Biophysical Chemistry 4 (1976) 79–92

INTERACTION OF NUCLEIC ACIDS WITH ELECTRICALLY CHARGED SURFACES
II. CONFORMATIONAL CHANGES IN DOUBLE-HELICAL POLYNUCLEOTIDES* ‡

Viktor BRABEC and Emil PALEČEK
Institute of Biophysics, Czechoslovak Academy of Sciences, 612 65 Brno, Czechoslovakia

Received 23 June 1975

The influence of adsorption of double-stranded (ds) DNA, ds RNA and homopolymeric pairs at a mercury electrode on conformation of these polynucleotides was studied. Changes in the polarographic reducibility of polynucleotides, which were followed by means of normal pulse polarography and linear sweep peak voltammetry at the dropping mercury electrode were exploited to indicate conformational changes. It was found that, as a consequence of adsorption of ds polynucleotides on the negatively charged electrode conformational changes similar to denaturation take place in a narrow potential region around −1.2 V (the region U). After sufficiently long time of the contact with the electrode (under our conditions about 10 s) these changes reach limiting values, which can approach total denaturation. Upon adsorption of ds polynucleotides on the electrode charged to more positive potentials than the region U either (1) no conformational changes occur or (2) only a small part of the polynucleotide (probably labile regions of the ds molecule) is very quickly denatured – the remainder of the molecule preserves its ds structure. Conformational changes of adsorbed ds polynucleotides are influenced by factors which change the stability of ds polynucleotides in solution. It is supposed that denaturation of ds polynucleotides in the region U might result from the strains connected with the repulsion of certain segments of the molecule anchored on the electrode from the negatively charged surface.

1. Introduction

Over the past several years an increasing body of evidence has suggested that interaction of DNA with electrically charged membranes plays an important role in many basic biological processes. However, for the present there exists little information on what conformational changes take place as a consequence of these interactions, even though the conformation of DNA can exert a great influence on the course of processes in which it takes part.

Certain information has been obtained by experiments investigating the influence of electric field on conformation of various polynucleotides in solution [1–4]. Electric fields in biological systems are limited to lengths of the order of 100 Å, i.e. much shorter than is the length of a DNA molecule. In the vicinity of an electrode immersed into a solution of an electrolyte an electric field is formed, which is also limited to distances of tens or hundreds Å from the electrode. An electrode immersed into a solution of DNA could thus be a suitable model for studies of interactions of DNA with electrically charged surfaces. In 1961 Miller [5,6] measured the differential capacity of the double layer of the dropping mercury electrode (DME) immersed into a solution of DNA. He concluded on the basis of his results that in the region of positive potentials a partial unwinding of double-stranded (ds) DNA took place on the electrode, whereas at negative potentials DNA preserved its ds structure. More recent studies of Flemming [7], who used a hanging mercury drop electrode (HMDE) did not confirm the original conclusion of Miller [5,6]. Flemming concluded that DNA preserves its ds structure over the whole range of potentials [7] and that an association of DNA molecules takes place on the surface of the electrode [8,9]. It has recently been found in our laboratory, on the basis of pulse-polarographic measurements [10], that conformational changes in ds DNA occur in a relatively narrow range of negative potentials as a consequence of interaction of ds DNA with the electrode. Similar conclusions

* Part I: E. Paleček, Collection Czech. Chem. Commun. 39 (1974) 3449.
‡ On the occasion of the 80th birthday of Professor Dr. V. Morávek.

have been reached independently by Valenta et al. [11,12], who used a different experimental arrangement.

In the present study the interaction of ds DNA, ds RNA and homopolymeric pairs with the electrode at neutral pH and various ionic strengths is investigated in a greater detail by means of different techniques. It follows from the results that the investigated polynucleotides are at least partially denatured as a consequence of the contact with the electrode. Denaturation of these ds polynucleotides on the electrode is dependent on factors influencing the stability of DNA in solution.

2. Experimental

2.1. Materials

Calf thymus DNA was isolated and characterised as in previous papers [13,14]. The content of denatured DNA estimated by pulse polarography [14] was lower than 0.3%. If not stated otherwise, denaturation was performed by heating DNA at the concentration of 84 μg ml^{-1} in 0.015 M NaCl with 1.5 mM sodium citrate, pH 7 (SSC/10) at 100°C for 10 min and subsequent quick cooling in an ice bath. Sedimentation coefficients $s_{20,w}$ of ds and denatured DNA (table 1) were kindly estimated by Dr. J. Šponar. $s_{20,w}$ were determined in 0.3 M ammonium formate with 0.1 M sodium phosphate, pH 7.0, and for denatured DNA also in 0.9 M NaCl with 0.1 M NaOH; concentration of both forms of DNA was 16 μg ml^{-1}. Molecular weights (m.w.) of both forms of DNA were calculated using empirical equations according to Studier [15] and the corresponding diffusion coefficients D (table 1) determined from Svedberg's equation [16]. Ds DNA was X-irradiated in the presence of atmospheric oxygen with a TUR T 250 apparatus [17] at a concentration of 840 μg ml^{-1}. The radiation dose was 0.16 eV/P. Irradiation of ds DNA with this dose did not cause formation of a detectable amount of denatured DNA [17]. If we took into consideration the decrease of m.w. of ds DNA after the above dose of radiation [18], we calculated that the increase of D of ds DNA after the irradiation did not exceed 5%. Ds RNA of phage $f2$ (the replicative form), which was kindly submitted to us by Dr. J. Doskočil, was prepared, characterised and denatured in the same way as in the previous paper [19]. The content of single-stranded (ss) RNA in the sample of ds RNA estimated by means of differential pulse polarography [14] was lower than 0.3%. Polyribocytidylic (poly(rC)), polyriboinosinic (poly(rI)) and polyriboguanylic (poly(rG)) acids were purchased from Schwarz, Orangeburg, N.Y. The double-helical complexes poly(rI)·(rC) were prepared in the same way as in the previous paper [20]. The concentration of poly(rC) and of polynucleotide complexes (related to the phosphorus content), as well as DNA and RNA concentrations were estimated spectrophotometrically using a Zeiss VSU 2-P apparatus. Spermine was obtained from Calbiochem. Chemicals used for preparation of the background electrolytes for the electrochemical measurements were all of analytical grade. pH values were measured with a Compensator E 388 Metrohm apparatus.

2.2. Methods of electrochemical analysis

Direct current (d.c.), differential pulse-, and alternating current (a.c.) polarographic measurements were carried out as described earlier [13,21,22]. In these measurements the d.c. ramp potential of DME (figs. 1a, 2a, b) was changed continuously (independent of the dislodgement of the drop) and linearly; the potential of the drop changed during the life of one drop maximally by 5 mV; the peaks (steps) on polarograms informed us thus only

Table 1
Sedimentation coefficients ($s_{20,w}$), molecular weights (m.w.) and diffusion coefficients (D) of DNA samples used in the present study.

Sample	$s_{20,w}$ (Svedberg)	m.w. × 10^{-6}	$D \times 10^9$ (cm^2 s^{-1})
ds DNA	29.4	17.9	8.9
denatured DNA, pH 7.0	40.2	3.4 [a]	63.0

[a] This m.w. was calculated using $s_{20,w}$ determined for DNA in 0.9 M NaCl with 0.1 M NaOH.

