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INTERACTION OF NUCLEIC ACIDS WITH ELECTRICALLY CHARGED SURFACES.

VI. A COMPARATIVE STUDY ON THE ELECTROCHEMICAL BEHAVIOUR OF NATIVE AND DENATURED DNAs AT GRAPHITE ELECTRODES

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Adsorption and electrochemical oxidation of deoxyribonucleic acid (DNA) at a pyrolytic graphite electrode (PGE) and a paraffin wax-impregnated spectroscopic graphite electrode (WISGE) were studied using differential pulse voltammetry. DNA is adsorbed at the surface of the graphite electrodes in a broad range of potentials including the potentials of electrochemical oxidation of DNA. Both native and denatured DNAs yield two single, well-defined and separated peaks, G and A, on the differential pulse voltammograms at the PGE and WISGE. The more negative peak, G, corresponds to electrochemical oxidation of guanine residues, whereas the more positive peak, A, corresponds to electrochemical oxidation of adenine residues. Peaks G and A of native DNA occur at the same potentials as peaks G and A of denatured DNA. However, electrochemical oxidation of adenine and guanine residues at graphite electrodes is markedly suppressed in native DNA. The heights of the peaks G and A represent a sensitive indicator of the helix-coil transition of DNA.

An analysis of the product of interaction of a sample of native DNA with a large pyrolytic graphite electrode in the presence of formaldehyde at approximately neutral pH did not prove changes in the secondary structure of native DNA due to its interaction with the graphite electrode. It is suggested that the decreased differential pulse-voltammetric activity of native DNA is connected with its decreased flexibility.

1. Introduction

In the last twenty years methods of electrochemical analysis have been used successfully in studies of nucleic acids. The use of these methods proved to be effective both in investigating the interactions of nucleic acids with electrically charged surfaces [1—5] and in studying the properties of nucleic acids in solution [6].

We have shown recently [7,8] that differential pulse voltammetry (DPV) at graphite electrodes can be used in studies of electrochemical oxidizability of polynucleotides. Single-stranded polynucleotides containing guanine and adenine yield two very well defined and separated oxidation peaks on differential pulse voltammograms, which are easily identified over large pH ranges (e.g., between pH 2.7 and 8.2) [7]. Oxidation of guanine residues is responsible for the more negative peak, whereas the more positive one corresponds to the oxidation of adenine residues. Polynucleotides that contain neither guanine nor adenine residues are not electrochemically oxidizable at graphite electrodes.

Recently two separated oxidation peaks on the voltammograms of DNA were independently observed by Yao et al., who used a glassy carbon electrode [9]. These authors did not, however, describe the nature of these peaks.

Preliminary measurements with double-helical DNA [7] showed that the electrochemical oxidizability of guanine and adenine residues incorporated in its structure is markedly suppressed as compared with thermally denatured DNA. We suggested that this property could be exploited for probing individually the adenine—thymine (A—T) and guanine—cytosine (G—C) regions in the course of structural and conformational changes of polynucleotides and for investigating their interactions with other solution species.

In the present communication we report results of a more detailed study of differential pulse-voltammetric

oxidation of double-helical and thermally denatured DNAs at a pyrolytic graphite microelectrode (PGE) and a paraffin wax impregnated spectroscopic graphite electrode (WISGE). We have shown that interaction of the double-helical DNA with the surface of a graphite electrode does not lead to pronounced changes in its secondary structure and that the heights of the well-defined differential pulse-voltammetric oxidation peaks provide a sensitive monitor for following the helix-coil transition of DNA.

2. Experimental

2.1. Material

Calf thymus DNA was isolated and characterized as described in our previous papers [2,10]. Contents of RNA, proteins and denatured DNA were less than 1%. Unless stated otherwise, the denaturation of DNA was performed under the conditions described previously [2,7]. Before using DNA for voltammetric measurements it was dialyzed for 48 h against sodium phosphate of ionic strength 0.01 and pH 7.0. The co-polymer polyribo (adenylate, guanylate) (adenine : guanine ratio 1.6 : 1.0) (poly (A, G)) from Schwarz, Orangeburg, N.Y. was kindly donated by Dr. E. Paleček. Deoxyadenosine-5'-monophosphate (dAMP) was obtained from Calbiochem, San Diego, Ca.

