was shown to be of use even in DNA analysis (Trnková et al. 1980; Paleček et al. 1981).

In the present study we have investigated the behaviour of synthetic polynucleotides, apurinic acid, and DNA by means of CV and found this method very suitable for studies of the anodic peak yielded by guanine residues in DNA. Also, it was found that the measurements need not necessarily be carried out at high scan rates as it has been believed until recently.

Materials and Methods

Poly(G), poly(U) and poly(A, G, U) were supplied by Sigma, poly(C) by Serva and poly(A) and guanosine by Calbiochem. For details of calf thymus DNA isolation and characterization see a previous paper (Lukášová et al. 1982). Thermal denaturation and determinations of DNA concentration were also carried out as described earlier (Paleček and Jelen 1980). Irradiation of DNA was performed with a ^{60}Co source. Native DNA (200 $\mu g.cm^{-3}$) was irradiated in 7.5×10^{-3} mol.dm^{-3} sodium chloride with 7.5×10^{-4} mol.dm^{-3} sodium citrate in the presence of atmospheric oxygen. Apurinic acid was prepared by the method of Tamm (Tamm et al. 1952).

CV measurements were carried out with a Polarographic Analyser PAR 174A connected to a Universal Programmer PAR 175 and an Omnigraphic 9002 XY recorder. A three-electrode system was used, including a working hanging mercury drop electrode, a platinum counter electrode, and a saturated calomel electrode. The working electrode was a Metrohm HMDE, type E410, with a surface area of 2.2 mm^2. All measurements were made at 25 °C. Solutions were deoxygenated by passing a slow stream of argon through them and, during the measurements, over their surface.

Results

DNA and synthetic polyribonucleotides were measured by means of CV in a medium of 0.6 mol.dm^{-3} ammonium formate with 0.1 mol.dm^{-3} sodium phosphate, pH 6.8; this medium has proved to be suitable for the analysis of nucleic acids by means of oscillographic polarography (Paleček 1983) or other electrochemical methods. First, we tried to test whether CV measurements could be carried out at usual scan rates, i.e. substantially below ~4 V.s^{-1}, as recommended for CV of guanosine (Janík 1969). Our measurements showed that even at these lower scan rates a well developed anodic peak could be obtained in guanine-containing polynucleotides.

Synthetic polyribonucleotides and apurinic acid. CV of poly(A), poly(U), poly(C), poly(G), poly(A, G, U) and apurinic acid at a concentration of 1×10^{-4} mol.dm^{-3} (related to the base content) was performed with a fresh mercury drop and 10 s

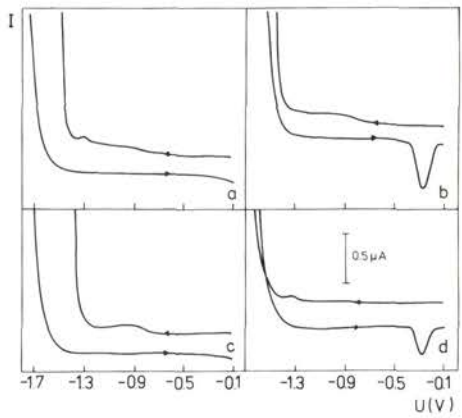

Fig. 2. Cyclic voltammograms of polyribonucleotides. *a*, poly(A); *b*, poly(A, G, U); *c*, poly(C); *d*, poly(G). The concentration of all the polynucleotides were 1×10^{-4} mol.dm^{-3} (related to monomer content). CV measurements were performed with a hanging mercury drop electrode (surface area 2.2 mm^2) in 0.6 mol.dm^{-3} ammonium formate with 0.1 mol.dm^{-3} sodium phosphate (pH 6.8). Scan rate 200 mV.s^{-1}, initial potential −0.1 V, switching potential −1.85 V, waiting time 10 s (at −0.1 V).