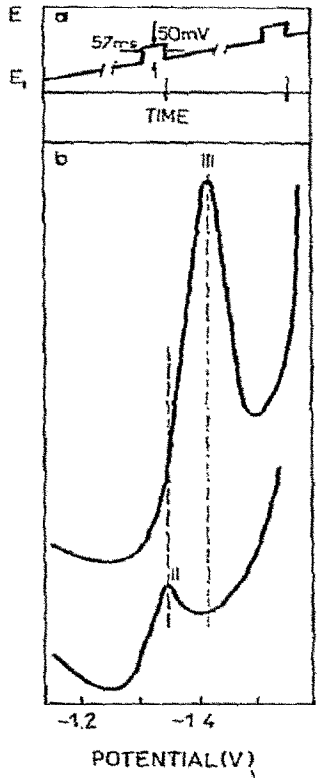

Fig. 1. Differential pulse polarography. (a) Signal applied. Arrows on the time axis indicate the drop fall. (b) Response obtained: ds DNA at the concentration of 200 $\mu g\ m^{-1}$ (lower curve) and denatured DNA at a concentration of 50 $\mu g\ ml^{-1}$ (upper curve). Both forms of DNA in 0.3 M ammonium formate with 0.1 M sodium phosphate, pH 7.0. The curve of ds DNA was obtained at 5 times higher sensitivity of the apparatus.

about electrode processes occuring at the potentials of the peak (step).

Ss polynucleotides containing adenine and cytosine yield reduction currents at potentials about −1.4 V [23] (fig. 1 — peak III). Ds DNA-like polynucleotides in which the adenine and cytosine reduction sites are hidden in the interior of the molecule (and form a part of the system of hydrogen-bond), are not reducible or yield relatively low currents (in the case of DNA approximately hundred times lower) at more positive potentials (fig. 1b — peak II) as compared with the corresponding ss polynucleotides (fig. 1b — peak III) [23].

The marked difference in behaviour of ss and ds polynucleotides represents a basis for the exploitation

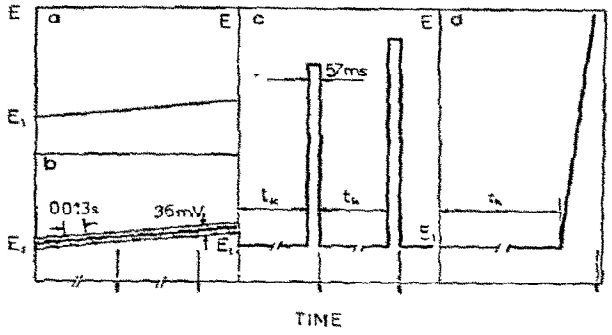

Fig. 2. Summary of signals applied to the DME in this study. Arrows on the time axis indicate the drop fall. (a) d.c. polarography; (b) a.c. polarography; (c) normal pulse polarography; (d) linear sweep peak voltammetry.

of methods of electrochemical analysis for studies of conformational changes in ds polynucleotides. In the present paper the differences in the values of reduction currents served to indicate changes in ds polynucleotides, which occured as a consequence of interaction of the polynucleotide with the electrode. For this purpose the linear sweep peak voltammetry at DME [24] was used together with normal pulse polarography, analogous to our previous study [10]. In these measurements the DME was first held for a definite time t_k (several seconds) at an initial potential E_i (figs. 2c, d), the value of which was preselected so that E_i was more positive than potentials of polynucleotide reduction. During the time t_k polynucleotide was adsorbed at the DME charged to E_i. In the case of normal pulse polarography, after the time t_k one rectangular voltage pulse was applied to the DME for 57 ms before the drop was dislodged (fig. 2c). The current was sampled for the final 16.7 ms of the pulse. After the dislodgement of the drop the cycle was repeated, but the height of the pulse was increased (fig. 2c). Thus the pulse potential became more negative. As soon as it reached values, at which bases bound in the polynucleotide were reduced a peak (step) appeared on current-pulse potential curves in the vicinity of −1.4 V (figs. 3a, 4a, b), which indicated eventual changes in the accessibility of the bases for the electroreduction induced by the several seconds contact of the polynucleotide with the electrode charged to E_i. In the case of linear sweep voltammetry, after the time t_k a single negative rapid and linearly changing ramp potential (scan rate $\geq 0.1\ V\ s^{-1}$) was applied to the DME

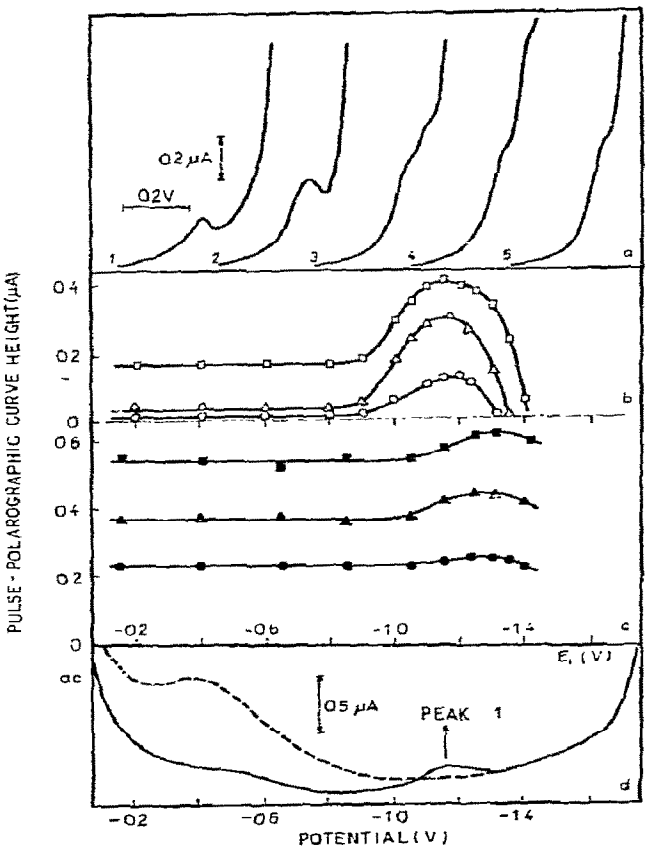

Fig. 3. (a) Normal pulse polarograms of ds DNA. DNA at the concentration of 420 μg ml^{-1} in 0.1 M sodium phosphate, pH 7.0, containing ammonium formate in concentrations: 0.1 M (curve 1), 0.3 M (curve 2), 0.6 M (curve 3), 1.0 M (curve 4), and 4.9 M (curve 5). Curve 5 was recorded with 2.5 times lower sensitivity than other curves. E_i = −1.2 V. (b) Dependence of the height of the normal pulse-polarographic curve of ds DNA on E_i. DNA at the concentration of 420 μg ml^{-1} in 0.1 M sodium phosphate, pH 7.0, containing ammonium formate in concentrations: (○—○) 0.1 M, (△—△) 0.3 M, (□—□) 1.0 M. (c) Dependence of the height of the normal pulse-polarographic curve of denatured DNA on E_i. DNA at the concentration of 42 μg ml^{-1} in 0.1 M sodium phosphate, pH 7.0, containing ammonium formate in concentrations: (●—●) 0.1 M, (▲—▲) 0.3 M, (■—■) 1.0 M. (d) A.c. polarograms of ds DNA. DNA at the concentration of 420 μg ml^{-1} in 0.1 M sodium phosphate with 1.0 M ammonium formate, pH 7.0 (– – –) background electrolyte.

