# Nucleotide Sequence-Dependent Opening of Double-Stranded DNA at an Electrically Charged Surface

F. Jelen and E. Paleček

*Institute of Biophysics, Czechoslovak Academy of Sciences, Královopolská 135, 612 65 Brno, Czechoslovakia*

**Abstract.** It has been shown earlier that the DNA double helix is opened due to a prolonged contact of the DNA molecule with the surface of the mercury electrode. At neutral pH, the opening process is relatively slow (around 100 s), and it is limited to potentials close to −1.2 V (against SCE). The opening of the double helix has been explained by strains in the DNA molecule due to strong repulsion of the negatively charged phosphate residues from the electrode surface where the polynucleotide chain is anchored via hydrophobic bases. Interaction of the synthetic ds polynucleotides with alternating nucleotide sequences /poly(dA-dT) . poly (dA-dT), poly (dA-dU) . poly (dA-dU), poly (dG-dC) . poly (dG-dC)/ and homopolymer pairs /poly (dA) . poly (dT), poly (rA) . poly (rU) and poly (dG) . poly (dC)/ with the hanging mercury drop electrode has been studied. Changes in reducibility of the polynucleotides were exploited to indicate opening of the double helix. A marked difference in the behaviour was observed between polynucleotides with alternating nucleotide sequence and homopolymer pairs: opening of the double-helical structures of the former polynucleotides occurs at a very narrow potential range (<100 mV) (region U), while with the homopolymer pairs containing A . T or A . U pairs, the width of this region is comparable to that of natural DNA (>200 mV). In contrast to natural DNA, the region U of homopolymer pairs is composed of two distinct phases. No region U was observed with poly (dG) . poly (dC). In polynucleotides with alternating nucleotide sequence, the rate of opening of the double helix is strongly dependent on the electrode potential in region U, while in homopolymer pairs, this rate is less potential-dependent. It has been assumed that the difference in the behaviour between homopolymer pairs and polynucleotides with alternating nucleotide sequence is due to differences in adsorbability of the two polynucleotide chains in the molecule of a homopolymer pair (resulting from different adsorbability of purine and pyrimidine bases) in contrast to equal adsorbability of both chains in a polynucleotide molecule with alternating nucleotide sequence. It has been shown that the mercury electrode is a good model of biological surfaces (e.g. membranes), and that the nucleotide sequence-dependent opening (unwinding) of the DNA

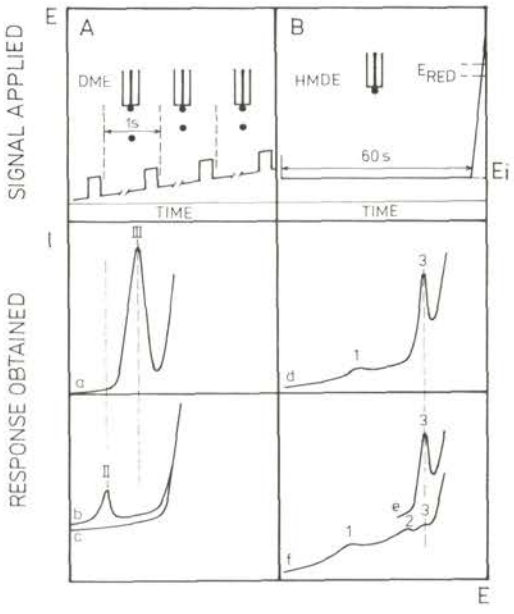


**Fig. 1.** Signals applied and responses obtained (*A*): in differential pulse polarography (DPP) in connection with the dropping mercury electrode (DME) (representing a technique working with small voltage excursion during the drop life time; (*B*): in linear sweep voltammetry (LSV) in connection with hanging mercury drop electrode (HMDE). (*A*): in DPP a single voltage pulse (usually of 10—50 mV) is applied to each drop of mercury dropping from the DME in 1—2 s intervals. The voltage ramp is scanned 1—2 mV/s. (*B*): in LSV, HMDE is kept for a certain time interval *t* at the initial potential *Ei*. During this waiting time *t*, DNA is adsorbed at the electrode and may undergo certain changes in its secondary structure due to its prolonged interaction with the electrode surface. After the waiting time *t*, the electrode potential is rapidly changed (usually 0.5—5 V/s) to more negative values at which ss DNA is reduced (*Ered*). The height of the voltammetric peak 3 is proportional to the amount of ss DNA reduced at the electrode surface, and it serves as an indication of the extent of surface denaturation of ds DNA. (*a*, *d*) thermally denatured DNA (*b*, *c*, *e*, *f*) ds DNA (in the bulk of solution); (*c*) at a concentration as usual for measurments of denatured DNA (20—30 μg/ml); (*b*) at higher concentrations (300—400 μg/ml) and a high sensitivity of the instrument; (*d*, *f*) *Ei* in the region T (e.g. −0.6 V); (*e*) *Ei* in the region U (e.g. −1.2 V), — for regions U and T see Fig. 2.

double helix at electrically charged surfaces may play an important role in many biological processes.

**Key words:** Double-stranded DNA — Homopolymer pairs — Alternating sequence polynucleotides — Electrically charged surface — Opening of the double helix — Voltammetry

## Introduction

Interaction of nucleic acids with various types of membranes plays an important role in living processes in the cell. Mercury electrodes the charge of which can be easily changed and controlled in a relatively wide range, have proved to be a suitable model of similar interactions (for reviews, see Nürnberg and Valenta 1978; Paleček 1980, 1983). It has been shown that the dropping mercury electrode (DME) in connection with the polarographic (voltammetric) methods, working with small voltage excursions during the drop life time (Fig. 1), is suitable for the study of DNA conformation in the bulk of solution (Paleček 1969, 1976, 1980, 1983). Measurements with a stationary mercury electrode yielded, on the other hand,information about changes in the polynucleotide conformation to which DNA is subject due to its interaction with the electrode surface charged to certain potentials (Nürnberg and Valenta 1978; Paleček 1983).

