On the adsorption and electrochemical oxidation of DNA at glassy carbon electrodes *

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Abstract

New experimental results concerning the adsorption and anodic oxidation of DNA denatured in acid at glassy carbon electrodes using differential pulse, square wave and cyclic voltammetry, and impedance have been obtained, in the pH range 0–12 and as a function of adsorption potential and time of adsorption. The assumption up until now of a completely irreversible oxidation of guanine and adenine residues is shown not to be true. An explanation for these and previous results, based on desorption of the oxidation products and their diffusion away from the electrode surface, physically impeded for multilayer adsorption of DNA residues, is proposed. This situation corresponds to long adsorption times, as is the case here, and is corroborated by scanning electron microscopy.

1. Introduction

There has been much interest in recent years in studying the electrochemistry of nucleic acids and their adsorption at different types of electrodes. These studies have been particularly directed towards DNA and its components and are summarized in reviews, e.g. ref. 1. Included in these studies, the reduction of DNA at mercury electrodes, and interaction between mercury electrodes and native or denatured DNA-containing solutions has been the object of investigation. The electrochemical oxidation of DNA at glassy carbon (GC) [2] or graphite [3–5] electrodes using the same techniques was done and the groups undergoing oxidation were identified as guanine and adenine residues. However, potentials at which this oxidation occurred were in disagreement: this was ascribed to the degradation of the DNA samples that were employed. Further differences in the signals obtained at carbon electrodes were observed according to whether or not the DNA was denatured [6,7].

Recently the electrochemical reduction of DNA has been re-examined with a view to more fundamental studies of the adsorption phenomenon [8–10] and the possibility of using adsorption to preconcentrate DNA on a mercury electrode surface, thus increasing sensitivity and enabling lower detection limits [11]. This technique has been referred to as adsorptive transfer stripping cyclic voltammetry, by analogy to the more common adsorptive stripping voltammetry. No similar study of oxidation has been undertaken to our knowledge.

In this paper, the electrochemical oxidation of DNA denatured at room temperature in acid solution has been investigated at GC electrodes, with a view to elucidating further the processes occurring and to answering some of the questions posed in previous work such as the irreversibility of adsorption and of the oxidation of guanine and adenine residues, the potentials at which oxidation occurs, and so forth. Thus, previous deductions concerning the adsorption and oxidation of DNA on the electrode surface are examined. Techniques employed include cyclic, differential pulse (DP) and square wave (SW) voltammetry, electrochemical impedance and scanning electron microscopy. Previous investigations of DNA oxidation employed only DP and cyclic voltammetry.

The importance and usefulness of pulse and a.c. techniques in the elucidation of electrode processes has been admirably summarised in reviews by Sluyters-Rehbach and Sluyters (12,13], with applica-

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tions to more complex cases involving reactant adsorption, e.g. ref. 14, showing how these techniques can be used to clarify the mechanistic pathways of biological compounds.

2. Experimental

Calf thymus DNA (sodium salt, type I) was obtained from Sigma Chemical Co. and was used without further purification. Buffer solutions of ionic strength 0.2 were used in all experiments in the pH range 0–12, and were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system; they were prepared from hydrochloric acid, acetate, phosphate, borax, and hydroxide in their normal pH ranges. DNA (~ 3 mg) was weighed and either dissolved directly in 10 cm^3 of the buffer or denatured as described below, being freshly made on each day of experimentation. The pH of each solution was measured directly before its use. Unless specified, the experimental results were obtained with denatured DNA.

Denaturation of DNA was done by the following procedure. An accurately weighed sample of approximately 3 mg of DNA was treated with 0.5 cm^3 of pure perchloric acid and stirred to dissolve for 10 min. 0.5 cm^3 of 9 M NaOH was then added to neutralize the solution followed by ~ 9 cm^3 of the appropriate buffer, such that the final volume was 10 cm^3.

