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INTERACTION OF NUCLEIC ACIDS WITH ELECTRICALLY CHARGED SURFACES. VII. THE EFFECT OF IONIC STRENGTH OF NEUTRAL MEDIUM ON THE CONFORMATION OF DNA ADSORBED ON THE MERCURY ELECTRODE ‡

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Triangular-wave direct current (d.c.) voltammetry at a hanging mercury drop electrode and phase-selective alternating current (a.c.) polarography at a dropping mercury electrode were used for the investigation of adsorption of double-helical (ds) DNA at mercury electrode surfaces from neutral solutions of 0.05–0.4 M $HCOONH_4$. It was found for the potential region T (from −0.1 V up to ca. −1.0 V) that the height of voltammetric peaks of ds DNA is markedly influenced by the initial potential only at relatively low ionic strength (μ) (from 0.05 up to ca. 0.3). Also a decrease of differential capacity (measured by means of a.c. polarography) in the region T depended markedly on the electrode potential only at relatively low ionic strength. The following conclusions were made concerning the interaction of ds DNA with a mercury electrode charged to potentials of the region T in neutral medium of relatively low ionic strength ($\mu < 0.3$). (i) When ds DNA is adsorbed, a significantly higher number of DNA segments is anchored in the positively charged electrode surface than in the surface bearing a negative charge. (ii) In the region T, especially adsorbed labile regions of ds DNA are opened in the electrode surface, which are present in ds DNA already in the bulk of the solution. (iii) In the narrow region of potentials in the vicinity of the zero charge potential a higher number of ds DNA segments can be opened, probably as a consequence of the strain which could act on the ds DNA molecule in the course of the segmental adsorption/desorption process.

1. Introduction

In the last twenty years the attention of several laboratories has been devoted to the study of interactions of nucleic acids with the surface of the mercury electrode (see, e.g., papers [1–9]). In these investigations the mercury electrode was employed as a model of the electrically charged surfaces that are present in living cells. The mercury surface differs considerably, especially by its chemical nature, from biological electrically charged surfaces. However, it is inert towards aqueous solutions of nucleic acids in a broader range of potentials (e.g. ref. [7]). Therefore the use of mercury electrode enables some general information on interactions of nucleic acids with electrically charged surfaces to be obtained.

Results of our previous measurements carried out in neutral media of ionic strength corresponding to 0.4 M and higher concentrations of salts of monovalent cations [7,8] showed that upon adsorption of double-helical (ds) DNA-like polynucleotides at the mercury electrode an opening of the double helix could take place in dependence on the electrode potential. In a narrow range of potentials at about −1.2 V (against the SCE) — in the region U — the extent of this surface conformational change reaches a limiting value, which in some cases represents nearly the whole ds molecule [7]. A more rapid conformational change of this type, limited, however, only to some labile regions of the ds molecule, takes place in a time shorter than 0.5 s on an electrode charged to potentials in a broad region around the potential of zero charge (ECM) — i.e. in the region T [7,8].

In the present paper methods of electrochemical analysis were used to describe how the conformation of ds DNA was changed upon its adsorption at a mercury electrode charged to potentials of the region T in a dependence of the ionic strength of the neutral medium.