Opening of the double helix due to the interaction of the DNA molecule with the electrode was demonstrated in 1974 (Valenta et al. 1974; Paleček 1974). At neutral pH, the opening process takes place in a potential range (region U) around −1.2 V (against the saturated calomel electrode — SCE) (Fig. 1, 2) (Paleček 1980, 1983). The process is relatively slow (tens of seconds) and involves an appreciable part of the DNA molecule. In a wide range of more positive potentials (region T), faster changes occur which are, however, limited to several percents of the molecule in the vicinity of certain anomalies in the DNA primary structure (e.g. single-strand breaks — ssb). At potentials more negative than the region U, no changes in DNA conformation were observed (region W) (Paleček and Kwee 1979). Changes in the ionic strength influenced both the potentials of the region U and intensities of the voltammetric signals in the region T (Brabec and Paleček 1976; Brabec 1980; Paleček and Jelen 1980). Changes to acidic (Brabec and Paleček 1976; Malfoy et al. 1976, 1977; Sequaris et al. 1976; Paleček and Jelen 1980) or alkaline (Paleček and Jelen 1980; Paleček 1980) pH values (sufficiently distant from the neutral pH) resulted in an increase of the voltammetric signals in the region T due to ionization of the bases.

So far, the study of conformation changes at the mercury electrode surface has been carried out mainly with natural DNAs, and only a small number of papers have dealt with the synthetic polynucleotides (Brabec and Paleček 1976; Sequaris et al. 1976; Malfoy et al. 1977; Jelen and Paleček 1979), considering only their base content and not the nucleotide sequence. In this paper we have studied double-stranded (ds) polynucleotides with different nucleotide sequences and we have found maked differences in the behavior of homopolynucleotide pairs and polynucleotides with an alternating nucleotide sequence.

## Materials and Methods

Calf thymus DNA was isolated and characterized as in our previous papers (Paleček and Vetterl 1968; Lukášová et al. 1982). Poly (dA-dT) . poly (dA-dT) ($S_{20,w}$ 8.0), poly (dA) . poly (dT) ($S_{20,w}$ 12.7) and poly (dG) . poly (dC) were purchased from PL Biochemicals, poly (dA-dU) . poly (dA-dU) ($S_{20,w}$ 5.2) from Collaborative Res., poly (dG-dC) . poly (dG-dC) ($S_{20,w}$ 14.7), poly (rA) ($S_{20,w}$ 5.0), poly (rU) ($S_{20,w}$ 4.4), and poly (rC) from Sigma. Poly (rA) . poly (rU) ($S_{20,w}$ 9.4) was prepared by mixing solutions of parent polynucleotides at a concentration of $1 \times 10^{-4}$ mol/dm$^3$ in 0.01 mol/dm$^3$ sodium phosphate with $10^{-4}$ mol/dm$^3$ EDTA, (pH 7) 1 : 1. Sedimentation constants were kindly determined by Dr. J. Šponar from Institute of Organic Chemistry and Biochemistry, Czechoslovak Acad. Sci., Prague. Polynucleotides were used for the electrochemical measurements without further purification steps. Polynucleotide samples were irradiated using $^{60}$Co source at a concentration of $1 \times 10^{-4}$ mol/dm$^3$ in the presence of atmospheric oxygen. Molecular weight of polynucleotides was decreased by sonication using a Model B-12 Sonifier (Brason Sonic Power Company, Danbury, Conn.) under conditions as described previously (Brabec and Paleček 1978). Sonicated poly (dA-dT) . poly (dA-dT) sample having in average 100 base pairs was kindly provided by Dr. M. Vorlíčková. Linear potential sweep voltammetric measurements were carried out with a PAR 174 Polarographic Analyzer in connection with an Automatic Electroanalysis Controller PAR 315 A and a PAR 303 Static Mercury Drop Electrode in a hanging mercury drop electrode mode using size L of the drop (with a surface of 2.6 mm$^2$). Saturated calomel electrode K77 with a bridge tube K65 was used as a reference electrode and Pt wire served as a counter electrode in the three-electrode system. A.c. polarographic measurements were performed with a GWP 563 Polarograph (Akademie Werkstätten für Forschungsbedarf der DAW zu Berlin). The amplitude of a.c. avoltage of 80 Hz superimposed on the d.c. voltage was 20 mV. DME used for the a.c. polarographic measurements had a drop time of 2.2 s at a mercury column height $h = 60$ cm.

The analysed solution was deoxygenated by passing a slow stream of pure argon through it and (during the measurements) over its surface.

*Principles of measurements of conformational changes in the ds polynucleotide due to its interaction with the electrode surface.* It has been shown earlier (Paleček 1969, 1980, 1983) that adenine and cytosine residues contained in a single-stranded (ss) polynucleotide are polarographically (voltammetrically) reducible. On the contrary, in double-helical DNA adenine and cytosine reduction sites are hidden in the interior of the molecule (and form a part of the hydrogen bonding system), are not reducible or yield relatively low polarographic currents at more positive potentials as compared with the corresponding ss polynucleotides. These results have been obtained by means of polarographic methods working with a small voltage excursion during the drop life time in connection with the dropping mercury electrode, i.e. techniques in which the resulting signal cannot be influenced by changes in the polynucleotide conformation, which may occur at potentials more positive than the reduction potential. In the present paper a hanging mercury drop electrode was used, instead of the dropping mercury electrode. The former electrode was first held for a definite time $t$ (usually 60 s) at an initial potential $E_i$ (Fig. 1) (the value of which was preselected so that $E_i$ was more positive than potentials of polynucleotide reduction), and subsequently followed by a single voltage sweep (0.5 V/s) reaching over the polynucleotide reduction potentials. Values of the observed voltammetric reduction peaks served to indicate changes in ds polynucleotides, which occurred as a consequence of interaction of the polynucleotide with the electrode charged to the potential, $E_i$, for the time, $t$.