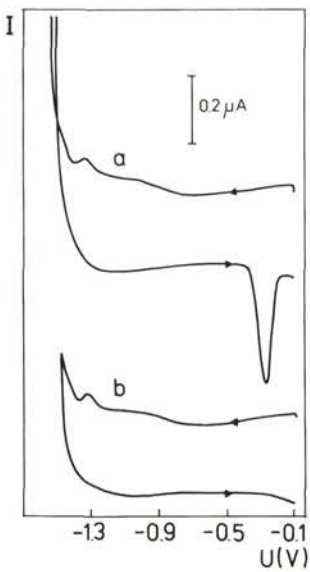

Fig. 3. Cyclic voltammograms of poly(G) at two different switching potentials. *a*, −1.85 V; *b*, −1.50 V. For other conditions, see Fig. 2.

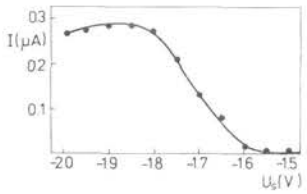

Fig. 4. Relationship between the height of anodic voltammetric peak of poly(G) and the switching potential U_s. For other conditions, see Fig. 2.

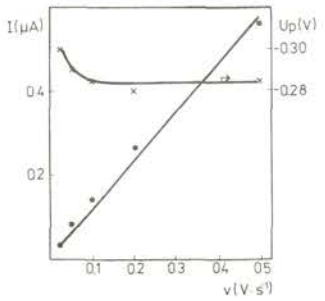

Fig. 5. Relationship between the height (I) and potential (U_p) of the anodic voltammetric peak of poly(G), and scan rate (v). ×——×, U_p; ●——● I. Concentration of poly(G) 1×10^{-4} mol.dm^{-3}. For other conditions, see Fig. 2.

waiting at the initial potential of −0.1 V in the above medium; the switching potential was −1.85 V and the scan rate 200 mV.s^{-1}. Under these conditions, poly(G) and poly(A, G, U) yielded an anodic peak at −0.27 V (Fig. 2*b*). Other polynucleotides (Fig. 2*a*, *c*) or apurinic acid (not shown) did not yield this anodic peak.

The height of the anodic peak of poly(G), similarly to that of the guanosine peak (Janík 1969; Trnková 1983), was dependent on the switching potential. At switching potentials more positive than about −1.6 V this peak did not appear (Figs. 3 and 4); in a range between −1.6 and −1.8 V it increased with the shift of the potential to more negative values, and in a range between −1.8 and −2.0 V the peak heigh was nearly independent of the switching potential (Fig. 4). The height of the peak of poly(G) was linearly dependent on scan rate over a range of 20—500 mV.s^{-1} (Fig. 5) and the peak potential shifted with the increasing scan rate to more positive values. As contrary to report by Janík (1969), at these relatively low scan rates the anodic peak was also yielded by guanosine. The dependence of the guanosine peak height on scan rate (not shown) was similar to that of poly(G). The scan rate dependence and the symmetry of the poly(G) peak suggest involvement of adsorption in the electrode process.

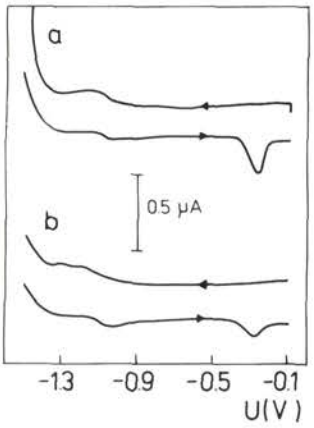

Fig. 6. Cyclic voltammograms of (*a*) denatured and (*b*) native DNA. DNA concentration 100 $\mu g.cm^{-3}$. For other conditions, see Fig. 2.