The concentrations of DNA and dAMP were estimated spectrophotometrically. The concentration of the copolymer poly (A, G) (expressed as the phosphorus content) was estimated by determining the phosphorus content in a poly (A, G) sample according to the method of Martin and Doty [11].

Formaldehyde used in this study was obtained from Lachema, Czechoslovakia; it was pretreated according to the procedure described by von Hippel and Wong [12]. Chemicals used for preparation of background electrolyte solutions were analytical grade.

The PGEs were machined from small rods of pyrolytic graphite to a diameter of 2 mm and length ca. 15 mm with the a,b plane forming the base of the cylinder. They were sealed into a length of 2 mm bore glass tube with Epoxy Resin 110 (Chemické závody, Plzeň, Czechoslovakia). A large area PGE (LPGE) was made from a graphite disc (diameter ca. 2.5 cm, thickness 3 mm). Pyrolytic graphite was a kind gift from Pfizer Minerals, Easton, Pa.

The WISGEs were made from rods of Spectroscopic graphite (Kablo, Topoľčany, Czechoslovakia) of diameter 6 mm according to the method of Morris and Schempf [13]. The paraffin used for the wax impregnation was obtained from Fluka, Switzerland. The rods of impregnated graphite were sealed into lengths of 6 mm bore glass tube with Epoxy Resin 110. Between runs, the PGE and WISGE were resurfaced by polishing the end with a waterproof carbide paper (600 grade, Carborundum, Kolín, Czechoslovakia) mounted on a rotating disc sander. The electrodes were ground flush with the rim of the glass tube. Electrical contact was made by placing a small amount of mercury in the tube and inserting a piece of platinum wire.

2.2. Methods

Voltammograms at the PGE and WISGE were obtained in a 2 ml capacity thermostated cell containing a mercury pool counter electrode. In most experiments a saturated calomel reference electrode (SGE) was employed. A fine Luggin capillary positioned close to the tip of the working electrode (e.g. PGE) was used to maintain electrolytic contact with the SCE.

Differential pulse-voltammetric measurements at the PGE and WISGE were carried out with a PARC Model 174 polarographic analyzer. Unless stated otherwise, differential pulse voltammograms were obtained with a pulse amplitude of 25 mV and a sweep rate of 5 mV s^{-1}. The current sampling for DPV at the graphite electrodes was set with the drop time control of the PARC Model 174 set at 0.5 s.

Voltammograms were recorded on a BAK 4T Aritma Praha $X-Y$ recorder. All voltammetric measurements were carried out with the voltammetric cell maintained at 25 ± 0.1°C. If not specified otherwise, all potentials quoted in this report are given versus the SCE at 25°C.

The basic procedures for DPV have been described previously [7,8]. We would remark here only that once the graphite electrode was inserted into the test solution contained in the electrochemical cell, it was allowed to stand for 10 s without any applied potential. Then, unless stated otherwise, an initial potential of 0.2 V was applied for 120 s, after which time the voltammetric sweep was commenced. In order to check the reproducibility of the curves, each voltammogram was recorded at least three times.

It has been shown in our previous communications [7,8] that it is necessary to use DPV in studies of electrochemical oxidizability of polynucleotides at graphite electrodes, because the sensitivity of the conventional linear sweep voltammetry is not sufficient. Moreover, investigations of voltammetric oxidizability of polynucleotides containing adenine should be carried out in background electrolytes containing only phosphate or acetate buffer [7,8]. If not stated otherwise, all voltammetric results given in this paper were obtained in acetate buffer (HOAc/NaOAc) of ionic strength 0.2. Most of the results reported here were obtained at the PGE, but essentially identical results were obtained at the WISGE.

DNA solutions for voltammetric and spectrophotometric measurements were prepared in the following way: A solution of suitable buffer was pipetted dropwise into an equal volume of a solution of DNA (in phosphate buffer of ionic strength 0.01 and pH 7) to obtain the desired composition and pH (pH was measured after mixing).