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2. Material and methods

Calf thymus DNA was isolated and characterized as in our previous papers [7,10]. Thermal denaturation and measurements of DNA concentration were also carried out as described earlier [7,10]. The native DNA samples at a concentration of 700 μg ml^{-1} were irradiated in the presence of atmospheric oxygen in 0.015 M NaCl with 1.5 mM sodium citrate pH 7 using γ-radiation from a Co source with a dose rate of 1240 rad min^{-1}. The adsorbed dose was measured by means of a Fe^{2+}/Fe^{3+} chemical dosimeter. The radiation dose was 7000 rads. Irradiation of native DNA with this dose did not cause formation of a detectable amount of denatured DNA [11]. Chemicals used for preparation of the supporting electrolytes were all of analytical grade. pH values were measured with a Compensator E 388 Metrohm apparatus. Triangular-wave voltammetry (TWV) at the hanging mercury drop electrode (HMDE) and phase-selective alternating current (a.c.) polarography at the dropping mercury electrode (DME) were used for detecting changes in the properties of the mercury/solution interphase. Differences in values of currents recorded by means of TWV were employed for investigating changes in ds DNA induced by its interaction with the electrode, as described in our preceding paper [7]. Briefly, DNA was adsorbed at the electrode during the time t_k, when HMDE was kept at the initial potential E_i (its value was always chosen so that it was more positive than potentials of DNA reduction – at about −1.4 V). After the time t_k the forward sweep, which was single negative rapid and linearly changing ramp potential (scan rate 1 V s^{-1}), was first applied to the HMDE. We have shown already [7] that data giving evidence for the appearance of conformational changes in ds DNA as a consequence of its interaction with the electrode were provided by the appearance of the voltammetric peak III (fig. 1a). Interaction of the mercury electrode with bases in DNA segments that have single-stranded (ss) structure in the adsorbed state is responsible for the appearance of this peak III. The fact that even solutions of ds DNA yielded the peak III was explained by surface denaturation of DNA [7,8]. The height of peak III of ds DNA served as a measure of the extent of surface denaturation of ds DNA on the electrode charged to a particular E_i. It has also been shown in our previous communications [7,8] that the voltammetric peak II

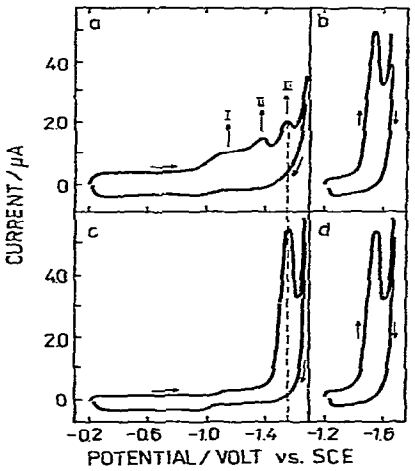

Fig. 1. Triangular-wave voltammetry at the HMDE of DNA in 0.05 M ammonium formate with 0.01 M sodium phosphate, pH 7.0 . Ds DNA at a concentration of 100 μg ml^{-1} and denatured DNA at a concentration of 50 μg ml^{-1}. Curves a, c, E_i = −0.2 V; curves b, d, E_i = −1.2 V. (a, b) Ds DNA; (c, d) thermally denatured DNA. Waiting time at E_i was 60 s.

appearing at pH 7 (fig. 1b) is connected with the existence of bases available for reaction with the electrode in DNA segments that have ds structure in the adsorbed state. After the potential had reached the value of −1.70 V, the voltage sweep was immediately reversed (the scan rate was again 1 V s^{-1}) so that E_i might be reached. TWV was carried out with a GWP 673 Multimode Polarographic Analyzer in connection with a Metrohm HMDE E 410 with a surface area of 3.5 × 10^{-2} cm^2. Voltammetric measurements were performed at the following settings: scan rate 1 V s^{-1}; presweep delay 60 s at initial potential E_i. Other settings were the same as in our previous study [7]. Phase-selective a.c. polarography was also carried out with a GWP 673 Multipurpose Polarographic Analyzer. A phase angle of 90° with respect to the applied alternating voltage was employed. For a.c. polarography a d.c. ramp of 0.004 V s^{-1} and a modulating voltage of 80 Hz and 0.020 V peak to peak was employed in all experiments; drop time control of the GWP 673 was set at 4.0 s. Thus under the conditions of our a.c. polarographic measurements, a.c. was directly proportional to the differential capacity of electrode double layer, C [12]. C is a sensitive indicator of changes taking place in the double layer; e.g., the adsorption