## Results

All polynucleotides (Table 1) were measured in the medium of 0.3 mol/dm$^3$

**Table 1.** Peak potentials ($Ep$) and sedimentation constants ($S_{w,20}$) of ds and ss polynucleotides

| Polynucleotide | $S_{w,20}$ | $Ep$, V | |
|---|---|---|---|
| | | peak 2 | peak 3 |
| poly (dA) . poly (dT) | 12.7 | — | −1.57 V |
| poly (dA-dT) . poly (dA-dT) | 8.0 | — | −1.55 V |
| poly (rA) . poly (rU) | 9.4 | −1.38 V | −1.57 V |
| poly (dA-dU) . poly (dA-dU) | 5.2 | −1.38 V | −1.48 V |
| poly (dG) . poly (dC) | — | — | −1.51 V |
| poly (dG-dC) . poly (dG-dC) | 14.7 | −1.37 V | −1.52 V |
| poly (rA) | 5.0 | — | −1.57 V |
| poly (rC) | — | — | −1.52 V |
| poly (rU) | 4.4 | — | — |

ammonium formate with 0.05 mol/dm$^3$ sodium phosphate (pH 6.8) (previously used in the work with natural and synthetic polynucleotides (Paleček 1974, 1980, 1983; Brabec and Paleček 1976)). At an initial potential ($Ei$) of −0.6 V poly (dA-dU) . poly (dA-dU) and poly (rA) . poly (rU) yielded, under the given conditions, peaks analogous to those of natural DNA (Fig. 2), i.e. in addition to the small nonspecific peak 1, a peak 2 (typical for helical regions) and peak 3. In poly (dA-dT) . poly (dA-dT) and poly (dA) . poly (dT), instead of two separated peaks 2 and 3, only one peak was observed at potential close to those of peak 3 of poly (dA-dU) . poly (dA-dU) and poly (rA) . poly (rU); the former peak will be further referred to as peak 3. Peak 2 was absent in poly (dG) . poly (dC) as well. Peak potentials ($Ep$) of all polynucleotides are summarized in Table 1.

*Dependence of heights of peak 2 and 3 on the initial potential (Ei)* was studied at a waiting time $t = 60$ s. In principle, the results agreed with those obtained with natural DNA (Paleček 1974, 1980, 1983; Brabec and Paleček 1976), i.e. both peak heights were almost independent of $Ei$ (Fig. 2) in a wide potential range (region T); in a relatively narrow range of negative potentials (region U), peak 2 sharply decreased while peak 3 increased and, after reaching its maximum value around −1.3 V, it decreased below its original value. The behaviour of poly (dG) . poly (dC) was an exception from the behaviour of the polynucleotides analysed (Table 1): the height of peak 3 of this polynucleotide was in the range of −0.4 to −1.3 V independent of $Ei$ and no increase of this peak (region U) was observed (Fig. 3*b*). This behavior of poly (dG) . poly (dC) thus agreed with that of poly (rG) . poly (rC) observed earlier (Jelen and Paleček 1979).

In agreement with the behavior of denatured DNA (Paleček 1974, 1980, 1983; Brabec and Paleček 1976) peak 3 of ss poly (rA) and poly (rC) was almost

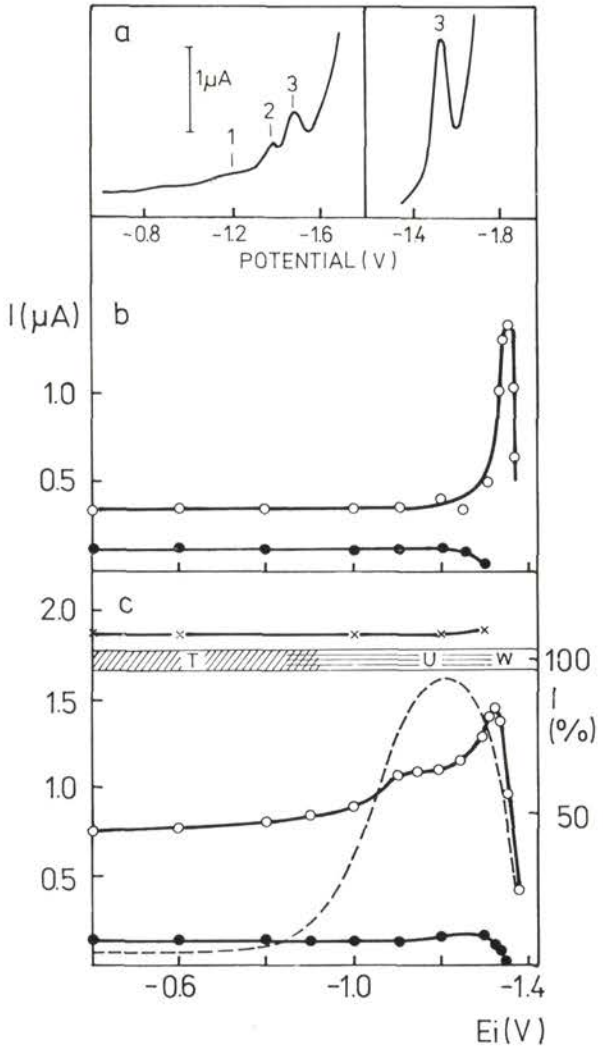


**Fig. 2.** Dependence of the voltammetric behaviour of polynucleotides on the initial potential (*Ei*). (*a*): voltammetric peaks of poly (dA-dU) . poly (dA-dU). *Ei* = −0.6 V (left), *Ei* = −1.35 V (right); (*b*): ●——●, height of peak 2: ○——○, height of peak 3; (*c*): poly (rA) . poly (rU), ●——●, height of peak 2; ○——○, height of peak 3; x——x, poly (rA); – – – –, calf thymus DNA (data extracted from Paleček and Kwee (1979)), peak height expressed in per cents of the height of peak of thermally denatured DNA. DNA at a concentration of 100 μg/ml, concentration of other polynucleotides was $5 \times 10^{-5}$ mol/dm$^3$ (related to phosphorus content). Background electrolyte: 0.3 mol/dm$^3$ ammonium formate with 0.05 mol/dm$^3$ sodium phosphate (pH 6.9). HMDE, scan rate 0.5 V/s, waiting time 60 s, potentials were measured against the SCE. U is the potential region in which relatively slow opening of the DNA double helix occurs, involving an appreciable part of the molecule (provided the time of DNA