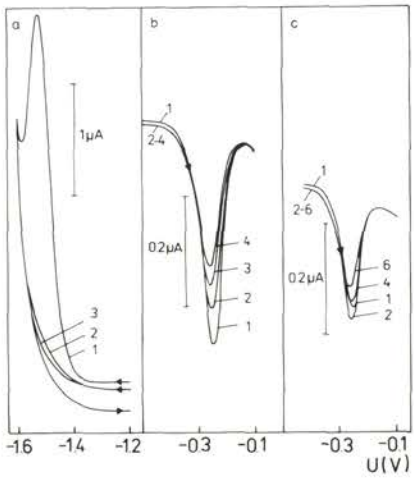

Fig. 7. Sections of cyclic voltammograms of denatured (*a*, *b*) and native (*c*) DNA with repeated voltage cycles. *a*, cathodic peak (due to reduction of adenine and cytosine residues), switching potential −1.6 V. *b*, *c*, anodic peaks, switching potential −1.85 V. Denatured DNA at a concentration of 100 μg/ml, native DNA 300 μg/ml, waiting time 120 s (at −0.1 V). For other conditions, see Fig. 2.

Native and denatured DNA yielded an anodic peak of similar characteristics (Fig. 6) as did poly(G) (Figs. 2, 3). In accordance with earlier oscillopolarographic measurements, the peak of thermally denatured DNA was substantially higher than that of native DNA (Fig. 6). The peaks of both native and denatured DNAs

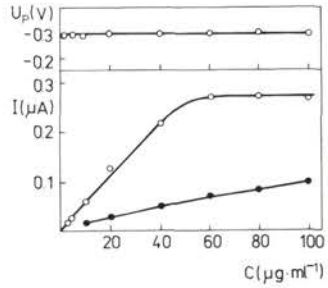

Fig. 8. Relationship between the height (I) and potential (U_p) of the anodic CV peak, and DNA concentration. ○———○ denatured DNA, ●———● native DNA. For other conditions, see Fig. 6.

were dependent on the switching potential, similarly as did the peak of poly(G) (Fig. 4).

If repeated voltage cycles were applied at the electrode the anodic peak of denatured DNA decreased gradually with the number of the cycles applied (*Fig. 7b*), while the peak of native DNA increased following the second cycle (Fig. 7*c*) and started to decrease after the third or fourth cycle. In agreement with previous studies (Valenta and Nürnberg 1974) the cathodic peak disappeared following the application of a second cycle (Fig. 7*a*).

The height of the anodic peak increased linearly with the concentration of denatured DNA within a range of 2.5—50 $\mu g.cm^{-3}$ at a waiting time of 10 s (Fig. 8); within a concentration range of 80—100 $\mu g.cm^{-3}$ the height of the peak of denatured DNA was independent of the concentration. Under the same conditions the peaks of native DNA increased linearly with the concentration within a range of 10—100 $\mu g.cm^{-3}$. If the measurements were performed at longer waiting time (120 s), a full coverage of the electrode surface was reached at native DNA concentrations higher than 200 $\mu g.cm^{-3}$ (the peak height did not depend on DNA concentration). Peak potentials of native and denatured DNAs were independent of the concentration. A relationship between the denatured and native DNAs peak heights on the scan rate is shown in Fig. 9.

Our earlier results have shown that double-helical DNA is denatured after a longer contact with the electrode charged to potentials of about −1.2 V (region U) (Paleček 1983; Paleček and Jelen 1984). The extent of the surface denaturation is negligible at scan rates of 1 $V.s^{-1}$ and higher if linear sweep voltammetry is used. In CV, DNA is exposed to these potentials twice during one cycle, so that even higher scan rates would be necessary to neglect the conformational changes resulting from the contact of DNA with the electrode in the region U. Thus, at the scan rates used in the present study, it should be considered that the height of native DNA peak is affected by conformational changes secondary to the interac-

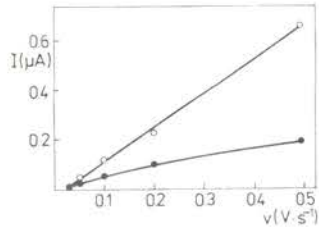

Fig. 9. Relationship between the height of the anodic CV peak of DNA and the scan rate (v). ○——○ denatured DNA, ●——● native DNA. For other conditions, see Fig. 6.

tion of double-helical DNA with the electrode. This conclusion is strengthened by the observation that the peak height ratio of denatured and native DNAs increase with the scan rate (from about 1 at 20 $mV.s^{-1}$ to 3.5 to 500 $mV.s^{-1}$ under conditions specified in Fig. 9) (at higher scan rates a smaller influence of surface denaturation on the native DNA peak height is expected), and by changes in the peak heights of native and denatured DNA during repeated voltage cycles (Fig. 7).