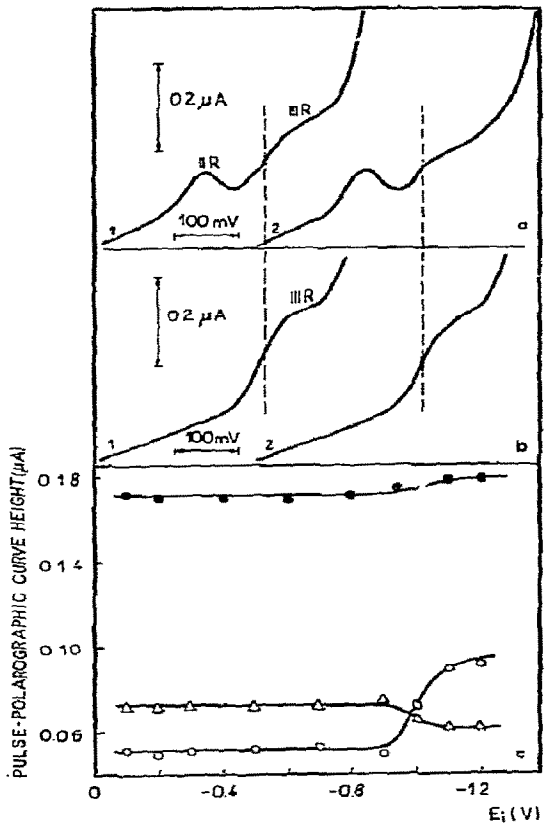

Fig. 4. Normal pulse polarography of ds and denatured RNA. Medium: 0.6 M ammonium formate with 0.1 M sodium phosphate, pH 6.8. Ds RNA at the concentration of 180 μg ml^{-1}, denatured RNA in a concentration of 18 μg ml^{-1}. (a) Polarograms of ds RNA; E_i = −1.1 V (curve 1), E_i = −0.2 V (curve 2). (b) Polarograms of denatured RNA; E_i = −1.1 V (curve 1), E_i = −0.2 V (curve 2). (c) Dependence of the height of the normal pulse-polarographic curves of the both forms of RNA on E_i. (○—○) step IIIR of ds RNA, (△—△) peak IIR of ds RNA, (●—●) step IIIR of denatured RNA.

(fig. 2d). The resulting current was measured as a function of this potential. Changes in the value of reduction current measured either by normal pulse polarography or by voltammetry thus yielded information on changes in the properties of the interfacial region in the dependence on E_i. Data giving evidence for appearance of conformational changes in a ds polynucleotide as a consequence of its interaction with the electrode were provided by the appearance of the voltammetric peak III (fig. 5a) (or normal pulse-polarographic step – fig. 4a) or by changes in its height (the voltammetric peak III appears at potentials about −1.42 V and is analogous

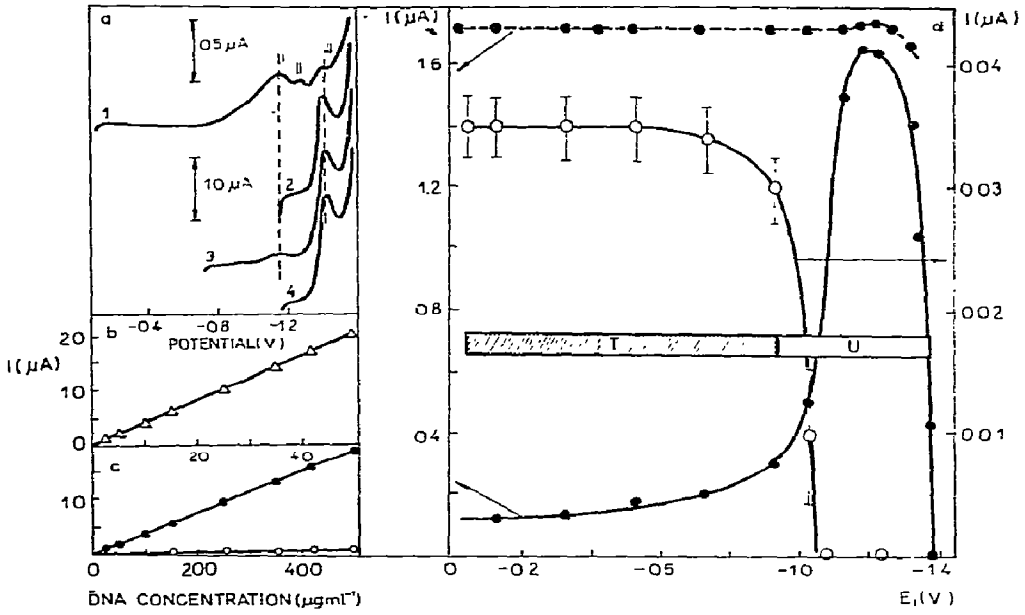

Fig. 5. Linear sweep peak voltammetry of ds and denatured DNA. Medium: 0.3 M ammonium formate with 0.1 M sodium phosphate, pH 7.0. Ds DNA at the concentration of 420 μg ml^{-1} and denatured DNA in a concentration of 42 μg ml^{-1}. Scan rate 1 V s^{-1}. (a) Voltammograms: Curve 1: ds DNA, E_i = −0.1 V; curve 2: ds DNA, E_i = −1.17; curve 3: denatured DNA, E_i = −0.7 V; curve 4: denatured DNA, E_i = −1.17 V. (b) Dependence of the height of the peak III of denatured DNA on its concentration. E_i = −0.2 V. (c) Dependence of the height of the peak III of ds DNA on its concentration. (○—○) E_i = −0.2 V, (●—●) E_i = −1.17 V. (d) Dependence of the peak heights of the both forms of DNA on E_i. (●—●) the peak III of ds DNA, (○–○) the peak II of ds DNA (estimated maximum experimental error in peak height measurement is indicated), (●– – –●) the peak III of denatured DNA.

to the differential pulse-polarographic peak III, which is yielded only by ss DNA (fig. 1b)). Background electrolytes contained ammonium formate, which enables measurement of the reduction currents of polynucleotides even in the vicinity of neutral pH [13].

Normal pulse-polarographic and linear sweep voltammetric measurements at the DME were carried out with a Model 174 Polarographic Analyzer in connection with Linear Sweep Module Accessory Model 174/51, supplied by Princeton Applied Research Corporation. If not stated otherwise a three electrode system was used including a DME, Pt-counter electrode of appreciable area, and a saturated calomel electrode (SCE). Normal pulse-polarographic measurements were performed at the following settings: scan rate 1 mV s^{-1}, negative scan, scan range 1.5 V, drop time 2.0 s, and low pass filter off. Voltammetric measurements: Model 174 – negative scan, scan range 1.5 V or 3.0 V and low pass filter off; Model 174/51 – IR compensation set below the point of oscillation of the system, current range multiplier 1, presweep delay was (if not stated otherwise) 10.0 s at E_i = −1.45 V; if E_i was more positive, presweep delay was decreased by subtracting (E_i + 1.45)/scan rate. Voltammograms were recorded with a OG 2–21 Speicheroszilloskop, VEB Messelektronik Berlin, GDR in the X–Y mode. Heights of voltammetric peaks were measured by the method proposed by Valenta and Nürnberg [25]. The mercury flow rate, m, of the capillary used for a.c. and pulse-polarographic measurements was 1.70 mg s^{-1} at a mercury column height (h) of 40 cm; m of the capillary used for voltammetry at the DME and d.c. polarography was 0.27 mg s^{-1} at h = 65 cm. The value of m was measured in distilled water at open circuit. If not stated otherwise, all electrochemical measurements were carried out at 25°C.