We also investigated the interaction of DNA with the graphite electrodes in the presence of formaldehyde. The aim of this experiment was to fix possible reversible conformational changes in DNA occurring in the vicinity of the electrodes by hydroxymethylating the DNA bases [12,14]. This approach could be used only for studies of the interactions of DNA with the graphite electrodes charged to potentials 0—0.6 V. At more positive potentials our sample of formaldehyde yielded a peak on the conventional linear sweep voltammogram recorded in the medium used in these experiments. A product of the interaction of DNA with the surface of the graphite electrode was obtained in a single-compartment cell currently used in macroscale electrolytis. The bottom of this cell represented the LPGE. Electrical contact to this working electrode was achieved with a large alligator clip. Two fine Luggin capillaries positioned close to the LPGE were used to maintain an electrolytic contact to the SCE and to a coiled platinum wire in 1.0 M KCl used as a counter-electrode. For the experiments we took 5 ml of DNA in a concentration of 20 μg/ml. DNA was dissolved in 0.2 M sodium acetate, pH 6.5. Throughout the experiment a magnetic stirrer rotated at the LPGE surface at a speed of approximately 50 rpm. Before each experiment the LPGE was resurfaced with carbide paper. The potential of the LPGE was controlled by the potentiostat of the PARC 174. The reaction of DNA was observed spectrophotometrically. Optical densities of the DNA samples at 252, 260 and 278 nm were measured by means of a Zeiss VSU-2P spectrophotometer.

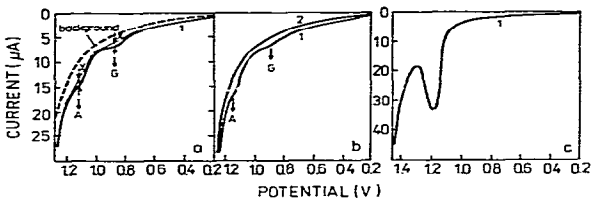

Fig. 1. Differential pulse voltammograms at the PGE in 0.2 M sodium acetate, pH 6.44 of (a) 300 μg of denatured DNA per ml, (b) 300 μg of native DNA per ml, (c) 0.28 mM dAMP. Curves 1 represent the first voltammetric trace at a clean, resurfaced PGE. Curve 2 (in fig. 1a) represents the second voltammetric trace obtained at the same PGE used to obtain curve 1 without resurfacing the electrode. The values x and y were taken to represent the heights of peak G and peak A respectively.

3. Results

Typical differential pulse voltammograms of denatured DNA at the PGE at pH 6.44 are shown in fig. 1a. Two rather well-defined peaks are clearly observable: peak G of guanine residues at about 0.9 V, and peak A of adenine residues at about 1.1 V [7]. A voltammogram of the same concentration of native DNA is shown in fig. 1b. The peak currents observed for native DNA are considerably lower than those of denatured DNA. For comparison a differential pulse voltammogram of monomeric dAMP in a concentration, corresponding to the content of adenine residues in the DNA samples of figs. 1a and 1b is presented in fig. 1c.

In our previous communication [7] a differential pulse voltammogram of native DNA at the PGE was also presented. On the voltammogram presented in that paper [7], the peak A of native DNA was (in accord with fig. 1a of the present paper) significantly lower than peak A of denatured DNA. However, peak G on the voltammogram of native DNA presented in our previous communication [7] was (contrary to the present paper (fig. 1b)) not observable. In order to ex-

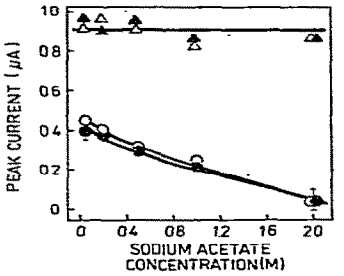

Fig. 2. Variation of the differential pulse voltammetric peak current of native and denatured DNAs with ionic strength at the PGE in sodium acetate, pH 6.44. DNA concentration was 300 μg/ml. (•) peak G of native DNA, (○) peak A of native DNA, (▲) peak G of denatured DNA, (△) peak A of denatured DNA.