We investigated the influence of the composition of the background electrolyte on the height of the peak of denatured DNA at a concentration of 100 $\mu g.cm^{-3}$ (waiting time 10 s) at pH 6.8 (Table 1). The highest peaks were obtained in media containing NH_4^+ or Mg^{2+} ions. Concentrations of at least 0.5 $mol.dm^{-3}$ of NH_4^+ or Cs^+ were required to obtain the maximum peak height (Fig. 10); the same effect was obtained with 0.1 $mol.dm^{-3}$ $MgCl_2$ (Table 1). The anions showed little effect on the peak height. The height of the native DNA peak also increased with the salt concentration (Fig. 10). If measurements were carried out under conditions when no adsorption equilibrium was reached at the electrode surface (concentration 40 $\mu g.cm^{-3}$, waiting time 10 s) (the peak height was linearly dependent on both the DNA concentration and the waiting time), the peak height ratio of denatured and native DNA, I_{den}/I_{nat}, was 12.6 and 4.3 in a media containing CsCl and ammonium formate, respectively (Table 2). Under the conditions of a full coverage of the electrode surface (the peak height did not depend on the DNA concentration or the waiting time) the ratio I_{den}/I_{nat} was 2.7 and 2.0 in media with CsCl and ammonium formate, respectively.

The dependence of the peaks of native and denatured DNA on pH was investigated in a medium containing 0.5 $mol.dm^{-3}$ CsCl with Britton-Robinson buffer in a pH range of 3.2—8.5. Denatured DNA yielded the highest peaks in a pH region of 5.5—6.8, with the peak height being only little dependent on pH; a pH increase above 7 or a decrease below pH 5 resulted in a steep decrease of the peak height. Native DNA behaved in a similar way, with the region of the relative independence of the peak height on pH being shifted to more acid values by about 0.5 pH units. Peak potentials of native and denatured DNA were shifted in

Table 1. Effects of ion type and concentration in the background electrolyte on the height (I) of the denatured DNA anodic CV peak

Salt	$I(\mu A)$	c_{MgCl_2} (mol.dm^{-3})	$I(\mu A)$
NaCl	0.14	0.100	0.30
$NaClO_4$	0.15	0.010	0.20
CsCl	0.12	0.005	0.18
NH_4ClO_4	0.31		
$HCOONH_4$	0.26		
$Mg(ClO_4)_2$	0.29		

Measurements were carried out in a medium containing 0.6 mol.dm^{-3} monovalent salts with 0.1 mol.dm^{-3} sodium phosphate, pH 6.8; in the case of magnesium salts Tris-HCl buffer, pH 6.8, was used instead of phosphate 0.05 mol.dm^{-3}. $Mg(ClO_4)_2$ was used in a concentration of 0.1 mol.dm^{-3}, $MgCl_2$ concentration (c_{MgCl_2}) is given in the Table. Scan rate 200 mV.s^{-1}, initial potential −0.1 V, switching potential −1.85 V, waiting time 10 s. DNA concentration 100 μg.cm^{-3}.

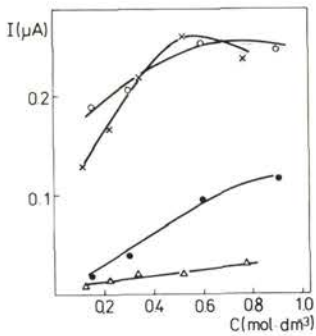

Fig. 10. Relationship between the heights of the anodic CV peaks of denatured and native DNA and salt concentration. ○——○ denatured DNA in $HCOONH_4$, ×——× denatured DNA in CsCl, ●——● native DNA in $HCOONH_4$, △——△ native DNA in CsCl. For other conditions, see Fig. 6.