3. Results

3.1. Normal pulse and a.c. polarography of DNA and RNA

Normal pulse polarography of DNA in neutral media containing various concentrations of ammonium formate yielded results which were qualitatively identical with our previous measurements in the medium of 0.6 M ammonium formate, pH 6.8 [10]: (1) The height of the curve of ds DNA depended on E_i (fig. 3b). (2) The height of the curve of denatured DNA was nearly independent of E_i (fig. 3c). (3) Same values of half-step potential ($E_{1/2}$) were observed for ds and denatured DNA's.

Depending on the concentration of ammonium formate the height* and shape of the normal pulse-polarographic curve of both forms of DNA were changed (fig. 3a) in a similar way to the changes of the height and shape of the d.c. polarographic step of denatured DNA in the vicinity of −1.4 V [26]. At pH 7.0 $E_{1/2}$ of ds DNA was shifted to more negative potentials with increasing concentration of ammonium formate up to the concentration of 0.5 M (fig. 3a); at higher ammonium formate concentrations $E_{1/2}$ did not change any more. A similar shift of $E_{1/2}$ was observed with denatured DNA (not shown). In the region of potentials of the a.c. polarographic peak 1 a maximum appeared on the curve of the dependence of the pulse-polarographic curve height of ds DNA on E_i (at $E_i = E_{max}$) (fig. 3b, d), which was shifted to more positive potentials with increasing ammonium formate concentration (table 2). The potential of this maximum was always identical with the summit potential of a.c. polarographic peak 1 ($E_{a.c.1}$) (table 2). At potentials of the a.c. polarographic peak 1 evidently a segmental desorption of the ds DNA from the DME surface takes place; an evidence for this process is given by the following two results: (1) Ds DNA yields lower or no reduction current measurable with normal pulse polarography at E_i more negative than potentials of the a.c. polarographic peak 1 (fig. 3b). (2) A.c. polarographic curves of ds DNA and background electrolyte merged at potentials more negative than the potential of the a.c. polarographic peak 1 (fig. 3d).

Numerous irregularities appeared on normal pulse polarograms of ds DNA recorded at E_i more positive than −0.8 V. Therefore it was impossible to decide by means of normal pulse polarography whether the current of ss DNA (peak III) or ds DNA (peak II) was measured, as it is possible when using differential (derivative) pulse polarography (fig. 1b).

The difference between the potentials of the differential pulse-polarographic peaks of a ds and the corresponding ss polynucleotide is higher for RNA than for DNA [19] so that the peaks of ss and ds RNA can be well distinguished. We tried to exploit this fact in the normal pulse-polarographic measurements and to find out whether the curves of ds RNA of phage $f2$ (which is more stable to thermal denaturation than calf thymus DNA [19] and exists in solution in the form A, in contrast to the B form of DNA) will exhibit similar dependence on E_i as the curves of DNA. We ascertained that in the medium of 0.6 M ammonium formate, pH 6.8 the curve IIR of ds RNA can be well distinguished from the curve IIIR of denatured RNA even by means of normal pulse polarography (figs. 4a, b). Similarly as for DNA the dependence of the heights of the normal pulse-polarographic curves on E_i was followed (fig. 4c). The step IIIR yielded by denatured RNA alone almost did not depend on E_i (fig. 4c). The heights of the peak IIR and the step IIIR yielded by ds RNA did not change to any significant extent on varying E_i from −0.1 to −0.9 V. A further negative shift of E_i caused a marked increase of the height of the step IIIR accompanied by a decrease of the peak IIR (fig. 4c), which gives evidence for a qualitative change of the properties of ds RNA, most probably for its denaturation on the electrode surface. Approximate data on the influence of the time of contact of ds RNA with the electrode on the above mentioned changes in ds RNA were obtained in a similar way as in the previous study for DNA [10]. The range of t_k at which our pulse-polarographic measurements could be carried out was between 0.44–4.94 s. In this range of t_k the dependences describing the kine-

* In those cases when a double-step appeared on the normal pulse polarograms (fig. 3a, curves 3–5), analogous results were obtained if the height of the more positive or more negative steps or even the sum of the heights of both steps were measured. In the present study the height of the more positive step was taken as a measure of the reduction of both forms of DNA. This way of measurement is justified by our preliminary results on the study of the electrode process which is responsible for the appearance of the normal pulse-polarographic reduction peak (step), exhibiting certain analogy between the shapes of curves of ss polynucleotides obtained by means of normal pulse and d.c. polarography [26].

Table 2
Summit potentials of the a.c. polarographic peak I of ds DNA ($E_{a.c.1}$) and potentials of maximum on the curve of dependence of normal pulse-polarographic curve on E_i (E_{max}) at different concentrations of $HCOONH_4$.

Concentration of $HCOONH_4$ (M)	0.1	0.3	0.6	1.0	2.0	4.9
$E_{a.c.1}$ (V)	−1.21	−1.17	−1.16	−1.15	−1.12	−1.10
E_{max} (V)	−1.21	−1.17	−1.17	−1.15	−1.13	−1.09

tics of changes of properties of RNA adsorbed on the DME had a course qualitatively identical to that of the dependences measured for DNA [10]: at E_i = −1.1 V the extent of the changes increased with increasing t_k, but did not reach the limiting value; at E_i = −0.2 V virtually no dependence on t_k was observed. Thus at t_k = 4.94 s the above mentioned changes in ds DNA or ds RNA adsorbed in the vicinity of E_{max} did not take place to the maximally possible extent.

3.2. Single-sweep peak voltammetry of DNA

It follows from the results of the preceding paragraph that it would be desirable to measure at longer t_k values, at which the changes in ds polynucleotides could reach a limit. Such measurements were made possible by using the single-sweep peak voltammetry. In addition, it was also possible to change the value of E_i during the presweep time by a jump and to change the voltage sweep rate. Voltammograms of DNA in 0.3 M ammonium formate, pH 7.0, at a scan rate of 1 V s^{-1} are shown in fig. 5a. A process similar to that which is responsible for the a.c. polarographic peak 1 can be probably considered as the cause of the voltammetric peak I; the voltammetric as well as a.c. polarographic peak I are yielded by ds and denatured DNA's at approximately identical potentials. The voltammetric peak I was not however used to follow the properties of adsorbed DNA in the present study. The voltammetric peak II is yielded only by ds DNA, whereas denatured DNA yields only the more negative peak III. The peaks I, II, and III were yielded by DNA on voltammograms at scan rates of 0.1–5.0 V s^{-1}. The dependence of the heights of the voltammetric peaks II and III of ds and denatured DNA's on E_i corresponded qualitatively to the analogous dependences obtained by means of normal pulse polarography with DNA as well as RNA (fig. 5d).