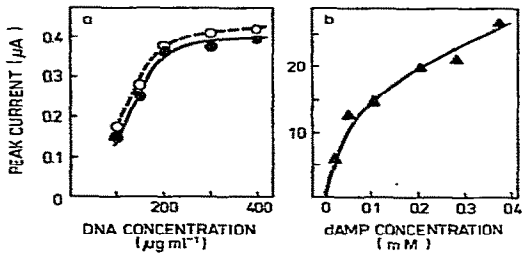

Fig. 3. Variation of the differential pulse voltammetric peak current with concentration of (a) native DNA and (b) dAMP at the PGE in 0.2 M sodium acetate, pH 6.44. The presweep time at 0.2 V was 120 s. (•) peak G, (○) peak A, (▲) dAMP.

plain this discrepancy, we studied the dependence of the heights of peaks A and G of native DNA as a function of ionic strength (fig. 2). One of the difference between voltammograms of native DNA reported in the present paper and the previous one [7] consists in the fact that the voltammogram shown in fig. 1b of the present paper was recorded in a medium of markedly lower ionic strength (0.2 as compared with 0.5 used in the previous paper [7]). As can be seen in fig. 2, the heights of both peaks of native DNA decrease with increasing ionic strength, whereas the heights of peaks A and G of denatured DNA practically do not depend on ionic strength. A PGE machined from pyrolytic graphite produced by General Electric Co. was used in the previous study [7]. Numerous irregularities occurred in the vicinity of the potential of peak G on differential pulse voltammograms recorded with the latter electrode. It is possible that a very small peak G of native DNA in a medium of ionic strength of 0.5 could be obscured by these irregularities. It can thus be summarized that native DNA yields both peaks A and G, but their heights are markedly reduced as compared with denatured DNA. Moreover, the potentials of peaks A and G of DNA are influenced neither by the secondary structure of DNA (figs. 1a and 1b) nor by ionic strength (not shown).

It was found that the heights and potentials of the differential pulse-voltammetric oxidation peaks G and A of native DNA were unaffected by the initial presweep potential over the range 0–0.85 V, similarly as in the case of denatured DNA [7] or polyadenylic acid (poly(A)) [8]. Using a 120 s presweep time at 0.2 V, the relationship between the peak current and native DNA concentration for both peaks G and A was clearly nonlinear (fig. 3a). Similar relationships were obtained for denatured DNA [7] and poly(A) [8]. At native DNA concentration greater than ca. 200 μg/ml the peak currents tend towards a limiting value. The lower level of detection for native DNA by differential pulse voltammetry at the PGE and WISGE is ca. 0.1 mg/ml. Under similar conditions the height of the voltammetric oxidation peak of dAMP also exhibited a nonlinear dependence on concentration (fig. 3b) although the effect was much less pronounced than in the case of native DNA (fig. 3a) or denatured DNA [7].

The effect of presweep time on the peak current of both peaks G and A (fig. 4) indicates that with increasing presweep time at 0.2 V both peaks G and A increase in a nonlinear fashion and approach a maximal value at presweep times >200 s (for native DNA concentration

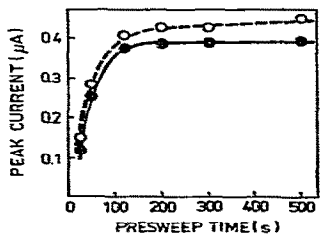

Fig. 4. Variation of the differential pulse voltammetric peak current with presweep time at 0.2 V for native DNA (concentration 300 μg/ml) at the PGE in 0.2 M sodium acetate, pH 6.44. (•) peak G, (○) peak A.

of 0.3 mg/ml). The peak current of dAMP was independent of presweep time.

The nonlinear relationships between the voltammetric peak current and DNA concentration (fig. 3a and ref. [7]) on the one hand and presweep time (fig. 4 and ref. [7]) on the other hand suggest that both forms of DNA are adsorbed at the PGE at 0.2 V as postulated on the basis of our alternating current (a.c.) voltammetric measurements [7]. Similar behaviour was observed at potentials between 0.0 and 0.85 V. The potentials of both peaks of denatured DNA [7] and native DNA were unaffected by either DNA concentration of presweep time.