Table 2. Height ratio of anodic CV peaks of denatured and native DNA I_{den}/I_{nat} at different degrees of coverage of the electrode surface

Medium	I_{den}/I_{nat}	
	i	ii
0.6 mol.dm^{-3} ammonium formate	4.3	2.0
0.5 mol.dm^{-3} CsCl	12.0	2.7

i, measurements performed under conditions before the adsorption equilibrium was reached; DNA concentration 40 μg.cm^{-3}, waiting time 10 s. ii, measurements performed under conditions of a full coverage of the electrode surface; native DNA concentration 300 μg.cm^{-3}, denatured DNA concentration 100 μg.cm^{-2}, waiting time 120 s. In both cases the medium contained 0.1 mol.dm^{-3} sodium phosphate, pH 6.8. For other conditions, see Table 1.

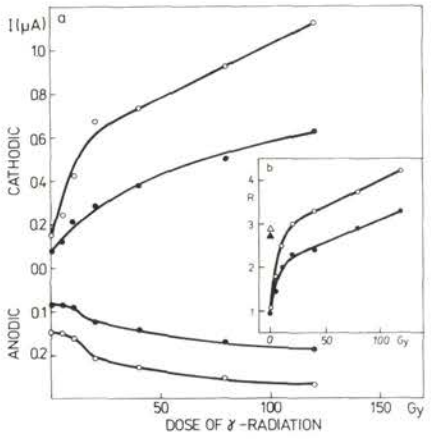

Fig. 11. Relationship between the height of the cathodic (adenine and cytosine) and anodic (guanine) CV peaks of double-stranded DNA and the gamma radiation dose. *R*, height ratio of the cathodic and anodic peaks ($I_{cathodic}/I_{anodic}$). ○——○ waiting time 10 s (electrode not fully covered); ●——● waiting time 120 s (adsorption equilibrium) at a potential of −0.1 V. *R* in thermally denatured DNA △, waiting time 10 s and ▲, 120 s. For other conditions, see Fig. 6.

negative direction by about 60 mV.pH^{-1} with the increasing pH, similarly as observed with guanosine (Trnková et al. 1980).

It follows from the measurements performed in the present study that denatured DNA always yields a higher anodic CV peak than does native DNA (Fig. 9, Table 2) under indentical conditions, i.e. differences in DNA properties due to different conformation features are manifested by differences in the heights of the anodic peaks. We tried to test whether the anodic peak would reflect changes in DNA conformation even substantially smaller than those occurring due to thermal denaturation. We used relatively low doses of ionizing radiation to induce local structural changes in the molecule of double-stranded DNA. Earlier, ionizing radiation induced damage to DNA was successfully investigated by means of DPP (Lukášová and Paleček 1971; Vorlíčková and Paleček 1974, 1978) and linear potential sweep voltammetry (Sequaris et al. 1978, 1982), which monitored cathodic signal of DNA. Using CV we measured both the cathodic and anodic peaks of DNA in dependence on the gamma-radiation dose over a range of 5—120 Gy (500—12,000 rad). The measurements were performed in a medium containing 0.6 mol.dm^{-3} ammonium formate with 0.1 mol.dm^{-3} phosphate, pH 6.9, at a scan rate of 200 mV.s^{-1} and a switching potential of −1.85 V under conditions (i) with no adsorption equilibrium reached; (ii) at a full coverage of the electrode surface; (iii) with adsorption equilibrium obtained at incomplete coverage of the electrode (the peak height did not depend on the waiting time, and it