We attempted to find out whether the changes in ds DNA caused by its adsorption on the DME charged to E_{max} are reversible. The mercury drop was immersed into the ds DNA solution continuously for 10 s. After this time the voltammogram was recorded (scan rate 2 V s^{-1}) and the height of the peak III measured in the dependence on potentials applied to the electrode during preceding 10 s. If the DME was kept for the whole time (10 s) only at E_i = −1.4 V, ds DNA did not yield any peak (fig. 5d). However, if the DME was first kept 5 s at E_{max} and then the potential of −1.4 V was applied to the same drop for another 5 s, ds DNA yielded the peak III (not shown). A high peak III was observed in a similar experiment (fig. 6), when DME was first charged to E_{max} for 5 s and then for further 5 s to the potential of the region T (fig. 5d). The appearance of the peak III as well as its height indicated that at least a part of the changes resulting from ds DNA interaction with the electrode surface charged to E_{max} remaind preserved even after removal of the changes-inducing potential. Thus it can be concluded that the changes in ds DNA appearing upon its interaction with DME charged to E_i in the vicinity of E_{max} (the region U — fig. 5d) are at least partially irreversible.

In standard voltammetric analysis the peak height is proportional to the concentration of a depolariser in the bulk of solution. In our case (fig. 5a) the peak III was formed even though the concentration of the depolariser responsible for its appearance was virtually zero in the solution. The depolariser (denatured DNA) was transported to the electrode in its inactive form (ds DNA), the diffusion coefficient of which was lower than that of the active form, which was formed as a consequence of the interaction of DNA with the electrode We characterised the height of the peak III of ds DNA by the concentration of denatured DNA in the solution, A, which was necessary for the appearance of the peak III of the given height, in order to be able to compare the heights of the peaks III yielded by ds DNA of the same

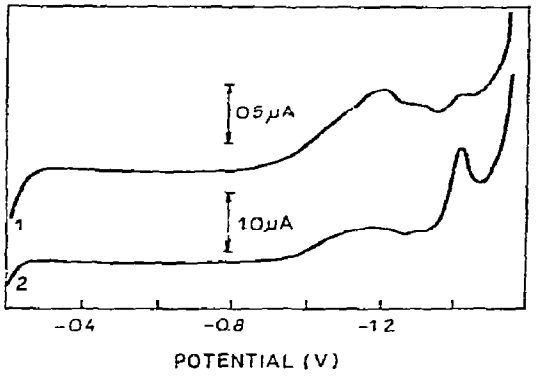

Fig. 6. Influence of a change of E_i before the application of voltage sweep on the course of voltammograms of ds DNA. DNA at the concentration of 420 μg ml^{-1} in 0.3 M ammonium formate with 0.1 M sodium phosphate, pH 7.0 Scan rate 2 V s^{-1}. The curves were recorded from −0.2 V. Presweep time 10 s. Curve 1: the DME charged for 10 s to potential −0.2 V; curve 2: the DME first kept for 5 s at −1.15 V and then for the same time at −0.2 V.

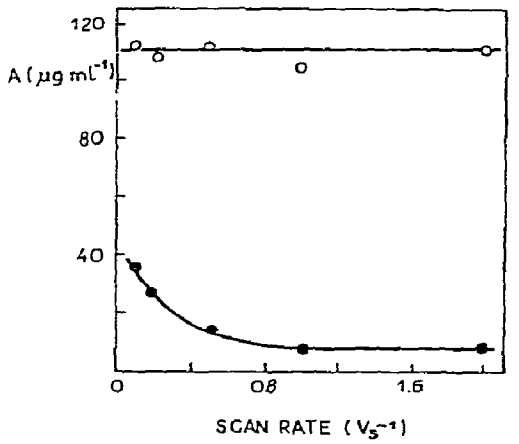

Fig. 7. Dependence of A on scan rate. Ds DNA at the concentration of 420 μg ml^{-1} in 0.3 M ammonium formate with 0.1 M sodium phosphate, pH 7.0. (●—●) E_i = −0.7 V, (○—○) E_i = −1.15 V.

concentrations under different conditions. Differences in diffusion coefficients of ds DNA (D_{nat}) and denatured DNA (D_{denat}) were accounted for: the slope of the linear dependence of the height of the peak III was divided by the ratio $(D_{denat}/D_{nat})^{1/2}$ and the concentration A read from thus corrected dependence.

We followed A in a dependence on scan rate at various E_i values. Whereas at $E_i = E_{max}$ A was practically independent of the scan rate, at E_i more positive than the region U, i.e. in the potential region T (fig. 5d) A decreased with increasing scan rate up to the scan rate 1 V s^{-1} (fig. 7). Further increase of the scan rate did not lead to any appreciable change of A. Thus, it follows from the preceding dependences that a longer contact of ds DNA with the electrode charged to potentials corresponding to the region U even in the course of voltage sweep can lead to conformational changes in DNA. If the time for the voltage to sweep through the region U (of the width of approximately 0.4 V), is shortened to 0.4 s, the results of measurements are no longer influenced by changes in scan rate (fig. 7).

We further investigated how the properties of adsorbed DNA were changed by action of factors which influence the stability of the double helix of DNA in solution. In the vicinity of pH 7 the stability of the double helix is increased, if the ionic strength [27] or the concentration of a polyamine in the solution are raised [28]. On the contrary damage to the DNA double helix by X-rays leads to a decrease of its stability [17,29]. We followed the influence of these factors on the dependence of the ratio X (i.e. the ratio of the heights of the peaks III of ds and denatured DNA's) on t_k. The ratio X first grew with t_k and then reached a limiting value (fig. 8). The value of t_k at which X just reached the limiting value (t_{lim}) was taken as a measure of the rate of changes. It follows from fig. 8 and table 3 that changes for native DNA at $E_i = E_{max}$ took place more rapidly the lower the concentration of CsCl (in the range 0.3–1.0 M) or the higher the dose of X-radiation. It is evident that t_k value of approximately 8 s was sufficient for the changes in the structure of ds DNA (adsorbed on the DME at E_{max}) to reach the limit at pH 7.0 (table 3). We determined the extent of these changes from the values A. As follows from table 3, A decreased if the stability of ds DNA in solution increased and vice versa.

At potentials corresponding to the region T the ratio X almost did not depend on t_k under conditions of our measurement (fig. 8). The concentration A measured at these potentials was changed in the dependence on fac-

Table 3
Influence of factors determining the stability of DNA in solution on the extent and rate of conformation changes in ds DNA adsorbed at the DME.

Sample of DNA [a] in	Adsorbed at E_{max}		Adsorbed at −0.2 V	
	A(μg ml^{-1}) at $t_k \geq t_{lim}$	t_{lim}(s)	A(μg ml^{-1}) at $t_k \geq 1$ s	$\sigma \times 10^{-3}$ [b]
0.6 M $HCOONH_4$, pH 7.0	103	8	16	1.1
0.6 M $HCOONH_4$ with 8 μM spermine, pH 7.0	62	8	16	1.1
0.6 M $HCOONH_4$, pH 7.0 [c]	128	5	24	1.6
1.0 M CsCl, pH 6.8	121	5	16	1.1
0.3 M CsCl, pH 6.8	163	3	20	1.3

a) Ds DNA at the concentration of 420 μg ml^{-1}; all samples were buffered by 0.1 M sodium phosphate.
b) σ is the number of dissociated nucleotide pairs in ds DNA of m.w. 1.8×10^7 adsorbed at the DME ($\sigma = 2.8 \times 10^4$ for A = 420 μg ml^{-1}).
c) Ds DNA X-irradiated with a dose of 4000 rads.

tors influencing the stability of DNA in the same manner as if measured at E_{max} (table 3).