The rather strong and apparently irreversible adsorption of native DNA could be also demonstrated by immersing a PGE into a solution of 300 μg of native DNA per ml and applying a potential of 0.2 V for 120 s. After this time the electrode was removed from the solution and washed with a solution of appropriate acetate buffer. After 30 s the electrode was inserted into an identical electrochemical cell which contained only pure acetate buffer solution. A potential of 0.2 V was again applied for 120 s and then a differential pulse voltammogram was recorded. Well-formed voltammetric oxidation peaks G and A were observed, the heights of which were almost identical with those expected for a solution containing 300 μg of native DNA per ml. The results presented in fig. 1b show that the product of electrochemical oxidation of native DNA at the PGE is also strongly adsorbed on the electrode. The first trace (curve 1 in fig. 1b) represents the differential pulse voltammogram of a solution of native DNA at a clean, freshly resurfaced electrode. The second trace (curve 2 in fig. 1b) was obtained under the same conditions as the first trace except that the PGE was not resurfaced. It can be observed from curve 2 (fig. 1b) that both oxidation peaks are absent. This suggests that the product of electrooxidation of adsorbed native DNA remains adsorbed at the PGE surface, so that fresh native DNA molecules cannot reach the electrode surface. Irreversible adsorption at the PGE charged to potentials of 0.2–1.3 V was also observed for denatured DNA [7].

Considering the possible utility of the differential pulse-voltammetric oxidation of native DNA for analytical purpose, it might be noted that the sensitivity of the technique could be enhanced by increasing

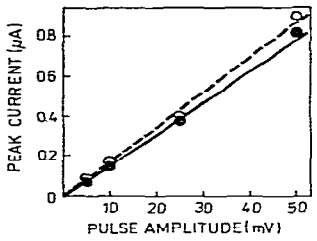

Fig. 5. Variation of the differential pulse voltammetric peak current with pulse amplitude for voltammetric oxidation of native DNA at the PEG in 0.2 M sodium acetate, pH 6.44 with 120 s presewep time at 0.2 V. (•) peak G, (○) peak A. Concentration of native DNA was 300 μg/ml.

the amplitude of the applied potential pulse (fig. 5). However, similarly as for denatured DNA [7], the voltammetric peaks become increasingly distorted at pulse amplitudes higher than about 50 mV. Accordingly, for most purposes the optimum pulse amplitude appears to be 25 mV. Potentials of peaks A and G of native DNA were shifted to more negative values with increasing amplitudes. A change of the amplitude from 5 mV to 50 mV induced a shift of the peak potential by approximately 30 mV.

Acid denaturation of DNA was studied by means of DPV at the PGE (fig. 6). Denaturation curves were observed which resembled the curve constructed on the basis of spectrophotometric measurements at 260 nm (fig. 6). Melting pH values, pH_m (defined here as pH at which half of the total increase in absorbance or peak height occurred), determined by means of electrochemical and optical methods, were identical (fig. 6). Peaks G and A of thermally denatured DNA were practically independent of pH (not shown).

For the study of conformation of DNA during its interaction with a mercury electrode it proved to be advantageous to study this interaction in the presence of formaldehyde [3]. In the presence of formaldehyde even a partial denaturation, which can be a reversible process, can be detected (for details see ref. [3] and experimental part of this report). We investigated the interaction of native and denatured DNAs at a concentration of 20 μg/ml with 3.3 mM formaldehyde in the presence of the LPGE charged to potentials of 0.2–0.6 V. Optical densities at 252 and 278 nm ($O.D._{252}$ and $O.D._{278}$) were measured to reflect conformational changes in DNA and its formylation re-

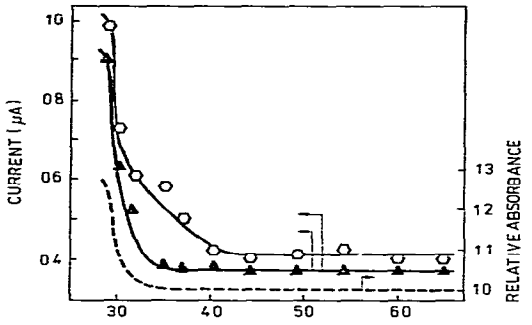

Fig. 6. Course of acid denaturation of DNA as studied by differential pulse voltammetry at the PGE and by u.v. spectrophotometry. The spectrophotometric and voltammetric measurements were carried out at 25°C in a medium of 0.2 M sodium acetate at pH values indicated in the diagram; the DNA concentration was 300 μg/ml in voltammetric measurements and 35 μg/ml in spectrophotometric measurements. Voltammetry: (▲) peak G, (○) peak A. Spectrophotometry: (– – – –) absorbance at 260 nm.