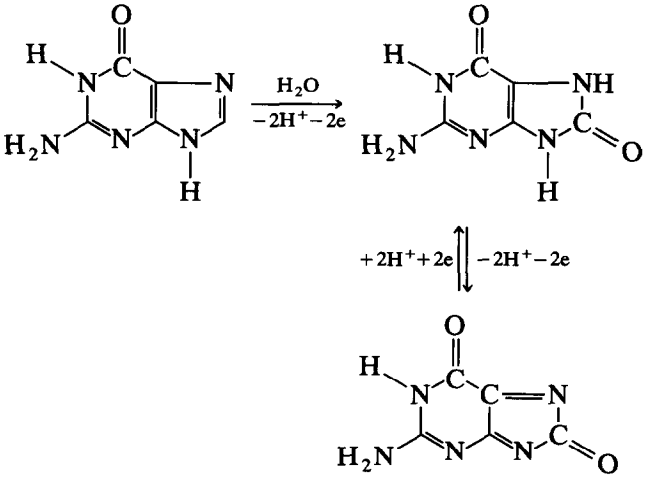
A one-compartment cell contained a (GC) electrode (Tokai, GC-20, area 0.07 cm^2) a Pt gauze counterelectrode and a saturated calomel electrode (SCE) reference electrode. The working electrode was polished with diamond paste down to 1 μm particle size on a polishing table before use and thoroughly cleaned; it was repolished as necessary, and always between adsorption experiments.

Voltammograms were recorded using either a PAR 174 polarographic analyzer or a PC-driven PAR 273A potentiostat/galvanostat running with model 270 electrochemical analysis software. Impedance measurements were made with a Solartron 1250 frequency response analyser coupled to a Solartron 1286 electrochemical interface. The potential range studied was from 0 to +1.3 V vs. SCE.

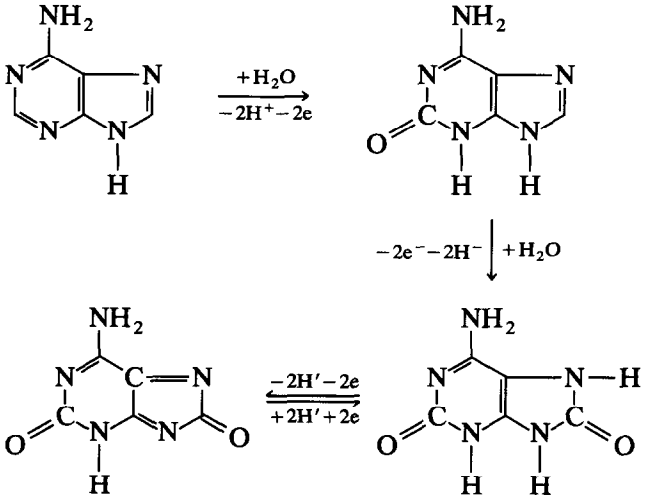
Scanning electron microscopy was done with a Jeol T330 scanning electron microscope on DNA-adsorption-coated tin dioxide electrodes which were prepared by DNA adsorption following the same procedures as for GC electrodes, and then sputter coated with a thin gold layer.

3. Results and discussion

A variety of experimental conditions were employed in this work, particularly regarding the adsorption of DNA on the GC electrode. In general, experiments were done with acid-denatured DNA, although some comparisons were made with native DNA (see below). Thus, for each pH value, different DNA adsorption times were tested as were different applied potentials for adsorption in the range from 0 to +1.3 V vs. SCE. Many of the results are qualitatively similar and the peak currents, but not peak positions, are quantitatively similar, being almost pH independent. Thus, the voltammograms presented in the figures are not confined to only one pH value. The oxidation of guanine and adenine themselves has been shown to follow a two-step mechanism involving the total loss of four electrons and four protons [1]. For guanine the mechanism is



and for adenine



In both cases, the first $2e^-$ oxidation is rate determining.

Questions unanswered in previous work, and to which we seek to respond, have to do with the irreversibility of DNA adsorption and the irreversibility of oxidation of adsorbed guanine and adenine residues.

These points are illustrated by the family of DP voltammograms at pH 5.3 in Fig. 1. These were obtained by first holding the electrode at 0.2 V vs. SCE for 15 min in a denatured DNA solution, before initiating the DP scans. As can be seen, with each scan the current becomes progressively lower, and the peak position becomes slightly more positive.

In Fig. 2 are shown forward and reverse DP scans in pH 4.5 acetate buffer. The forward scans are shifted from their positions in Fig. 1 owing to the decrease in pH. There is a small displacement of the peak positions between the forward and reverse scans (~30 mV), and the currents obtained in the negative-going direction are much smaller. These reverse scans show evidence of residual guanine and adenine residue oxidation. Additionally, immediately restarting a DP scan from 0 V in the positive direction, gives large peaks once more, similar to Fig. 1.

The deduction that can be made from results such as those in Figs. 1 and 2 is that fresh guanine and adenine bases can reach the electrode surface, suggesting movement of the partly adsorbed DNA strands over the surface. In addition to these two peaks, there is a small peak at +0.5 V on the forward scan. Although this peak always appeared in our experiments, sometimes hardly distinguishable from the background, it was always larger when the adsorption potential was very positive, around +1.3 V. The peak position suggests strongly that it is due to free guanine base as described in ref. 15, and its appearance has important implications for understanding the processes occurring, as will be discussed.