of organic molecules results as a rule in a decrease of C and an adsorption/desorption process or reorientation of the adsorbed molecules may cause the formation of peaks on curves of the dependence of C versus the electrode potential [13,14]. The mercury flow rate, m, of the capillary used in a.c. polarographic measurements (measured in distilled water at open current circuit) was 1.17 mg s^{-1} at a mercury column height of 45 cm. A.c. polarographic measurements were performed with the electrode surface not fully covered by adsorbed DNA molecules. However, qualitatively identical results were obtained at the HMDE, which made it possible to measure with the electrode surface fully covered by DNA molecules. D.c. voltammetric results at the HMDE were thus qualitatively comparable with the a.c. polarographic results obtained at the DME. All potentials quoted in the present report are given versus the saturated calomel electrode (SCE) at 25°C. All electrochemical measurements were carried out with an electrochemical cell maintained at 25°C. Other details of the electrochemical measurements reported in the present paper are given in our preceding communication [7].

3. Results

In a neutral medium of relatively low ionic strength (in 0.05 M ammonium formate with 0.01 M sodium phosphate) triangular-wave voltammograms at the HMDE were recorded for ds DNA. At E_i more positive than ca. −1.0 V the forward (cathodic) sweep gives three well distinguishable peaks I, II and III (fig. 1a), similarly as in a neutral medium of higher ionic strength (at concentrations of ammonium formate or cesium chloride higher than 0.4 M) used in our earlier studies [7,8]. Fig. 1 shows that, in accord with the earlier results [7,8], even in the neutral medium of low ionic strength peak II is yielded only by ds DNA, whereas thermally denatured DNA yields only peak III (peak I is yielded by both forms of DNA, but its nature will not be discussed in the present paper). In the course of the reverse part of the current–potential curve corresponding to the descending (anodic) branch of the triangular sweep, only peak I has an anodic counterpart situated about 0.1 V more positively (fig. 1). The same result (not shown) was obtained even in the case when the descending branch of the triangular sweep

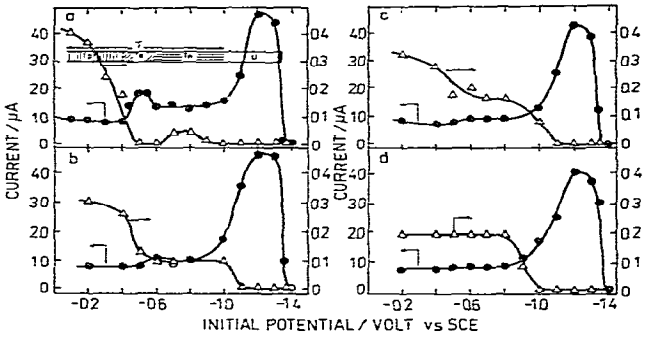

Fig. 2. Dependence of the voltammetric peak heights of ds DNA on E_i (•) peak III of ds DNA; (△) peak II of ds DNA. Medium: (a) 0.05 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0; (b) 0.1 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0; (c) 0.2 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0; (d) 0.4 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0. Other conditions of the measurement were the same as in fig. 1.

started at −1.43 V, i.e. at the potential corresponding to the more negative foot of peak II (fig. 1a). These results suggest that the appearance of peaks II and III (fig. 1) is probably caused by irreversible electrochemical reduction of adenine and cytosine residues [10] rather than only a reorientation or an adsorption/desorption processes [13,14].

In a medium of 0.05 M ammonium formate, pH 7.0, dependences of the heights of peaks II and III (I_{II} and I_{III}) on E_i were recorded for ds DNA (fig. 2a). I_{III} was nearly independent of E_i in the region of E_i from −0.1 V to −0.4 V (in the region T_p – fig. 2a). In the vicinity of potential of −0.5 V, i.e. in the vicinity of the potential of zero charge ‡, a maximum appeared on the dependence I_{III} versus E_i (in the region T_o – fig. 2a). In the region of potentials from −0.6 V to ca. −1.0 V (in the region T_n) I_{III} was again independent of E_i. At potentials more negative than ca. −1.0 V (in the region U – fig. 2a) I_{III} first grew markedly, and after reaching a maximum in the vicinity of −1.2 V, again decreased. Quite a different course was exhibited by the dependence of I_{II} versus E_i. In the region T_p I_{II} decreased with decreasing E_i; in the region T_o peak II

‡ The potential of ECM in the DNA solutions used in the present study measured by the drop time method [15] was in the region of potentials of −0.5 – −0.6 V.

quite disappeared, but in the region T_n ds DNA again yielded a small peak II (fig. 2a). At E_i corresponding to the more positive end of the region U, peak II disappeared completely.