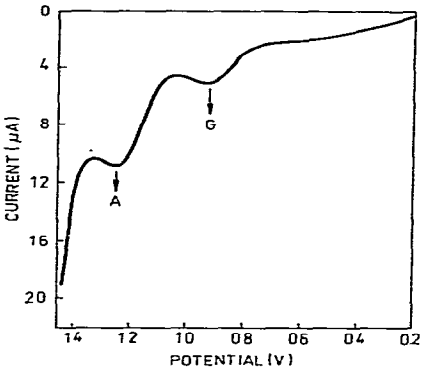

Fig. 7. Differential pulse voltammogram at the PGE of 4×10^{-4} M copolymer polyribo(adenylate, guanylate) (adenine : guanine ratio 1.6 : 1.0) in 0.2 M sodium acetate, pH 6.44. The potentials are given against the mercury on the bottom of polarographic vessel.

spectively [12,14]. After 20 min contact of a solution containing denatured DNA and formaldehyde with the LPGE O.D.$_{252}$ increased approximately by 20%, whereas O.D.$_{278}$ increased approximately by 40%. O.D.$_{252}$ and O.D.$_{278}$ practically did not change at all in the absence of the LPGE under otherwise identical conditions. If the sample of thermally denatured DNA was replaced by native DNA in this type of experiment, O.D.$_{252}$ and O.D.$_{278}$ practically did not change even after a 30 min contact of the DNA solution with the LPGE. This result obtained with native DNA could be explained by complete inhibition of the exchange between adsorbed and dissolved native DNA molecules due to a strong adsorption of native DNA on the electrode [7]. However, the following argument indicates that this exchange did take place. Many more molecules of denatured DNA reacted with formaldehyde at the LPGE/solution interface than would correspond to the number of molecules that covered the LPGE surface during the initial period of the experiment. As shown previously by means of e.g. a.c. voltammetry [7], denatured DNA is adsorbed on the PGE surface at least with the same strength as native DNA.

The influence of the roughness of the graphite electrode surface on the relative heights of the differential pulse-voltammetric oxidation peaks of denatured and native DNAs was also investigated. This was done by measuring the heights of voltammetric peaks for two solutions containing DNA in the concentration of 300 μg/ml. The first solution contained thermally denatured DNA, the other native DNA. The ratio of the peak height of denatured DNA (I_{denat}) to that of native DNA (I_{nat}) was approximately 2.4 for peak G and 2.3 for peak A, if the PGE was resurfaced with carbide paper (figs. 1a, 1b). However, if the graphite electrode was resurfaced by the procedure described, followed by further polishing with smooth paper until the electrode had a smooth mirror-like finish, the ratio I_{denat}/I_{nat} decreased by 20–25% to the value of ca. 1.8 for both peaks G and A. The increase of the ratio I_{denat}/I_{nat} with increasing roughness of the electrode surface was also observed at the WISGE.

In order to obtain further information on the mechanism of the electrode process by which adenine and guanine residues bound in polynucleotides are oxidized at the PGE, we recorded differential pulse voltammograms of a copolymer poly (A, G). The single-stranded copolymer under investigation contained 1.6 times more adenine residues than the guanine residues; the base sequence of this polynucleotide was not known. As shown in fig. 7, the ratio of the heights of peaks A and G corresponded roughly to the ratio of the number of adenine and guanine residues in this copolymer. This suggests that the consumption of electrones in the electrochemical oxidation at the PGE of an adenine residue and a guanine one bound in a polynucleotide are not very different. The height ratio of peaks G and A of this polynucleotide did not

change if voltammograms were recorded at 0° and 35°C.