It follows from our results that even for denatured DNA a slight increase of the reduction current takes place in the region U (figs. 3c, 5d). This increase might be connected with the presence of short helical regions which were formed owing to aggregation and partial renaturation. Measurements of samples of denatured DNA differing in the content of helical regions (the content of the helical regions was checked spectrophotometrically [30,31] confirmed this assumption. Whereas in the case of denatured DNA obtained under conditions that stimulate aggregation and renaturation the increase of the reduction current was maximal (fig. 9), this increase disappeared in denatured DNA which was free of helical regions (conditions minimizing the aggregation, measured at 70°C).

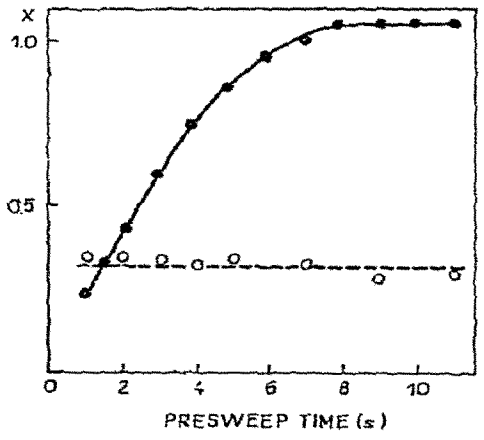

Fig. 8. Dependence of X on presweep time. (X is a ratio of the heights of the peaks III of native and denatured DNA's.) (●—●) $E_i = E_{max}$; (○- - - -○) $E_i = -0.75$ V. Ds DNA at the concentration of 420 μg ml^{-1}, denatured DNA at the concentration of 42 μg ml^{-1}. Medium: 0.6 M ammonium formate with 0.1 M sodium phosphate, pH 7.0. Scan rate 5 V s^{-1}.

Further the consequences of interaction of poly (rG)·(rC) and poly(rI)·(rC) with the mercury electrode were investigated. The measurements were carried out in a medium of 0.15 M ammonium formate with 0.1 M NaCl and 0.06 M sodium phosphate, pH 7.0, under conditions when the surface of the DME was fully covered by adsorbed polynucleotide molecules; it was thus possible to exclude the influence of unequal diffusion coefficients of the ds polynucleotides on the height of the voltammetric peak appearing at −1.45 V (fig. 10a). The height of this peak was changed with E_i (fig. 10b) in an analogous manner to changes of the peak III of DNA. In the case of the thermally more stable poly(rG)·(rC), however, the peak was very small even at $E_i = E_{max}$. On the contrary for the less stable poly(rI)·(rC) the height of the peak at $E_i = E_{max}$ corresponded to the reduction of all poly(rC) bound in the complex.

Valenta et al. [11,12], who also followed the conformational changes of ds DNA on the surface of the mercury electrode, did not observe the marked dependence of the conformational changes on E_i (the regions

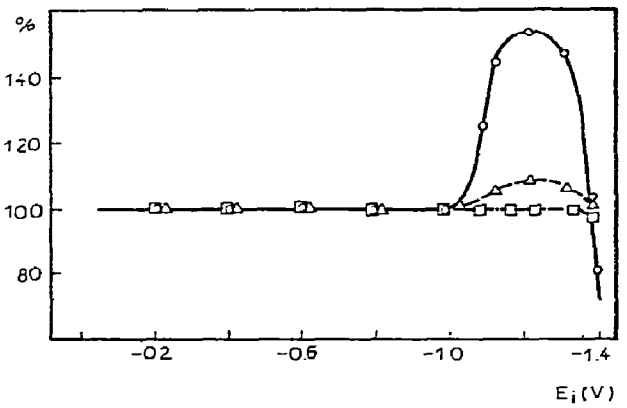

Fig. 9. Influence of aggregation of denatured DNA on dependence of the height of the voltammetric peak III on E_i. Denatured DNA in 0.3 M ammonium formate with 0.1 M sodium phosphate, pH 7.0. Scan rate 2 V s^{-1}. The peak height at E_i = −0.2 V was taken as 100%. (△—△) denatured DNA at the concentration of 42 μg ml^{-1}; the denaturation was carried out under conditions, when the aggregation of DNA was minimal, i.e. at the DNA concentration of 84 μg ml^{-1} in the manner described in sect. 2.1.; measured at 25°C. (□—□) denatured DNA at the concentration of 42 μg ml^{-1}; the denaturation was carried out at the DNA concentration of 84 μg ml^{-1} in the manner described in sect. 2.1.; measured at 70°C. (○—○) denatured DNA in a concentration of 126 μg ml^{-1}. The denaturation was carried out under conditions stimulating aggregation of DNA, i.e. at the DNA concentration of 756 μg ml^{-1} for 10 min in SSC at 100°C and then left for 2 hr at 37°C [30,31]; measured at 25°C.

T and U) because of a different experimental arrangement. In an effort to correlate their voltammetric measurements carried out with the HMDE with the d.c. polarographic inactivity of ds DNA [13,23] they put forward an assumption that this inactivity was caused by too short a contact of DNA with the electrode (in standard d.c. polarographic experiments approximately 3 s). They concluded that for the detectable denaturation at least 10 s contact of ds DNA with the electrode was necessary. We have carried out d.c. polarographic measurements with the DME having a drop time of 15 s. A scan rate was chosen so that the change of potential during the lifetime of one drop was about 5 mV, i.e. that this change should be roughly identical with the change in standard d.c. polarographic measurements. Ds and denatured DNA at the concentration of 420 μg ml^{-1} in 0.5 M ammonium formate with 0.1 M sodium phosphate, pH 7.0 were measured. Denatured DNA under these conditions produced usual d.c. polarographic step [26]. The extension of the drop time had no effect on the d.c. polarographic behaviour of ds DNA: no reduction step appeared.

4. Discussion

4.1. Denaturation of ds polynucleotides in a narrow region of negative potentials (the region U)

Adsorption of ds polynucleotides on the surface of the mercury electrode charged to potentials which correspond to the region U leads to an appearance of conformational changes in these polynucleotides. The results reported in the present study indicate that these changes have the character of denaturation. An evidence for this is given by the growth of the peak III (step IIIR) in the region U (figs. 4c, 5d) and the parallel decrease of the peak II (IIR), which is yielded only by ds DNA (ds RNA) As long as the conformation changes have the character of premelting changes [32–36], an increase of the peak II would be expected on the basis of the analogy with measurements carried out by means of differential (derivative) pulse polarography. In addition the irreversibility of the observed changes (fig. 6) is in agreement with the assumption on the denaturation of ds polynucleotides The possibility that the changes observed in the region U could be conditioned only by a higher surface concentration and re-orientation of the ds molecules in the electrode surface can be excluded on the basis of our results. The electroreduction of the adenine and cytosine residues which were liberated from the double helix and became accessible for the electrode process, is responsible for the appearance of the pulse-polarographic faradaic peak III. Thus, for the appearance of the faradaic peak III neither the increase of the surface concentration of the ds molecules nor their re-orientation are sufficient. Besides, our measurements were carried out under conditions when the rate of polynucleotide adsorption was diffusion controlled, under an incomplete coverage of the electrode surface. A change in the electrode potential could thus cause a re-orientation of the molecules, but could not increase their surface concentration so substantially, that the current increase observed in the region U (fig. 5a) might be explained by it. (On the other hand, our results do not exclude the possibility of a change of the surface concentration of DNA at full coverage of the electrode in the arrangement used by