Increasing concentration of ammonium formate up to the concentration of ca. 0.3 M did not influence qualitatively the courses of the dependences of I_{II} and I_{III} of ds DNA on E_i (figs. 2a–c). The maximum on the dependence I_{III} of ds DNA versus E_i, located in the vicinity of –0.5– –0.6 V, decreased with increasing ionic strength; it disappeared completely in the medium of 0.4 M ammonium formate (fig. 2d). Also the difference in values of I_{II} in the regions T_p and T_n decreased with increasing ionic strength, since I_{II} decreased in the region T_p, whereas it grew in the region T_n; in neutral medium of 0.4 M ammonium formate both I_{II} and I_{III} of ds DNA were independent of E_i in the whole region T (fig. 2d).

The behaviour of DNA at the mercury electrode charged to the potentials of the region U (fig. 2a) was discussed in greater detail in our previous paper [7]. Therefore this behaviour of DNA will not be described in the present paper.

Thermally denatured DNA yielded only peak III in neutral media of ammonium formate in concentrations ranging from 0.05 to 0.4 M (it did not yield peak II). The height of peak III of thermally denatured DNA was practically independent of E_i in the range from –0.1 to ca. –1.3 V (fig. 3). Only a small increase of this peak was observable at higher concentrations of ammonium formate in the vicinity of $E_i = -1.2$ V (fig. 3b). This increase is connected with aggregation and partial renaturation of thermally denatured DNA [7].

The results of the above described voltammetric measurements were compared with those of a.c. polarographic measurements performed in the same media (fig. 4). Ds DNA markedly decreased a.c., especially at potentials more positive than ca. –0.5 V. In the vicinity of this potential (where a maximum appeared on the curve of the dependence of I_{III} of ds DNA versus E_i – figs. 2a–c) ds DNA yielded peak O [3] on a.c. polarograms (figs. 4a–c). Its height decreased with increasing ionic strength; at ammonium formate concentrations higher than 0.4 M peak O practically disappeared (fig. 4d). Increasing of ionic strength also caused ds DNA to decrease more markedly a.c. even at potentials more negative than ca. –0.6 V (figs. 4a–d).

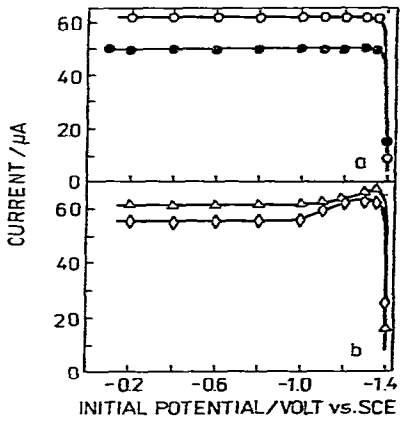

Fig. 3. Dependence of the voltammetric peak III height of thermally denatured DNA on E_i (a): (○) 0.05 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0; (●) 0.1 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0. (b): (△) 0.2 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0; (◇) 0.4 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0. Other conditions were the same as in fig. 1.

On the contrary, thermally denatured DNA decreased markedly a.c. in the range of potentials from –0.1 to –1.0 V at ammonium formate concentrations from 0.05 to 0.4 M, without yielding peak O in the vicinity of the potential of –0.5 V (figs. 4e–h). The adsorption behaviour of thermally denatured DNA in dependence on the ionic strength of the medium was studied in greater detail in our previous paper [3] and will be neither fully described nor discussed here.