4. Discussion

In a living cell nucleic acids interact with electrically charged surfaces of biological membranes [15–17]. Up to this time, these interactions have been studied indirectly, i.e. by means of various model surfaces in vitro. The surface of a mercury electrode has often been used for this purpose [1–5]. It follows from the results obtained in our laboratory [2,3] that, as a consequence of interaction of native DNA with the mercury electrode at pH of about 7, a conformational change takes place in native DNA. These conformational changes could be characterized as an opening of the double helix, when the bases become accessible for reaction with their environment. The extent of these changes depended significantly on the electrode potential [2,3]. On the other hand, Malfoy et al. [4, 18] investigated interactions of DNA with the mercury electrode mostly at acid pH (at pH of about 5.6). They interpreted their results as a surface denaturation of native DNA, which hit the major part of the DNA molecule and was not so markedly influenced by the electrode potential as at neutral pH [4,18,19]. In their recent paper [4] Malfoy et al. also suggest that the general course of interfacial events should be largely independent of the specific chemical nature of the respective charged interface, as far as it depends only on common physical interfacial parameters.

The results of the present study show that no pronounced surface denaturation of DNA takes place on the interaction of native DNA with the surface of graphite electrodes charged to potentials 0–1.4 V. The following three facts give evidence in favour of this conclusion: (1) Acid denaturation of DNA in the bulk of solution can be monitored by means of DPV at graphite electrodes (fig. 6); melting pH (pH_m) thus obtained was identical with that determined by means of spectrophotometric measurements. (2) The result of the experiment in which changes in DNA conformation due to its interaction with the LPGE in the presence of formaldehyde were followed was negative. The sensitivity of the determination of the conformational changes of DNA upon its interaction with the electrodes using formaldehyde (in the arrangement described in the experimental part of the present paper) can be estimated as units of per cent of nucleotide pairs in DNA molecules; this estimation follows from our pevious paper [3], in which the same procedure was used for investigating the interaction of DNA with a mercury electrode. (3) The different course of ionic strength dependences of the heights of peaks G and A of both forms of DNA (fig. 2).

The growth of peaks G and A of native DNA with decreasing ionic strength (fig. 2) could be connected with the fact that sugar-phosphate residues located at the surface of native DNA molecule play an important role in the adsorption of native DNA on the surface of the graphite electrode. In this type of native DNA adsorption attractive electrostatical forces between negative charges of DNA phosphate groups and positive charges of the graphite electrode surface might assert themselves more expressively. The increasing of ionic strength could cause neutralization of phosphate groups through screening of their charges by cations of the background electrolyte and thus decrease of the adsorbability of native DNA at the graphite electrode. The number of segments of native DNA occurring in direct contact with the electrode would thus be decreased. In the case of denatured DNA also residues of the more hydrophobic bases released from the inside of the DNA double helix by denaturation can freely react with the environment, and thus also with the electrode. At neutral pH the bases possess the character of electroneutral substances and also a more hydrophobic character than the sugar-phosphate residues [20]. The independence of the heights of peaks G and A of denatured DNA of ionic strength (fig. 2) can thus be explained by the fact that denatured DNA is adsorbed at the surface of graphite electrodes as an electroneutral substance, predominantly by means of the bases.

The oxidizability of native DNA is markedly decreased as compared with denatured DNA (figs. 1a, 1b); nevertheless it is not suppressed completely. The presence of peaks A and G on voltammograms of native DNA (fig. 1b) cannot be explained as being due to the presence of a small amount of denatured DNA in the sample of native DNA; the amount of denatured DNA in our sample of native DNA was so small (<1%) that it could not yield any oxidation current at the PGE under the differential pulse-voltammetric conditions [7]. The lower peak currents of

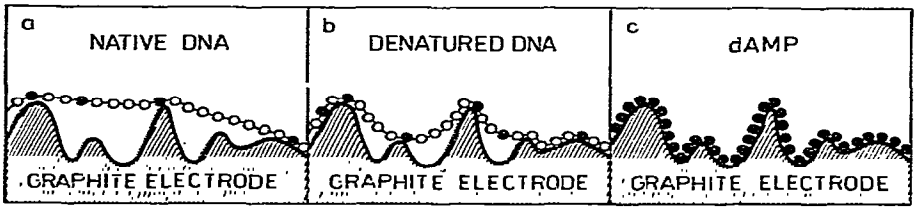

Fig. 8. Schematic representation of the adsorption of DNA and dAMP at the surface of graphite electrodes. (a) rigid native DNA; (b) flexible denatured DNA; (c) dAMP. Full circles represent dAMP, open circles other nucleotides present in DNA.