increased with the DNA concentration). Under either conditions (i, ii, and iii) both the cathodic and anodic peak increased with the radiation dose. However, in the dose range of 5—40 Gy the cathodic peak increased with the radiation dose more steeply than did the anodic one (Fig. 11). Differences in the dependence of the anodic and cathodic peak on the radiation dose in a lower dose range is well illustrated by Fig. 11*b*, where the anodic to cathodic peak height ratio (R) is plotted against the radiation dose. In a dose range of 40—120 Gy, R changed less steeply with the increasing dose. For non-irradiated native DNA, R was close to unity, for thermally denatured DNA R was 2.7 and 2.9 under conditions (i) and (ii), respectively. It thus follows from the results presented that even small changes in DNA conformation are reflected by changes in the anodic peak height, under conditions when the peak height may be affected by eventual changes in the DNA diffusion coefficient as well as when changes in the DNA transport rate to the electrode cannot be effective (at a full coverage of the electrode surface). On the other hand, one should bear in mind that the values of I_{den}/I_{nat} (Table 2) may be influenced by the presence of single-strand breaks and other anomalies in the primary structure of native DNA and are thus characteristic of the given DNA sample, and not generally of calf thymus DNA.

Discussion

It was shown in the present study that CV is a suitable method to investigate the anodic signal of DNA, which has earlier been studied by means of oscillographic polarography at controlled alternating current (Paleček 1958, 1961, 1966). Our measurement with synthetic polynucleotides (Fig. 2) and apurinic acid showed that the anodic CV peak is specific for guanine residues in DNA. This specificity is advantageous, since it allows to investigate the individual behaviour of guanine-cytosine pairs in DNA. This is impossible with the cathodic peak (Fig. 1), which is due to the reduction of both adenine and cytosine.

In addition, we could show that the anodic signal of guanine (Fig. 2*d*) may be investigated by means of CV over a wide range of scan rates, including the usual rates of about 100 $mV.s^{-1}$ (Fig. 5), at which no oscillograph is required and only a recorder, which is a standard equipment of common polarographic (voltammetric) analyzers can be used. The earlier claim (Janík 1969) that fast sweep rates would be necessary to obtain the anodic peak of guanosine might be associated with the fact the instrumentation used at the end of the sixties was not sensitive enough to detect small anodic currents of guanosine produced at lower scan rates.

The mechanism of the electrode process responsible for the appearance of the anodic peak of guanine has not yet been studied in such a detail as was electroreduction of adenine and cytosine (Elving 1976; Paleček 1983). It has been found so far (Trnková 1983) that, due to the reduction of guanine in the region of

negative potentials, its pyrimidine and imidazole rings are not disturbed and the reduction product is easily re-oxidized by atmospheric oxygen to guanine. During the oxidation of the reaction product at the electrode two electrons are consumed and an ionized species is involved in the electrode process. The results of the present study show a good agreement between CV of poly(G) (Figs. 3—5) and guanosine (Trnková 1983). It may thus be supposed that the electrode processes in the polynucleotide are in principle the same as in the monomer. It is known that as a result of the reduction of the adenine and cytosine residues in denatured DNA a product is formed at the electrode which blocks its surface (Valenta and Nürnberg 1974) and prevents, during the application of the next cycle in the CV regime, DNA reduction (Fig. 7*a*). On the contrary, the anodic peak of guanine appears on the CV curve even if a second and further cycles are applied (Fig. 7). This could be explained in such a way that the reduction product of guanine (formed in the region of the switching potential) is re-oxidized to guanine in the course of the anodic process, or that the electrode process of guanine can proceed through a layer of reduction products of adenine and cytosine blocking the electrode. However, a more detailed elucidation of the mechanism of the electrode process responsible for the appearance of the anodic peak of guanine will require further work.