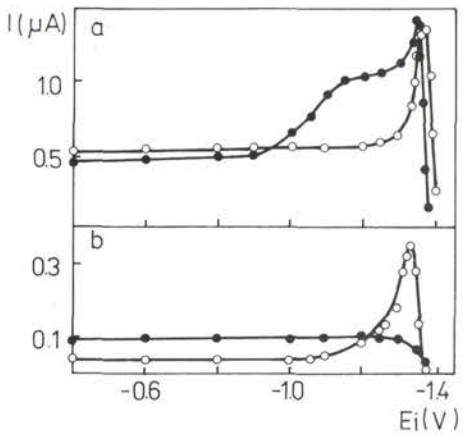


**Fig. 3.** Dependence of the height of peak 3 of ds polydeoxyribonucleotides on the initial potential *Ei*. (*a*): (●——●) poly (dA) . poly (dT); (○——○) poly (dA-dT) . poly (dA-dT). (*b*): (●——●) poly (dG) . poly (dC); (○——○) poly (dG-dC) . poly (dG-dC). For other conditions see Fig. 2.

independent of *Ei* (Fig. 2*c*). If the behaviour of poly (dG) . poly (dC) is not considered, a marked difference in peak height-*Ei* dependences of homopolymer pairs and ds polynucleotides with alternating nucleotide sequences on the other hand can be observed: in the latter polynucleotides, the region U is substantially narrower than in homopolymer pairs (Figs. 2, 3), which show, in addition to a sharp maximum close to *Ei* −1.35 V a shoulder at more positive potentials.

*Dependence of peak heights on time of interaction of polynucleotides with the electrode charged to potentials of the region U.* At polynucleotide concentrations used in this paper, the adsorption equilibrium in region T was reached in about 10 s. After reaching the adsorption equilibrium (in region T), the heights of peaks of ds polynucleotides were independen of time, at least for one minute. At longer time intervals, most of the polynucleotides showed a slight decrease of their peak heights. We attempted to follow changes in the heights of the peaks in dependence on time at *Ei* of region U. First, we let pass $t_i = 30$ s at $Ei_1 = -0.6$ V (region T) (Fig. 4). During this time, adsorption equilibrium was reached, and after the period $t_i$ the potential jumped to $Ei_2$ in region U. The electrode was kept at $Ei_2$ for a certain time $t_2$, followed by a rapid potential sweep to negative values (i.e. to potentials at which the adenine or cytosine moiety is reduced); $t_2$ usually varied

interaction with the electrode is sufficiently long). T is the potential region where fast opening of the DNA dobule helix takes place; it is limited to several per cents of the molecule in the vicinity of certain anomalies in the DNA primary structure (e.g. single strand breaks). W is the potential region where no changes in the DNA conformation were detected.

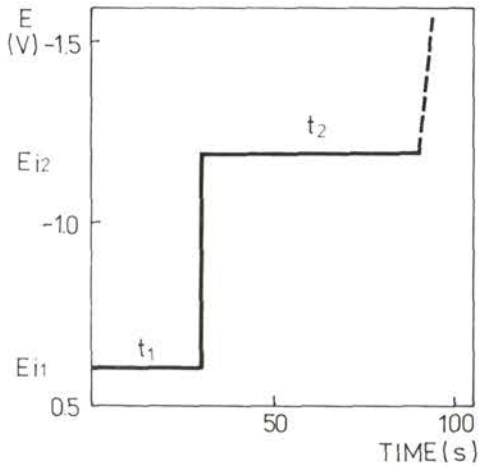


**Fig. 4.** Schematic presentation of polarization sequence of the mercury electrode with potentiostatic double step followed by single-sweep voltammetry. *Ei*, initial potential; *t*, waiting time; – – – –, sweep (in this paper, sweep rate was usually 0.5 V/s).

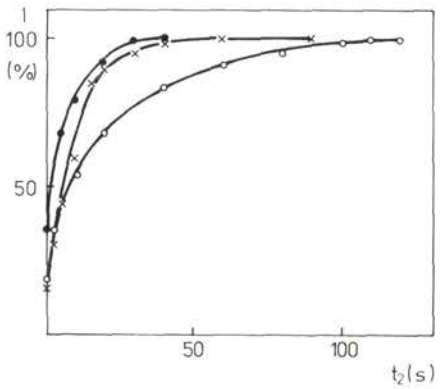


**Fig. 5.** Dependence of the height of peak 3 of ds polydeoxynucleotides on waiting time $t_2$. ●——●, poly (dA-dT) . poly (dA-dT); ○——○, poly (dA) . poly (dT); x——x, poly (dG-dC) . poly (dG-dC). The maximum height of peak 3 was taken as 100 percent. Potentiostatic double step sweep method (for explanation, see Fig. 4). For other conditions see Fig. 2.

from zero to 120 s. The dependence of peak 3 on $t_2$ were investigated for all the polynucleotides at $Ei_2$, corresponding to the maximum height of the peak ($Ei_{2MAX}$) found in the investigation of the dependence of peak 3 on *Ei* (Figs. 2 and 3), or at some other potentials of region U. Parallelly, the dependence of peak 2 on $t_2$ was investigated for those polynucleotides which yielded this peak. At $Ei_{2MAX}$, peak 3