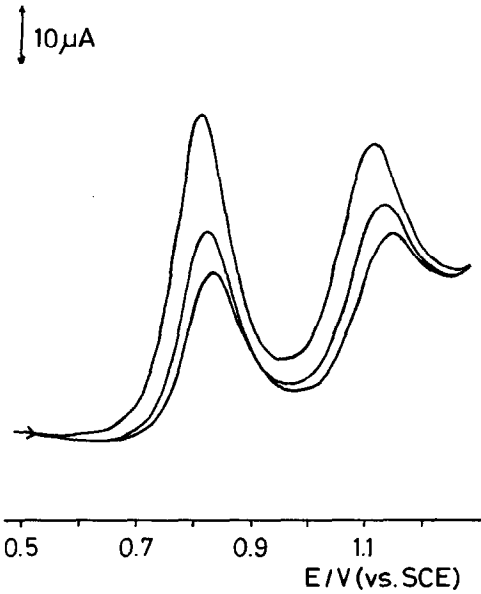


Fig. 1. Successive DP scans in pH 5.3 acetate buffer after 15 min adsorption of DNA at +0.2 V vs. SCE ([DNA] = 0.32 mg cm^{-3}). Pulse amplitude 25 mV, pulse width 50 ms, scan rate 5 mV s^{-1}.

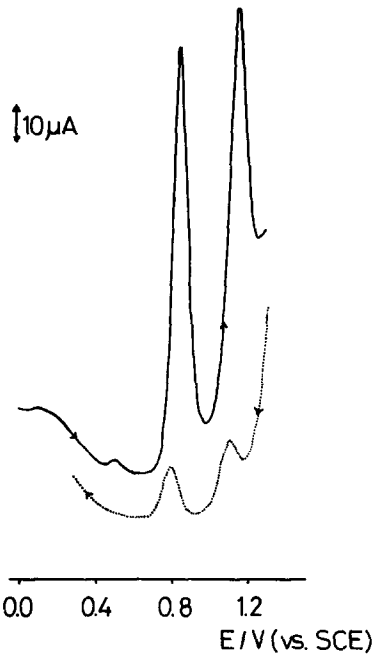


Fig. 2. Forward and reverse DP scans in pH 4.5 acetate buffer after 15 min adsorption at +1.3 V vs. SCE ([DNA] = 0.34 mg cm^{-3}). Other conditions as Fig. 1.

The use of SW voltammetry as an attractive alternative to DP voltammetry [16] was investigated: the faster scan should bring advantages in minimizing alterations in the state of adsorption of the DNA on the electrode, whilst also minimizing capacitive contributions. SW voltammograms were recorded in both positive and negative directions, such as the example of Fig. 3. This shows the peaks at a "high" effective scan rate where it was not possible to do DP or linear sweep (see below) voltammetry. Peak positions are almost exactly those obtained in DP scans; note also the small peak at 0.5 V appears again on the forward scan.

Considering the two main peaks, we find that the pH dependence of the peak potential from DP (or SW) voltammetry obeys the relations $E_p = 1.15-0.062$ pH (guanine residues) and $E_p = 1.42-0.067$ pH (adenine residues) which is in accordance with a ratio of one proton to one electron in the oxidation mechanism, as in the oxidation schemes for guanine and adenine previously proposed and shown above. The plots of E_p vs. pH are in Fig. 4.

Cyclic voltammetry shows clear evidence that the electrode kinetics are not completely irreversible. A typical cyclic voltammogram obtained at 10 mV s^{-1} scan rate is shown in Fig. 5: fast scan rates resulted in