native DNA (fig. 1) could be also connected with a slower transport of molecules of native DNA to the electrode [2]. The voltammetric measurements with native and denatured DNAs were, however, carried out under conditions when the coverage of the electrode surface with DNA molecules was close to the maximum (fig. 3a and ref. [7]), so that the influence of a difference in diffusion coefficients of the two forms of DNA on the peak currents was practically negligible. Moreover, native DNA is not homogeneous conformationally [6], so that the bases present in labile double-helical (ds) regions of DNA molecule could be responsible for the appearance of oxidation peaks A and G of native DNA. However, not even this possibility seems probable. As will be demonstrated in our following communication [21], increasing of the number of labile regions in ds DNA (e.g. by introducing single-strand breaks into a molecule of ds DNA or by increasing the number of bases damaged by γ-radiation) does not lead to significant changes in the heights of peaks A and G of ds DNA.

It follows from the results presented in this paper that the increase of voltammetric peaks A and G due to thermal denaturation of DNA (figs. 1a, 1b) depends on the roughness of the graphite electrode surface. The greater the roughness of the electrode surface, the more pronounced the increase. An explanation of this effect may be found in the probable different adsorption behaviour of native and denatured DNAs at graphite electrodes. Thus it follows from the results presented here (figs. 1a, 1b) as well as from those presented in our last communication [7] that both native and denatured DNA are adsorbed at graphite electrodes over a broad range of potentials including the potentials at which electrochemical oxidation occurs. It seems reasonable that the flexible polymeric chain of denatured DNA could better conform to or copy the uneven surface of a graphite electrode than could the more rigid molecule of double-helical DNA. The number of segments of DNA in direct contact with or in very close proximity to the electrode and which may be electrooxidized must, therefore, be higher in the case of denatured DNA than in the case of double-helical DNA. This idea is illustrated schematically in figs. 8a, 8b. Thus the increase of oxidation currents accompanying the denaturation of DNA (figs. 1a, 1b) appears to be largely connected with an increase of concentration of guanine and adenine residues in close proximity to the electrode surface. We explained in an analogous way the increase of oxidation peak of polyadenylic acid caused by its transition from the double-stranded rigid form to the flexible single-stranded form [8].

As is mentioned in the introduction the electrochemical oxidation of DNA at a glassy carbon electrode has quite recently been studied by Yao et al. [9]. Using conventional linear sweep voltammetry they obtained a curve which was qualitatively identical with curve 1 in fig. 1 of the present paper. Yao et al. used, however, a commercial sample of DNA isolated from herring sperm, which had not been further characterized, in particular concerning the content of denatured DNA. They assumed for their DNA sample a molecular weight of 1×10^6, i.e. roughly twenty times lower than was the molecular weight of our sample of DNA [2]. It means that their DNA sample was apparently strongly degraded and thus could contain denatured DNA of relatively low molecular weight. Flexible short molecules of single stranded DNA might cover the graphite electrode surface much more completely than e.g. thermally denatured DNA of molecular weight of approximately 10^7 used in

our studies. The DNA sample of Yao et al. could thus yield a significantly higher oxidation current measurable even by conventional voltammetry.

The decreased adsorbability at uneven and porous surfaces (as compared with smooth ones) is a known property of polymers [22]. This effect is due to difficult penetration of polymers into pores and grooves in the adsorbent surface. It is apparent that the more rigid the polymer, the lower the rate of penetration. Upon adsorption of both rigid native DNA and denatured DNA (the flexibility of which is not infinitely high) on the surface of graphite electrodes the whole surface of even a moderately rough electrode will therefore not be completely covered. Thus, the coverage of the electrode surface by denatured DNA must be somewhat less than that which could be accomplished by, e.g., monomeric dAMP molecules, which could penetrate narrow pores and grooves in the surface more readily than molecules of the same dimensions bound in a polymer. This idea is also illustrated schematically in figs. 8b, 8c. It is for this reason that the voltammetric oxidation peaks of dAMP (fig. 1c) and dGMP [7] are so much higher than the peaks A and G of native and denatured DNAs at the same concentration (related to the monomer content) (fig. 1 and ref. [7]).

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