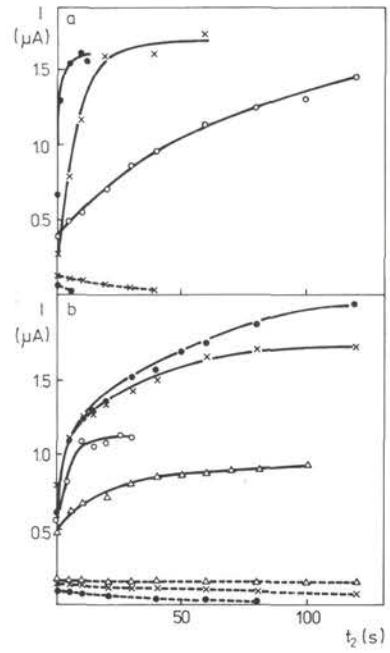


**Fig. 6.** Dependence of the heights of peak 2 and 3 on waiting time $t_2$ at various initial potentials ($Ei$). ($a$): poly (dA-dU) . poly (dA-dU). ●, $Ei = -1.38$ V; $Ei = -1.35$ ($Ei_{max}$); ○, $Ei = -1.32$ V. ($b$): poly (rA) . poly (rU). ●, $Ei = -1.34$; x, $Ei = -1.31$ V; ○, $-1.37$ V; △, $-1.20$ V. – – – –, peak 2; ——, peak 3. The maximum height of the given peak was taken as 100 percent. Potentiostatic double step sweep method, waiting time $t_1$ was 30 s (for explanation, see Fig. 4). For other conditions see Fig. 2.

of all ds polynucleotides increased in dependence on $t_2$ until it reached a limiting value (Figs. 5 and 6). On the contrary, peak 3 of ss poly (rA) (at $Ei = -1.34$ V, which corresponds to $Ei_{2MAX}$ of poly (rA) . poly (rU)) was initially independent of $t_2$ and it decreased after waiting times longer than 60 s. Peak 2 of ds polynucleotides decreased in dependence on $t_2$ up to complete disappearance (Fig. 6). The dependences of the peak heights on $t_2$ had different courses for ds polynucleotides with alternating sequence and for homopolynucleotide pairs (Figs. 5 and 6): The dependence of peak 3 on $t_2$ for polynucleotides with alternating sequence exhibited a steeper course, and the limiting values were reached in substanially shorter times (less than 40 s) than it was the case for homopolynucleotide pairs. Peak 2 of polynucleotides with alternating sequence (Fig. 6$a$) decreased more steeply and disappeared within a shorter time than in homopolynucleotide pairs (Fig. 6$b$).

The dependence of the peak height on $t_2$ at various $Ei_2$ was studied for poly (dA) . poly (dT) and poly (dA-dT) . poly (dA-dT) (not shown), poly (rA) . poly (rU) and poly (dA-dU) . poly (dA-dT) (not shown), poly (rA) . poly (rU) and poly (dA-dU) . poly (dA-dU) (Fig. 6). For poly (dA-dT) . poly (dA-dT), the limiting

value was reached in 6 s at $Ei_2 = -1.40$ V, in about 10 s at $-1.37$ V, and in 120 s at $-1.34$ V. A similar dependence was also observed for poly (dA-dU) . poly (dA-dU) (Fig. 6*a*). For poly (dA) . poly (dT) (not shown) and poly (rA) . poly (rU) (Fig. 6*b*) the influence of $Ei_2$ on the course of the dependence was substantially smaller. For both homopolynucleotide pairs an increase of peak 3 in dependence on $t_2$ could be observed at $Ei_2$ of about $-1.2$ V, i.e. at potentials at which the peak height was independent of $t_2$ for polynucleotides with alternating sequence (not shown).

*Influence of degradation of polynucleotides.* Polynucleotides used in this paper have different molecular weights (Table 1) and no one of them is expected to be monodisperse. The half-width of the region U in polynucleotides with an alternating nucleotide sequence varies in a narrow range from about 40 to 60 mV (Figs. 2 and 3), while the molecular weights show great differences (m.w. of poly (dA-dT) . poly (dA-dT) is $5.2 \times 10^5$ and that of poly (dG-dC) . poly (dG . dC) is $3.3 \times 10^6$). Similarly, results obtained with homopolymer pairs suggest that differences observed in widths of the region U (Figs. 2 and 3) between polynucleotides with an alternating nucleotide sequence and homopolymer pairs are not determined by differences in molecular weights of the polynucleotides. Degradation of polynucleotides with sonication and $\gamma$-irradiation has supported this suggestion. In agreement with the results of Sequaris et al. (1978) sonication and $\gamma$-irradiation of poly (dA-dU) . poly (dA-dU), poly (dA-dT) . poly (dA-dT) and poly (rA) . poly (rU) resulted in an increase in the height of peak 3 in region T without any significant change in the width of region U, even after a dose of 400 Gy (this dose causing breakage of several percents of all sugar-phosphate linkages) or 15 min of sonication. Even very short polynucleotide chains (about 100 base pairs) of poly (dA-dT) . poly (dA-dT) prepared by sonication yielded a narrow region U without any sign of a shoulder at more positive potentials. Heights of peak 3 at Ei of the region T in the strongly degraded polynucleotide reached about 70 % of peak heights found at $Ei_{max}$. It is thus evident that in these polynucleotides a substantial part of the double helix was opened before the interaction with the electrode, and further bases were released from the double helix at the electrode surface charged to potentials of the region T (where surface denaturation proceeds very fast (Paleček 1980, 1983; Brabec and Paleček 1976) and cannot be measured with the methods used in this paper). In agreement with this behaviour, the dependences of the peak 3 heights on $t_2$ (not shown) at $Ei_{max}$ were rather flat because the surface denaturation in region U started when most of the electrode surface was already covered with ss polynucleotides.