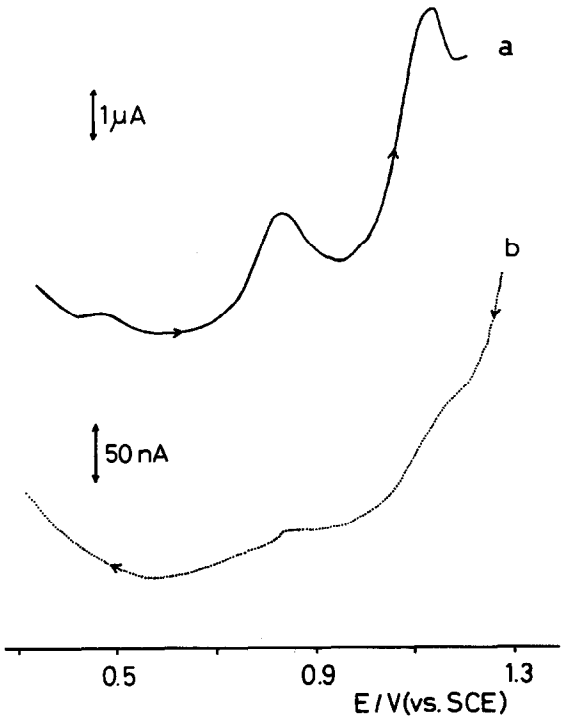


Fig. 3. Oxidation and reduction SW voltammograms in pH 5.5 acetate buffer; $f = 25$ Hz, scan increment 2 mV, amplitude 25 mV, adsorption at +0.6 V vs. SCE ([DNA] = 0.30 mg cm^{-3}). Scan a, from 0.0 V in positive direction, after 300 s adsorption. Scan b, from 1.3 V in negative direction, after 1800 s adsorption (cathodic difference current shown upwards).

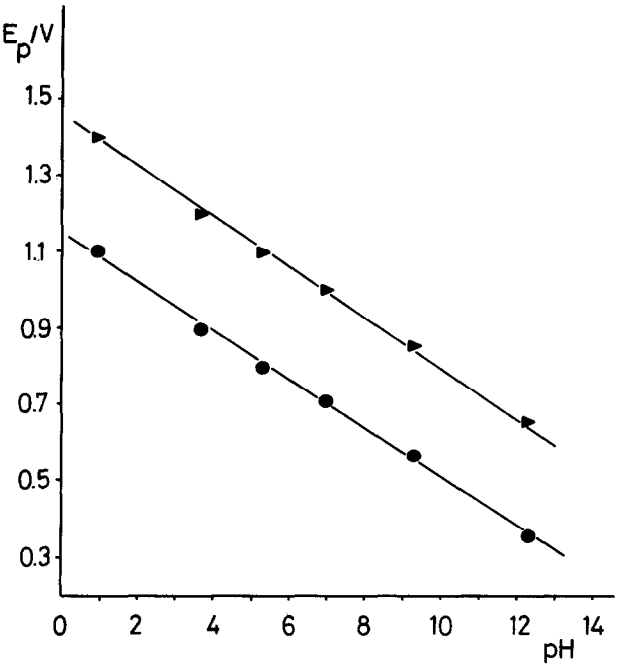


Fig. 4. Plot of E_p vs. pH for the DP peaks corresponding to oxidation of guanine (●) and adenine (▲) residues in the DNA chain.

no peak being registered. It was on the basis of cyclic voltammograms without cathodic peaks that previous workers [2,4] concluded that the oxidation was irreversible. However, in our experiments, small peaks can be found on the negative scan, but to obtain this it was necessary to adsorb DNA on the electrode for at least 10 min to accumulate DNA: previous work appears not to have included this fixed potential adsorption step. The midpoints between the corresponding anodic and cathodic peaks of 0.84 and 1.15 V correlate well with the potentials obtained in DP voltammetry. As in Fig. 2, a small extra peak is visible, especially on the reverse scan at around 0.3 V. The values of potential at half-peak height of 0.85 V and 1.17 V respectively are in fairly good agreement with those obtained by Yao et al. [2] and Kenley et al. [17], and in disagreement with Brabec [3].

The inset of Fig. 5 shows the background-subtracted voltammogram, from which it is clear that the peak shape, particularly obvious for guanine residue oxidation, does not conform to that expected for adsorbed species (see for example ref. 18). This implies that, although the DNA is adsorbed, the guanine and ade-