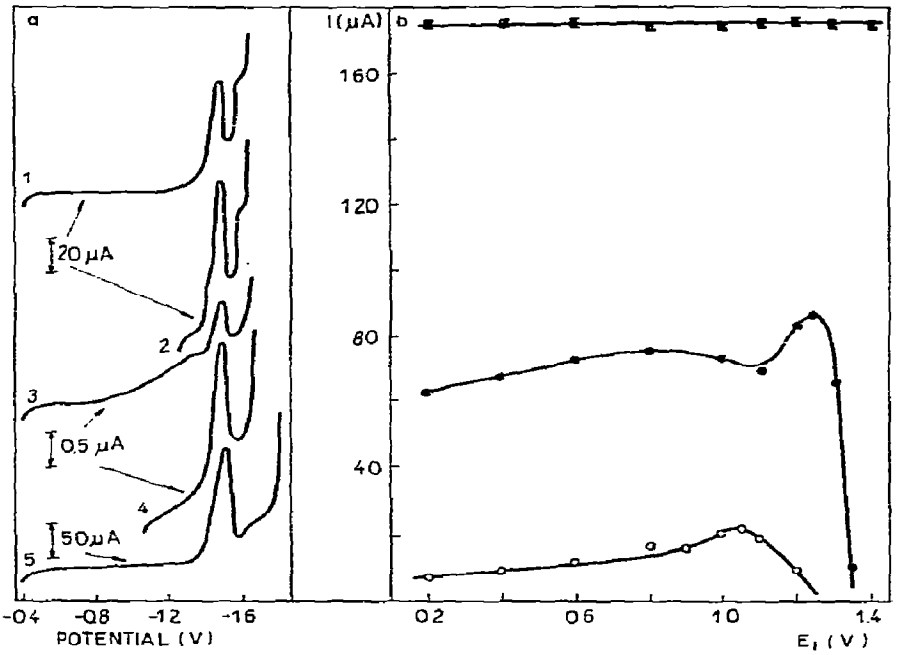

Fig. 10. Voltammetric behaviour of polyribonucleotide complexes and poly(rC) in 0.15 M ammonium formate with 0.06 M sodium phosphate and 0.1 M NaCl, pH 7.0. The complexes were at the concentration of 1×10^{-3} M, poly(rC) at the concentration of 5×10^{-4} M. Scan rate 2 V s^{-1}. All potentials are given against mercury on the bottom of polarographic vessel. (a) Voltammograms: Curve 1: poly(rI)•(rC), $E_i = -0.4$ V; curve 2: poly(rI)•(rC), $E_i = -1.25$ V; curve 3: poly(rG)•(rC), $E_i = -0.4$ V; curve 4: poly(rG)•(rC), $E_i = -1.05$ V; curve 5: poly(rC), $E_i = -0.4$ V. (b) Dependence of the height of the voltammetric peak yielded by the complexes and poly(rC) at potentials about −1.45 V (fig. 10a) on E_i. (o—o) poly(rG)•(rC), (•—•) poly(rI)•(rC), (▪—▪) poly (rC).

Flemming [8,9].) The surface denaturation of ds polynucleotides in the region U proceeds relatively slowly (in units of seconds) fig. 8). In some cases the complete or nearly complete denaturation can take place; in other words, after sufficiently long contact of a ds polynucleotide with the electrode the polynucleotide can yield the signal corresponding to the reduction of a ss polynucleotide in an equimolar concentration (poly(rI)•(rC) — fig. 10).

4.2. Interaction of ds polynucleotides with the electrode in the region from −0.1 up to −1.0 V (the region T)

In the region T, in which the electrode can bear a positive (at potentials more positive than the potential of the electrocapillary maximum (ECM)*, i.e. at roughly −0.5 V) or negative charge, the value of the measured current (characterising the amount of reducible groups released from the double helix) depended neither on the time of contact of the ds polynucleotide with the electrode (fig. 8) nor on the electrode potential (figs. 3, 4, 5). This fact could be explained in two ways: (1) in the region T the interaction of the ds polynucleotide with the electrode leads to none or only to very slow ($\gg$ 10 s) structural changes, or (2) the changes take place very quickly ($<$ 0.5 s), affect only a small part of the adsorbed molecule and do not proceed further. The explanation (2) is supported by the fact that the peak III (step IIIR), which is characteristic for a ss polynucleotide, was present on normal pulse-polarograms of ds RNA (fig. 4a) and on voltammograms of ds DNA (fig. 5a). The ds polynucleotides did not yield the peak III in diferential (derivative) pulse polarography (where the electrode is charged to a potential close to that of the reduction potential, i.e. more negative than the

* The charge on the electrode is zero at the potential of the ECM [24].

region U, in the time preceding the measurement); therefore it is improbable that the presence of the peak III on voltammograms (fig. 5a) and normal pulse-polarograms (fig. 3a, 4a) could be caused by a contamination of a ds polynucleotide by ss material. The height of the peak III of ds DNA corresponds to the presence of approximately 1.3% of thermally denatured DNA, i.e. it is approximately 2 times as high as the height of the peak II estimated in the same samples by means of differential (derivative) pulse polarography. It is thus possible that in the region T primarily labile regions of the double-stranded molecule (e.g. the vicinity of a single-strand break, adenine–thymine rich regions, etc. [33–36]) and their closest neighbourhood are denatured, while the major part of the molecule preserves its ds structure under these conditions.

4.3. Mechanism of formation of the conformational changes

In the vicinity of DME there exists a nonhomogeneous electric field, which has its highest strength (up to 10^6 V cm^{-1}) in the so-called inner or compact double-layer (its width corresponds to a few atomic diameters) adhering to the metal phase. In the direction from the electrode the electric field strength sharply decreases; on the boundary of inner and diffuse double-layer it has only roughly $\frac{1}{10}$ of its original value, which then decreases in the diffuse double-layer nearly to zero (the depth of diffuse double-layer depends on ionic strength [37]; in the medium of ionic strength 0.1 it can amount to approximately 90 Å). Previous theoretical calculations have suggested that an electric field strength of the order of magnitude 10^4 V cm^{-1} and higher can cause denaturation of DNA [38]. However, experiments carried out later [1] gave evidence for structural changes (strength of the field of the order of magnitude 10^4 V cm^{-1}, concentration of Tris buffer, pH 7 $1 \times 10^{-4} - 1 \times 10^{-3}$ M) different from the DNA denaturation (only a change in the angle of the bases to the longitudinal axis of the double helix was supposed).

In ds DNA sample with the m.w. above 3×10^5 certain degree of flexibility is presumed [39]. It can be thus expected that our ds DNA sample (m.w. 1.8×10^7 – see sect. 2.1) was adsorbed as a flexible polymer i.e. through two-dimensional trains of segments alternating with three-dimensional loops [40, 41]. If we thus consider the influence of the electric field on the DNA molecule adsorbed at the electrode surface, it is necessary to realise that probably only a small part of the ds DNA molecule is located in the inner part of double-layer and that long segments of the molecule extend beyond the diffuse double-layer and protrude into the bulk of solution. Fixation of the molecules on the electrode surface as well as the inhomogeneity of the electric field makes the orientation of molecules in the direction of the field difficult and thus reduces the ability of the field to cause a polarisation of the ionic atmosphere of the polynucleotide, which is proposed to be responsible for the appearance of conformational changes [2]. Consequently, a simple application of the conclusions on the effects of electric fields on DNA in the bulk of solution [1–4,38] does not suffice for explaining the conformational changes of a polynucleotide adsorbed on an electrode [11,12].