*Polynucleotide adsorption* was investigated by means of a.c. polarography in association with a dropping mercury electrode (i.e. by the method using small

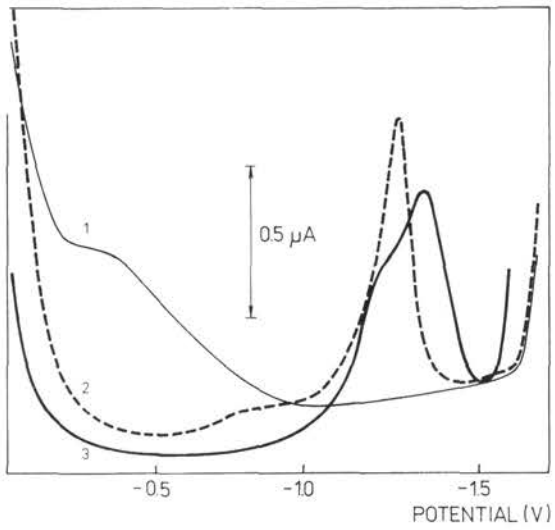


**Fig. 7.** Alternating current polarograms of ss 3, poly (rA) and 2, poly (rU) 1, background electrolyte. $6 \times 10^{-3}$ mol/dm$^3$ polynucleotides in 0.3 mol/dm$^3$ ammonium formate with 0.05 mol/dm$^3$ sodium phosphate (pH 6.9). DME, for conditions, see Materials and Methods.

changes of potential during the drop lifetime (Fig. 1)). A.c. polarograms of poly (rA) and poly (rU) in a medium of 0.3 mol/dm$^3$ ammonium formate with 0.05 mol/dm$^3$ phosphate, pH 6.8, showed that, at full electrode coverage, the differential capacity in the vicinity of the potential of zero charge (p.z.c.) decreased more for poly (rA) than for poly (rU) (Fig. 7) and that the potential of the tensammetric peak of poly (rA) was by about 60 mV more negative than the peak potential of poly (rU). This difference in potentials could be affected by a participation of faradaic current on the peak of poly (rA), which is polarographically reducible in the given medium (Paleček 1980, 1983). Therefore, additional measurements were carried out in 0.3 mol/dm$^3$ KCl with Britton-Robinson buffer, pH 8.05, where poly (rA) behaves as a nonreducible substance. Even under these conditions, a marked difference was observed in the peak potentials of poly (rA) and poly (rU) (Fig. 8*a*) in a broad concentration range including the regions of both the total and partial coverage of the electrode in the vicinity of p.z.c. (Fig. 8*b*). Our results show that poly (rU) is adsorbed at the electrode less firmly than poly (rA) and that the latter polynucleotide is desorbed at more negative potentials as compared with poly (rU).

## Discussion

*Influence of electric field.* During the interaction with the electrode the polynuc-

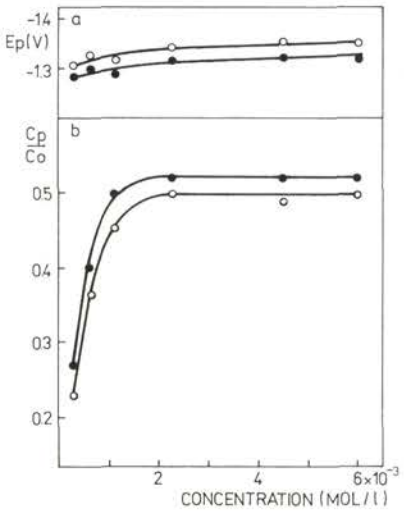


**Fig. 8.** Dependence of (a) the tensammetric (a.c. polarographic) peak potential, $Ep$ and ($b$) the relative differential capacity, Cp/Co on the polynucleotide concentration. ●——●, poly (rU), ○——○, poly (rA). Cp and Co are differential capacities of the electrode double layer at −0.6 V in the polynucleotide solution and in background electrolyte, respectively. Background electrolyte: 0.3 mol/dm$^3$ KCl with Britton-Robinson buffer (pH 8.05). DME, for conditions, see Materials and Methods.

leotide is subject of effects of various factors, including electric field. Effects of electric field on polynucleotides in solution has been studied in a number of papers (O'Konski and Stellwagen 1965; Neumann and Katchalsky 1972; Revzin and Neumann 1974; Pörschke 1974; Diekmann et al. 1982a, b; Charney and Yamaoka 1982; Che Hung Lee and Charney 1982). A strong orientation of DNA by electric fields was observed, and it was attributed to polarization of the ion atmosphere around the polyelectrolyte (Diekmann et al. 1982a). It has been assumed that the resulting dipole causes partial orientation of the molecules with their long axis parallel to the electric field. A strong electric field formed in the vicinity of the electrode is limited to distances of units or tens of nm from the electrode, it thus cannot cause orientation of a long DNA molecule with its long axis parallel to the field. Such an effect might be expected in duplexes not longer than 10—20 base pairs. The nonhomogeneous electrode has its highest strength (up to $10^6$ V/cm) in the so-called inner or compact double-layer (with a width corresponding to several atomic diameters) adhering to the metal phase. The field strength sharply decreases with the distance from the electrode. It has roughly one tenths of its original value at the boundary of the inner and the diffuse double-layer, and decreases nearly to zero in the diffuse double-layer (the depth of the diffuse double-layer at an ionic strength of 0.1 mol/dm$^3$ is approximately 9 nm).

Let us consider the interaction of a ds DNA molecule having 1000 base pairs (i.e. the contour length over 300 nm, and the persistence length roughly 45 nm)

with the mercury electrode. DNA is transported to the electrode by diffusion and it may be expected that in a distance over 10 nm from the electrode surface, the orientation of the DNA molecule towards the electrode will be accidental. If the electrode potential is close to the p.z.c. (in the medium used in the present study at about −0.5 V), the strength of the electric field of the electrode is close to zero and the DNA segment which is sufficiently close to the electrode surface is adsorbed via the sugar-phosphate backbone, or via bases, not included into the double-helix (if available). The nonadsorbed portion of the molecule in the solution is subject to thermal motion. The adsorption of the segment may be accompanied by changes in its hydration, its ionic atmosphere, as well as by certain conformation changes. These changes would occur, however, in connection with the adsorption an not solely due to the effect of the electric field. In accord with this assumption the heights of ds DNA peaks 2 and 3 are independent of Ei in a rather broad range of potentials in the vicinity of p.z.c. (Figs. 2 and 3).