Influence of the secondary structure of DNA. In the case of adenine and cytosine, the primary reduction site (1,6 double bond in adenine and 3,4 double bond in cytosine) is involved in the hydrogen bonding system and is not accessible to electroreduction in the intact double-helical B form of DNA (Paleček 1983). In single-stranded (denatured) DNA these sites are accessible and a reduction signal is obtained. The electroactive site of guanine residues is not yet known, but the double bonds which are the potential candidates for the electroreduction (most probably the 7,8 double bond) are not involved in the hydrogen bonding in the double helix. The smaller height of the anodic peak of native DNA (as compared to denatured DNA) (Fig. 6, Table 2) is thus not due to the participation of guanine residues in the formation of hydrogen bonds with cytosine ; rather, it may be due to a steric blockade of a fraction of guanine residues and/or to a different adsorbability of native DNA. Conformational changes in the double-helical DNA might thus be manifested in a different manner in changes of the heights of the anodic and cathodic peaks, which could provide a more detailed information about the nature of these conformational changes. Experiments of this type with model synthetic double-helical polynucleotides are in progress.

The type and the concentration of ions in the background electrolyte have considerable effects on the anodic CV peak of monomeric guanosine (Trnková 1983). These effects are mediated through the (buffering) ability of ions to

maintain constant pH in the vicinity of the electrode, and by their presence in the electrode double layer. In the case of DNA the effects of ions may be of an even more complex nature. Ions can affect DNA conformation in solution, its adsorption at the electrode, and, in the case of double-helical DNA, even the course of conformational changes occurring at the electrode. It may be supposed that ions with high screening efficiency (Mg^{2+}, NH_4^+) play a similar role as they do in the reduction of adenine and cytosine in the polynucleotide chain (Paleček 1969, 1983), i. e. they screen the negative charges of the polynucleotide and make possible its contact with the negatively charged electrode. The great differences in the peak height ratios of native and denatured DNA (Table 1) in dependence on the type of salt at incomplete coverage of the electrode surface cannot be explained satisfactorily as yet. Different salts may influence the adsorption of DNA at the electrode and the course of conformational changes of double-helical DNA at the electrode surface in a different way.

The applicability of CV for the detection of minor changes in the conformation of double-helical DNA. It has been shown earlier (Sequaris et al. 1982) that the disturbance in the double-helical DNA structure secondary to a longer contact with the mercury electrode does not prevent the application of linear potential sweep voltammetry (monitoring only cathodic processes) to detect lesions induced in the double-helix of DNA by radiation or other agents. The results with gamma-irradiated DNA presented in the present study (Fig. 11) indicate that the same is true for the application of CV (monitoring the anodic process of guanine). It also follows from these results that conformational changes, mainly in regions rich in adenine-thymine pairs, occur as a result of irradiation, as indicated by changes in the height ratio of the cathodic and anodic peaks (Fig. 11*b*). These results supplement the existing information on the damage to DNA by ionizing radiation obtained by means of electrochemical and other methods (Lukášová and Paleček 1971; Vorlíčková and Paleček 1974, 1978).

An attempt to obtain information about the extent of the damage to guanine-cytosine pairs has been made recently (Brabec 1980) by means of differential pulse voltammetry at the graphite electrode. This method makes it also possible to investigate separately the voltammetric peak of guanine, oxidized at the graphite electrode at a potential different from that of adenine. However, this technique has proved to be little sensitive to the DNA damage. Using this method no changes were found in the peak heights of guanine or adenine after DNA irradiation with doses up to 400 Gy (40 krad). Only in a higher dose range these peaks were observed to increase; the guanine peak increased with the dose less steeply than did the adenine peak (up to 3,000 Gy), and the difference in the slope of this increase was substantially smaller than that found in our measurements (Fig. 11).

We can thus conclude that CV in connection with the mercury electrode is a method suitable to investigate the signal of guanine residues in DNA, and can be employed (even at the usual scan rates of about 100 $mV.s^{-1}$) to detect lesions in the DNA double-helix induced by the action of mutagenic and carcinogenic agents.

Abbreviations

CV — cyclic voltammetry
DPP — differential (derivate) pulse polarography
DNA — deoxyribonucleic acid
poly(A) — poly(riboadenylic acid)
poly(C) — poly(ribocytidylic acid)
poly(U) — poly(riboridylic acid)
poly(G) — poly(riboguanylic acid)
poly(A,G,U) — poly(riboadenylic-guanylic-uridylic acid) (with a random nucleotide sequence)

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