We also studied the influence of γ-irradiation of ds DNA on its voltammetric and a.c. polarographic behaviour in neutral medium of relatively low ionic strength, i.e. in 0.05 M ammonium formate. We found that γ-irradiation with a dose that did not lead to the formation of denatured DNA, but caused an increase of the number of labile regions in ds DNA [16,17], induced a marked increase of I_{III} of ds DNA in the whole region T (fig. 5). Even the maximum on the curve of dependence of I_{III} versus E_i was more pronounced. On the contrary, I_{II} was lower in the region T_p after the irradiation. However, this decrease was substantially less pronounced than the increase in I_{III} of ds DNA. The irradiated ds DNA did not yield peak II in the region T_n (fig. 5). A.C. polarographic behaviour of ds DNA in this medium was influenced by γ-irradiation only at potentials more negative than ca. –0.5 V.

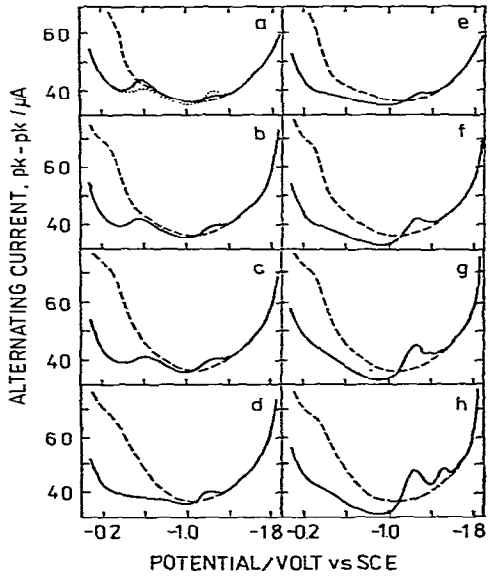

Fig. 4. A.c. polarograms of DNA. (a–d) ds DNA at a concentration of 350 μg ml^{-1}; (e–h) thermally denatured DNA at a concentration of 200 μg ml^{-1}. Medium: (a), (e) 0.05 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0; (b), (f) 0.1 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0; (c), (g) 0.2 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0; (d), (h) 0.4 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0. (– – – –) curve of supporting electrolyte; (· · · ·) curve of γ-irradiated DNA.

Peak O was decreased, and the irradiated ds DNA also decreased a.c. at potentials more negative than ca. −0.6 V more markedly than non-irradiated DNA (fig. 4a).

If the irradiated DNA was thermally denatured, it yielded only peak III on voltammograms, which was independent of E_i (not shown), similarly as did not non-irradiated thermally denatured DNA (fig. 3). The height of peak III of denatured irradiated DNA was slightly higher. The a.c. polarogram of thermall denatured irradiated DNA (not shown) was qualitatively identical with the polarogram of non-irradiated thermally denatured DNA (fig. 4e). The decrease of a.c. in the vicinity of the potential of ECM and also desorption peaks in the vicinity of the potential of −1.2 V were more pronounced.

We attempted to find out whether the changes in ds DNA caused by its adsorption on the mercury electrode charged to the potential of the region T_o (fig. 2a)

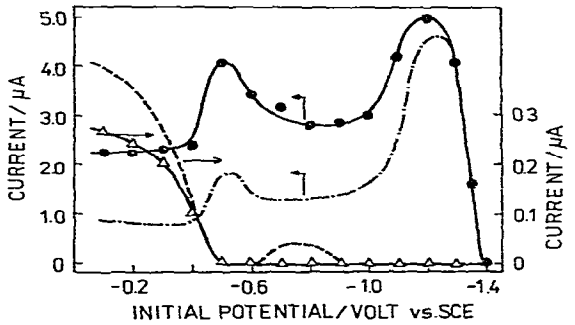

Fig. 5. Dependence of the voltammetric peak heights of γ-irradiated ds DNA on E_i in 0.05 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0. (△) peak II of γ-irradiated ds DNA; (●) peak III of γ-irradiated ds DNA; (– – – –) peak II of non-irradiated ds DNA; (– · –) peak III of nonirradiated ds DNA. Other conditions of measurement were the same as in fig. 1.