Let us now consider a case when the electrode potential is more negative than potentials of the region U (e.g., −1.6 V). As soon as a segment of a DNA molecule enters sufficiently deep into the diffuse double-layer, it probably becomes reoriented along with the polarization of the ionic atmosphere, due to the electric field action. However, repulsion forces between the negatively charged electrode and negatively charged phosphates are so strong that DNA adsorption does not take place and the segment probably diffuses off (as it is indicated by the a.c. polarographic behavior of DNA in connection with DME (Paleček 1980, 1983) and by the results of measurements using the double step sweep technique (Paleček and Kwee 1979).

*Surface denaturation.* If the electrode is charged to potentials of the region U, orientation and polarization effects probably occur as a result of the action of electric field on a segment of a molecule which approached sufficiently close to the electrode surface. At these potentials, the segment can, however, be anchored at the electrode surface if it contains bases accessible for an interaction with the electrode (e.g. owing to local changes in the conformation either in the vicinity of ssb or at the end of the molecule or due to a temporary opening of the double helix). In such a case, the segment is adsorbed relatively firmly via the bases (Paleček 1980, 1983; Janík and Sommer 1973; Brabec and Paleček 1972), whereas the neighbouring parts of the molecules are repulsed from the electrode (potentials of the region U correspond to desorption potentials found by a.c. polarography in connection with DME). It has been assumed (Paleček 1974, 1980, 1983; Brabec and Paleček 1976) that, due to the tension conditioned by strong repulsion of ds segments adjacent to the segment adsorbed through bases (the later segment has necessarily a ss character), the molecule unwinds. Bases released from the double helix during this process are adsorbed at the electrode and denaturation proceeds to further regions.

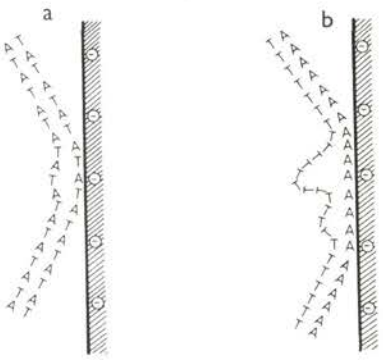


**Fig. 9.** Schematic representation of the interaction of ds polynucleotides (a) with alternating nucleotide sequence, poly (dA-dT) . poly (dA-dT) and (b) homopolymer pairs, poly (dA) . poly (dT) with the mercury electrode charged to a potential of −1.2 V (against SCE). The polynucleotide with alternating nucleotide sequence behaves like in the region of potential of zero charge; the secondary structure of the homopolymer pair is disturbed due to uneven adsorbability of the polypurine and polypyrimidine strands.

*Different adsorbability of bases.* Earlier it has been established (Vetterl 1965) that the surface activity of monomeric bases increases in the sequence cytosine < uracil < adenine < guanine, and this order is followed even in the dinucleoside monophosphates (Cummings et al.1977). Stronger adsorbability of poly (rA) as compare with poly (rU) found in this paper on the ground of a.c. polarographic measurements (Figs. 7 and 8) is in agreement with the adsorption properties of low molecular weight nucleic acid constituents (Vetterl 1965; Cummings et al. 1977). The differences in adsorbability of bases may influence the surface denaturation (in the region U) of DNAs in dependence on their nucleotide sequences. While in duplexes with alternating nucleotide sequences both polynucleotide chains are adsorbed with an equal firmeness, it can be expected that in homopolymer pairs one of the chains (probably the polypurine one) will be adsorbed more strongly. Thus segments of polynucleotides with alternating nucleotide sequence should be desorbed from the DME in a narrower potential range than homopolymer pairs. Because of the existing correlation between the potential of the desorption (tensammetric) peak and potentials of the region U (found earlier with calf thymus DNA (Brabec and Paleček 1976)), region U in polynucleotides with alternating nucleotide sequence should be narrower as well. Results of our experiments with polynucleotides containing A . T or A . U pairs (Figs. 2 and 3*a*) are in a good agreement with this assumptions. The presence of two phases in region U observed with poly (dA) . poly (dT) and poly (rA) . poly (rU) (Figs. 2 and 3) may tentatively be explained on the assumption that, at potentials close to −1.2 V, repulsion forces

between the negatively charged phosphates and the electrode are relatively weak and the double helix is only partially disturbed (peak 2 is not significantly influenced at these potentials), and the molecule is not fully unwound even in time intervals longer than 120 s. It may be expected that at these potentials segments of poly (U) and poly (T) tend to desorb from the electrode surface (Figs. 7, 9), while those of poly (A) adsorbed at the electrode via bases are anchored firmly at the surface. At potentials close to $Ei_{max}$ the double helix is disturbed in a way similar to that of the polynucleotides with alternating nucleotide sequence, but the molecule of poly (dA) . poly (dT) (or poly (rA) . poly (rU)) is anchored at the surface mostly, or exclusively, by poly (A) segments.

The dependence of the height of peak 3 on $t_2$, which show a weaker influence of $Ei_2$ (in the vicinity of $Ei_{max}$) in homopolymer pairs (Fig. 6b) than in polynuleotides with alternating nucleotide sequence (Fig. 6*a*), supports the above view. In polynucleotides with alternating nucleotide sequence, the shift in $Ei_2$ to negative potentials results in stronger repulsion forces between the polynucleotide and the electrode; due to this the double helix unwinds (at a certain potential, the repulsion is so strong that the polynucleotide molecule is no more held at the surface, and the process is stopped). In homopolymer pairs the shift of $Ei$ to more negative potentials causes elimination of chains of poly (dT) (or poly (rU)) as possible sites of anchoring of the molecule at the electrode surface.