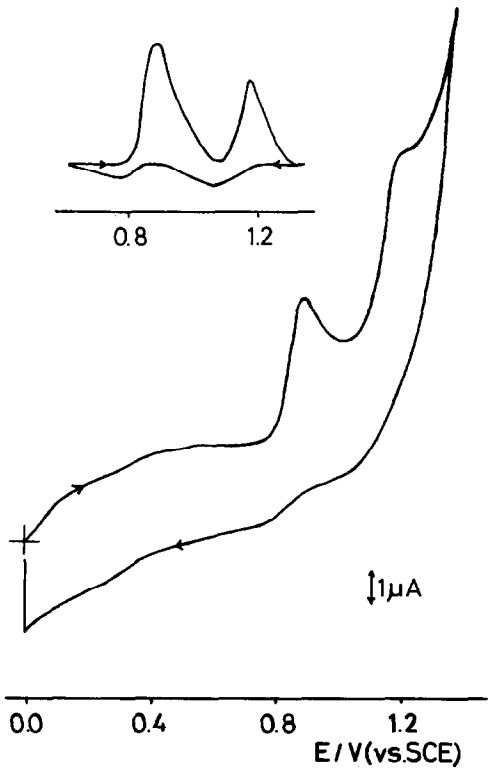


Fig. 5. Cyclic voltammogram recorded in pH 4.5 acetate buffer after 15 min adsorption at +1.3 V vs. SCE ([DNA] = 0.34 mg cm^{-3}). $v = 10$ mV s^{-1}. Inset shows background-subtracted cyclic voltammogram.

nine groups themselves have some freedom of movement, as suggested above in the DP measurements. Peak separation is approximately 100 mV for both peak pairs. Assuming a quasi-reversible system for these groups, we can estimate a rate constant using the expression [19]

$$\Psi = k_0 D^{-1/2} (vnF/RT)^{-1/2} \pi^{-1/2}$$

Putting $n = 2$ (see schemes above), estimating $D = 5 \times 10^{-6}$ cm^2 s^{-1}, then, assuming $\alpha = 0.5$, we find that $\Psi = 0.1$ for this peak separation, and obtain a standard rate constant k_0 of $\sim 4 \times 10^{-4}$ cm s^{-1}. The peak half-widths $W_{1/2}$ of the differential pulse peaks, for example Fig. 1, are > 100 mV; using the expression $W_{1/2} = 3.3RT/\alpha nF$ [20] for a standard rate constant in the range estimated by us from cyclic voltammetry leads to the deduction that $\alpha \sim 0.4$. This value is reasonable, as we would expect the activated complex to be closer to reagent than product. This smaller value of α would affect the rate constant estimated above from cyclic voltammogram data by 10% or so, but the assumptions made in the estimation are such that this is of no great consequence: what we wished to calculate is the range in which the rate constants lie.

Impedance spectra of GC electrodes, both bare and covered with DNA, were recorded, from which it was clear that the spectra of the covered electrodes were dominated by the DNA-adsorbed layer. The appearance of the spectra was capacitive at all potentials, typical of an adsorbed film, with evidence of some surface roughness (roughness factor of ~ 0.9 [21]): an example is given in Fig. 6. Over the potential range studied, there was little variation in the qualitative features of the spectra obtained, except in zones corresponding to electrochemical oxidation, where a small high frequency semicircle was seen, probably corresponding to charge transfer under the adsorbed layer. The calculated capacities between 30 and 50 μF cm^{-2} did not vary in a meaningful way within this range of values with pH or with applied potential, which can probably be ascribed to the rather irregular adsorption of the DNA itself.

The points which require discussion can be summarized as follows.

(i) Adsorption of DNA occurs throughout the potential range studied, but appears to be more effective when carried out at high applied potentials. The "extra" peak at less positive potentials, not found previously, appears more accentuated after adsorption at +1.3 V.

(ii) Successive DP or SW scans result in a progressive diminution of current peaks; peaks are obtained on the reverse scans though of much lesser height.

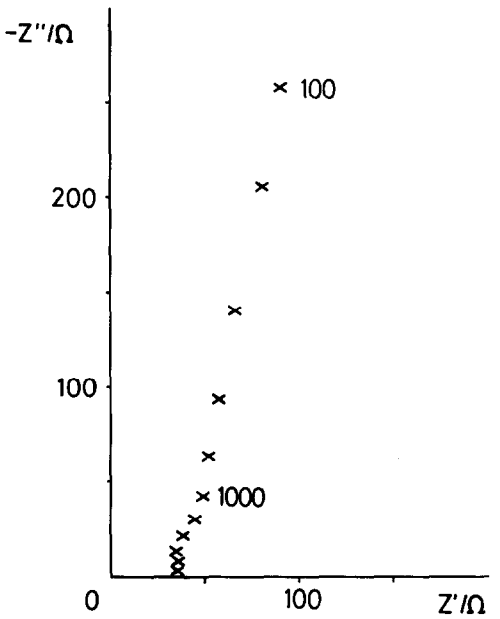


Fig. 6. Complex plane impedance spectrum at +1.1 V vs. SCE in pH 9.3 borax buffer after adsorption at +1.1 V vs. SCE during 20 min ([DNA] = 0.32 mg cm^{-3}). Frequencies in hertz.