are reversible in a medium of relatively low ionic strength. The mercury drop electrode was immersed in a solution of ds DNA in 0.5 M $HCOONH_4$ with 0.01 M sodium phosphate, pH 7.0, continuously for 120 s. After this time linear sweep voltammogram was recorded and the heights of peaks II and III were measured in the dependence on potentials applied to the electrode during the preceding 120 s. If the HMDE was kept for the whole time (120 s) only at $E_i = -1.4$ V, ds DNA did not yield any peak. However, if the HMDE was first kept for 60 s at −0.5 V (i.e. at the potential of the region T_o) and then potential of −1.4 V was applied to the same drop for a further 60 s, ds DNA yielded peak III. The height of this peak III was slightly lower (by about 10%) than the height of peak III yielded by ds DNA after 120 s of HMDE prepolarization only to $E_i = -0.5$ V. In a similar experiment the HMDE was first charged to −0.5 V for 60 s and then for a further 60 s to potential of −0.2 V (i.e. to the potential of region T_p). Ds DNA then yielded peak III, the height of which was even about 15% higher than the height of peak III yielded by ds DNA under conditions, when the HMDE was prepolarized for 120 s to $E_i = -0.5$ V. These results indicate that at least a part of the changes resulting from ds DNA interaction with the mercury electrode surface charged to E_i of the region T_o remains preserved even after the removal of the change-inducing potential. It may be thus concluded that the changes in ds DNA appearing upon its interaction with the HMDE charged to

E_i of the region T_o (in a neutral medium of relatively low ionic strength) are at least partially irreversible. A similar result was obtained for ds DNA upon its interaction with the mercury electrode charged to E_i of the region U [7].

4. Discussion

Our preceding papers [7,8] describe the extent of conformational changes that lead to an opening of the DNA double helix, which were formed in ds polynucleotides in the course of their adsorption on the mercury electrode (the extent D). It was shown in these studies [7,8] that in neutral media of higher ionic strength (higher than ca. 0.4) the extent D (monitored by means of I_{III}) did not depend on E_i in the region T. It is shown in the present study that in neutral medium of low ionic strength (lower than ca. 0.3) the extent D also did not practically depend on E_i in the region T with the exception of the region T_o (fig. 2a), where a significant increase in the extent D could be observed (figs. 2a–c). This increase was the higher the lower the ionic strength (figs. 2a–c). The fact that the potential T_o is identical with potentials of a.c. polarographic peak O (figs. 2 and 4) points at the connection of processes responsible for the increase of I_{III} of ds DNA in the region T_o (figs. 2a–c) and for the appearance of a.c. polarographic peak O. We have already shown [3] that segmental desorption of ds DNA is responsible for appearance of the non-faradaic capacitive a.c. polarographic peak O. This conclusion has been confirmed by other authors more recently [18,19]. This desorption takes place owing to the electrostatic nature of ds DNA adsorption in a medium of lower ionic strength [3,18], since the sign of the surface charge of the electrode is changed in the vicinity of the potential of peak O formation (peak O appears always in the vicinity of the potential of ECM [3]). Thus, in the region T_o a process analogous to that proceeding in the region U [7] can take place: Under conditions of formation of peak O some segments of DNA molecule are bound to the electrode surface by adsorption forces, whereas other non-adsorbed segments of the same DNA molecule can be repulsed from the electrode. The different adsorbability of ds DNA segments could be connected with the fact that ds DNA molecules are not homogeneous in their sequence and conformation [20,21]. Thus a ds DNA molecule can, under such conditions, be exposed to a strain which could lead to further disturbance of the regular DNA double helix. As a consequence of this disturbance a higher number of bases could become accessible for the reaction with the electrode.