Double helices containing G . C pairs are extremely stable (they are not thermally denatured even at 100 °C and low ionic strengths). It may thus be expected that their opening at the electrode surface is more difficult than that of less stable polynucleotides containing A . T and A . U pairs. In fact, in poly (rG) . poly (rC) (Jelen and Paleček 1979) and in poly (dG) . poly (dC) (Fig. 3*b*) region U is absent. On the contrary, poly (dG-dC) . poly (dG-dC) produces a distinct region U not very different from those of poly (dA-dT) . poly (dA-dT) or poly (dA-dU) . (poly (dA-dU) (Figs. 2 and 3*a*). The interaction of polynucleotides containing G . C pairs with the electrode remains not well understood. One may only speculate that the ability of poly (dG-dC) . poly (dG-dC) to open at the electrode surface might be associated with the known ability of this molecule to undergo $B \rightleftarrows Z$ transition (at room temperature and neutral pH) (Zimmerman 1982) and to show premelting (Paleček 1976; unpublished); these phenomena are observed neither with poly (dG) . poly (dC) nor with poly (rG) . poly (rC).

The area A occupied by a constituting nucleotide of the polynucleotide adsorbed at the mercury electrode surface was calculated by Miller (1961), Janík and Sommer (1973), and Nürnberg and Valenta (1978). Miller (1961) reported values of 0.35—0.86 $nm^2$ for native DNA and a value of 0.93 $nm^2$ for denatured DNA (Nürnberg and Valenta (1978) reported 0.7 $nm^2$ for the denatured DNA). Janík and Sommer (1973) gave 0.87 $nm^2$ for poly (rU) with a short chain (about 100 nucleotides) and 0.77 $nm^2$ for poly (rU), having approximately 1700 residues

in the chain. The area A for native DNA has thus been given by Miller (1961) only. It follows from his measuremnts that, at a full coverage of the electrode, the surface concentration of DNA is within the range of 0.065 to 0.16 μg/cm$^2$. Considering that conditions of Miller's measurements differred from those of the present work, and that the surface concentration may undergo changes in the course of surface denaturation, we can make only a rough estimate on the unwinding rate of ds polynucleotides at $Ei_{max}$ using Miller's (1961) data. We have shown that in the majority of the polynucleotides tested, surface denaturation occurs within 5—10 s in a half of the material adsorbed (Figs. 5 and 6); this would give unwinding rates in the range of $2—10 \times 10^{-11}$ mol/cm$^2$ . s.

If we suppose that the unwinding of a molecule of a ds polynucleotide starts in one point and that the molecule becomes unwound during the measurement, we could try to calculate the initial rate of unwinding, expressed as a number of base pairs per second. Values calculated for $Ei_{max}$ lie approximately within the limits of 100—300 base pairs per second, and no marked differences exist between homopolymer pairs and alternating sequence polynucleotides. The relatively large scatter of values is due to polydispersity of the samples and to the fact that the ds molecule may unwind at the electrode from its ends and perhaps from ssb (the number of which in the polynucleotide molecule is not known). At $Ei$ more negative than $Ei_{max}$ higher unwinding rates were found for alternating sequence polynucleotides than for homopolymer pairs. At $Ei$ more negative than $Ei_{max}$ (by 30 mV) the unwinding rate of poly (dA-dT) . poly (dA-dT) was about 400 base pairs per second, i.e. a more than a twofold increase in the rate as compared with $Ei_{max}$; this rate is approximately four times lower that the replication rate of *E. coli* DNA (Cairns 1963).

Our results show that the rate of surface denaturation decreases in dependence on time (Figs. 5 and 6). This retardation could be associated with differences in the way of adsorption of ds and ss polynucleotides. It can be supposed that, at potentials of the region U, the ratio of adsorbed trains/loops will be higher for ss polynucleotides than for ds DNA; in other words, after denaturation of a considerable amount of DNA adsorbed at the electrode, the electrode area available for adsorption of bases released from the double helix will become the limiting factor.

*Electrode as a model of biological surfaces.* There is a rich experimental evidence on interaction of complexes of nucleic acids with various kinds of membranes. Recently, an electric field-induced cell-to-cell fusion has been demonstrated (Zimmermann and Vienken 1982). The membrane electric fields are limited to lengths of the same order of magnitude as those of the electrodes, and many electrically charged membranes have hydrophobic character (similarly as the mercury electrode surface). The interaction of polynucleotides with the mercury

electrode thus represents a suitable model of nucleic acid interaction with various biological surfaces. The latter interactions play a significant role in many biological processes; e.g. it is known that DNA replication in prokaryotic cells proceeds within a complex of membrane and DNA (Harmon and Taber 1977; Voikov and Schevchenko 1977). Recently, it has been shown (Budker et al. 1980) that ds polynucleotides adsorbed on natural and model (hydrophobic) phospholipid membranes have their secondary structure significantly changed. Digestion of these polynucleotides with $S_1$ nuclease and the way of their methylation with dimethylsulfate suggest opening of the double helix with the bases adhering to the membrane. It is thus not unreasonable to expect that principles of interactions of ds polynucleotides with the electrode shown in this an earlier papers (Paleček 1980, 1983; Nürnberg and Valenta 1978) may come into play in biological processes *in vivo*. In can thus be assumed that interaction of DNA with the electrically charged membrane (or with some other surface) may facilitate opening of the DNA double helix, and influence the extent and rate of the opening process in dependence on the electric charge of the membrane and nucleotide sequence of the polynucleotide.

## Abbreviations

DPP — differential pulse polarography
LSV — linear sweep voltammetry
HMDE — hanging mercury drop electrode
DME — dropping mercury electrode
SCE — saturated calomel electrode
p.z.c. — potential of zero charge
ssb — single-strand break
ds — double-stranded
ss — single-stranded

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