In our previous communication [10] we formulated a hypothesis that the conformational changes of DNA in the region U are connected with a desorption of the polynucleotide. A good correspondence between the potentials of the desorption a.c. polarographic peak 1 (fig. 3) of ds DNA as well as ds RNA with the potentials of the region U under various conditions (table 2) is in agreement with this hypothesis.

In order to explain the mechanism of DNA denaturation on the electrode surface further progress in the experimental and theoretical investigation of polymer adsorption will be necessary. For the present we can suggest only a rough tentative scheme of this process under our experimental conditions: In the vicinity of the potential of the ECM, segments of ds DNA are anchored on the electrode surface by the sugar-phosphate backbone as well as by sporadic bases located in the labile regions of DNA [42]. As the electrode potential is made more negative a weakening of the adsorption of DNA by the sugar-phosphate backbone occurs and eventually desorption of some segments. On the contrary the regions anchored on the surface by means of the bases can remain adsorbed even at potentials corresponding to the region U. At these potentials a situation can arise, when DNA molecule is anchored on the surface by one segment S, while the adjacent segments are strongly repelled from the electrode; consequently an unwinding of the molecule takes place. In the segment S, which is specifically adsorbed by means of the

bases, hydrogen bonds have to be ruptured and the solvation sheet is probably disturbed; this segment has thus ss character. From the segment S the surface denaturation proceeds to further regions of the molecule, the main part of the unwinding taking place in the outer double-layer and in the bulk of solution. This way of unwiding is supported by the fact that the rate as well as the extent of denaturation are dependent on ionic strength and on the presence of a polyamine (table 3), i.e. on factors which influence the stability of DNA in solution. It is highly probable that ss regions formed in the vicinity of the segment S are immediately adsorbed via bases and their adsorption may stimulate further DNA unwinding.

If we thus carry out the whole measurements on a single drop (either with HMDE or a DME of a relatively long drop life in combination with a single-sweep technique) and proceed from potentials of the region T or U to more negative values, both ds and thermally denatured DNA's yield qualitatively identical signals, whether we follow the reduction of polynucleotides [10—12] (figs. 3,5) or their adsorption/desorption behaviour [8,9]. This is connected with the fact that the ds DNA is at least partially denatured due to its contact with the electrode charged to potentials of the region U (and possibly region T), even though it was transported to the electrode from the bulk of solution in the ds form. The degree of surface denaturation is strongly dependent on the time, for which ds DNA was in the contact with the electrode in the region U (fig. 8) as well as on further conditions (table 3, fig. 10). On the contrary, if we work with the DME under conditions usual in d.c. polarography (fig. 2a), a possible slight surface denaturation which may occur in the region U (and T) cannot be manifested by the appearance of the d.c. reduction step even in the case when the drop time is prolonged several times. This is caused by the fact that the intact DNA transported from the bulk of solution does not adsorb on fresh mercury drops charged to the potentials at which the reduction of the denatured DNA occurs (fig. 3d).

References

[1] C.T.O'Konski and N.C. Steilwagen, Biophys. J. 5 (1965) 607.
[2] E. Neumann and A. Katchalsky, Proc. Nat. Acad. Sci. USA 69 (1972) 993.
[3] A. Revzin and E. Neumann, Biophys. Chem. 2 (1974) 144.
[4] D. Pörschke, Nucleic Acids Res. 1 (1974) 1601.
[5] I.R. Miller, J. Mol. Biol. 3 (1961) 229.
[6] I.R. Miller, J. Mol. Biol. 3 (1961) 357.
[7] J. Flemming, Biopolymers 6 (1968) 1697.
[8] J. Flemming, Biopolymers 12 (1973) 1975.
[9] J. Flemming and H. Berg, Bioelectrochem. Bioenergetics 1 (1974) 460.
[10] E. Paleček, Collection Czech. Chem. Commun. 39 (1974) 3449.
[11] P. Valenta and H.W. Nürnberg, Biophys. Struct. Mechanism, 1 (1974) 17.
[12] P. Valenta, H.W. Nürnberg and P. Klahre, Bioelectrochem. Bioenergetics 1 (1974) 487.
[13] V. Brabec and E. Paleček, Biophysik 6 (1970) 290.
[14] E. Paleček, in: Methods in enzymology, vol. 21, eds. L. Grossman and K. Moldave (Academic Press, New York, 1971) p. 3.
[15] F.W. Studier, J. Mol. Biol. 11 (1965) 373.
[16] D. Lang and P. Coates, J. Mol. Biol. 36 (1968) 137.
[17] E. Lukášová and E. Paleček, Radiation Research 47 (1971) 51.
[18] U. Hagen, Biochim. Biophys. Acta 134 (1967) 45.
[19] E. Paleček and J. Doskočil, Anal. Biochem. 60 (1974) 518.
[20] A Bezděková and E. Paleček, Studia Biophysica 34 (1972) 141.
[21] V. Brabec and E. Paleček, Z. Naturforsch. 29c (1974) 323.
[22] V. Brabec and E. Paleček, J. Electroanal. Chem. 27 (1970) 145.
[23] E. Paleček, in: Progress in nucleic acid research and molecular biology, vol. 9, eds. J.N. Davidson and W.E. Cohn (Academic Press, New York, 1969) p. 31.
[24] L. Meites, Polarographic techniques (Interscience, New York, 1965).
[25] P. Valenta and H.W. Nürnberg, J. Electroanal. Chem. 49 (1974) 41.
[26] V. Brabec, J. Electroanal. Chem. 50 (1974) 235.
[27] J. Marmur and P. Doty, J. Mol. Biol. 5 (1962) 109.
[28] E.J. Gabbay and R. Glasser, Biochim. Biophys. Acta. 224 (1970) 272.
[29] D.T. Kanazir, in: Progress in nucleic acid research and molecular biology, vol. 9, eds. J.N. Davidson and W.E. Cohn (Academic Press, New York, 1969) p. 117.
[30] S. Basu, Biopolymers 5 (1967) 876.
[31] S. Basu, Z. Naturforsch. 24b (1969) 106.
[32] E. Paleček, J. Mol. Biol. 20 (1966) 263.
[33] E. Paleček, Arch. Biochem. Biophys. 125 (1968) 142.
[34] E. Paleček and I. Frič, Biochem. Biophys. Res. Commun. 47 (1972) 1262.

[35] E. Paleček, Studia Biophysica 42 (1974) 59.
[36] E. Paleček, Studia Biophysica 50 (1975) 177.
[37] D.M. Mohilner, in: Electroanalytical chemistry, vol. 1, ed. A.J. Bard (M. Dekker, Inc. New York, 1966) p. 241.
[38] T.L. Hill, J. Amer. Chem. Soc. 80 (1958) 2142.
[39] D.M. Crothers and B.H. Zimm, J. Mol. Biol. 12 (1965) 525.
[40] A. Silberberg, J. Polymer Sci., Part C, 30 (1970) 393.
[41] B. Janík and R. Sommer, Biopolymers 12 (1973) 2803.
[42] V. Brabec and E. Paleček, Biopolymers 11 (1972) 2577.