We have shown by means of a.c. polarographic measurements that in a neutral medium of lower ionic strength (lower than ca. 0.3) ds DNA is markedly more adsorbed at the positively charged electrode surface in the region T (figs. 4a–c and ref. [3]): The difference in the ds DNA adsorbability at the positively and negatively charged surfaces of the electrode decreased with increasing ionic strength of the medium. In the light of this markedly different adsorbability of ds DNA, the result showing that the extent D almost does not depend on the sign of the surface charge of the electrode in the region T (with the exception of the region T_o) is somewhat surprising. This voltammetric and a.c. polarographic behaviour of ds DNA could be explained on the basis of an assumption according to which the appearance of the peak III of ds DNA (fig. 1a, b) is connected with the presence of the labile regions existing in ds DNA molecules already in the bulk of solution [20,21]. Only these labile regions would be opened at the electrode surface charged to the potentials of the regions T_p and T_n (fig. 2a). The adsorbability of base residues at the mercury electrode is markedly higher than that of other components of DNA [22]. As bases in the labile regions of ds DNA (e.g. in the vicinity of single strand or ds break, damaged base etc.) are accessible for the reaction with the electrode [20,21], it is reasonable to expect that these labile regions of ds DNA are adsorbed at the mercury electrode similarly to thermally denatured DNA [3], i.e. predominantly via bases roughly in the same extent on the positively and negatively charged electrode surface even in the medium of relatively low ionic strength.

The results presented in this paper make also possible to suggest which groups in ds DNA are responsible for the appearance of the voltammetric peak II (fig. 1). In the neutral medium of low ionic strength the peak II of ds DNA was clearly dependent on the initial potential of the electrode (figs. 2a–c), qualitatively in the same way as the adsorbability of ds DNA (fig. 4). For explaining this result we suggest that the appearance of the peak II is connected with the presence of

labile regions in ds DNA, which would occur in DNA only as a consequence of the DNA adsorption at the electrode surface. The latter labile regions might have a quite different character than the labile regions existing in ds DNA already in the bulk of solution. Ds structure of DNA could be labilized in the electrode surface by e.g. an electric field (for review see, e.g., ref. [23]), as has already been outlined [5,7]. The electric field can have a strength of even 10^6 V cm^{-1} in the immediate vicinity of the mercury electrode [14]. The character of the conformational changes induced by the electric field in intact segments of adsorbed DNA could be of a kind that polarographically reducible bases in DNA (adenine and cytosine) [20] would become accessible for electroreduction without total separation of DNA chains in this labilized region. This explanation is also in a very good agreement with the results obtained with irradiated DNA (figs. 4a, 5). The voltammetric peak II would thus indicate the presence of adenine and cytosine residues accessible for electroreduction at the mercury electrode but still present in ds structure of DNA.

The influence of ionic strength on DNA adsorption at the mercury electrode was studied recently by Flemming [24]. He confirmed, in accord with results published earlier [3,18], that in the medium of relatively low ionic strength ds DNA is adsorbed to a greater extent at the positively charged electrode surface. He concluded that his results excluded the possibility of an opening of the DNA double helix in the mercury/solution interphase. He monitored the DNA adsorption by means of a.c. voltammetry at the HMDE and normal pulse polarography at the DME. His measurements were carried out in a medium of pH about 6 under conditions of incompletely covered electrode surface by the DNA molecules. However, at pH 6 under conditions used on Flemming's experiments [24], ds DNA does not yield separated peaks II and III specific for ds and single-stranded DNAs respectively in the electrode surface, as in the case of our voltammetric measurements performed at pH 7 and in the presence of either $HCOONH_4$ or CsCl (figs. 1, 2 and refs. [7,8]). At pH 6 peaks II and III merge [25], so that it is impossible to decide which part of normal pulse polarographic peaks observed by Flemming corresponded to single-stranded and ds DNA in the electrode surface. Flemming's paper [24] does not exclude the possibility of opening of the DNA double helix in the electrode surface. It thus demonstrates that some results of voltammetric investigations of DNA at mercury electrodes can be interpreted even without assuming conformational changes in adsorbed ds DNA, if the results of other authors are not taken into consideration [5,7,8,19,26].

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