(iii) Cathodic peaks are obtained in cyclic voltammetry, showing that the electrochemical oxidation is not completely irreversible.

(iv) The mode of adsorption of DNA, and to what extent it is degraded by acid denaturation.

The understanding of the first of these points may be aided by consideration of the cyclic voltammetry of guanine [15]: a pair of anodic–cathodic peaks appeared at around +0.4 V, but only after the first oxidation scan. Thus, we can attribute the "extra" peak to products of guanine oxidation, although we note that a tiny peak appears even after adsorption at 0.0 V: this could be due to the result of the acid denaturation of the DNA, and may be caused by the mode of adsorption of the DNA residues.

The irreversibility of the oxidation has already been questioned and will be further mentioned below. With regard to adsorption irreversibility, the DP scans of Fig. 7, done in DNA-containing and in buffer only solution, show that the loss of signal is relatively similar after an initial reduction in height. Figure 8 shows that denatured DNA leads to higher currents for guanine and adenine residue oxidation than does native DNA. This is in agreement with the suggestion of Brabec and Koudelka [4] that the breaking of the DNA strand into shorter chains allows them to be adsorbed following the contours of the electrode surface, increasing the contact area. Our results suggest that adsorption is of degraded rather than unravelled DNA. This point has been discussed previously [11] and may depend on the

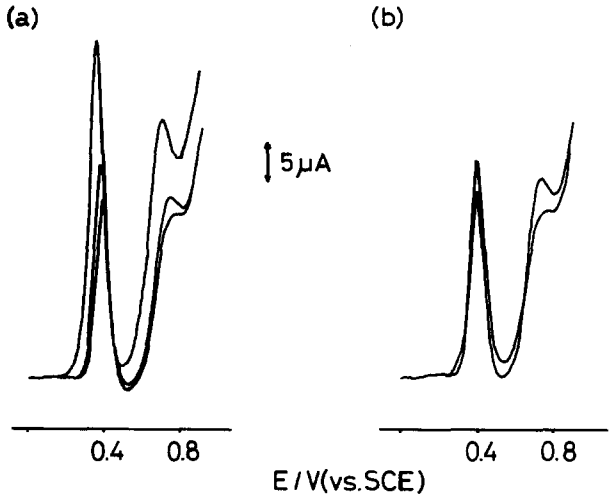


Fig. 7. Successive DP scans in pH 12.3 sodium hydroxide + potassium chloride solution after 6 min adsorption at +1.1 V vs. SCE ([DNA] = 0.36 mg cm^{-3}); other conditions as in Fig. 1. (a) In DNA-containing solution. (b) After transfer to buffer solution not containing DNA.

type of denaturation: acid or thermal denaturation could lead to different results. It is generally accepted that thermal denaturation tends to lead to an unravelling of the double helix: such long DNA strands would make relatively little contact with the electrode surface, as they would be unable to follow the contours of the rough (on a molecular and microscopic, i.e. impedance results) scale, whereas residues would be able to. It would thus appear that the latter of these is the more likely, as would be predicted from the rather drastic nature of acid denaturation.

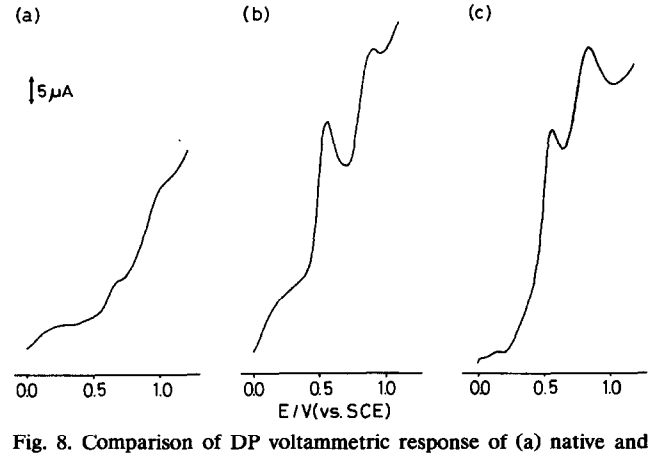


Fig. 8. Comparison of DP voltammetric response of (a) native and (b), (c) denaturated DNA in pH 9.3 borax buffer, after 15 min adsorption at +0.2 V vs. SCE ([DNA] = 0.32 mg cm^{-3}); other conditions as in Fig. 1. In (c), scan recorded, after adsorption, in buffer solution not containing DNA.

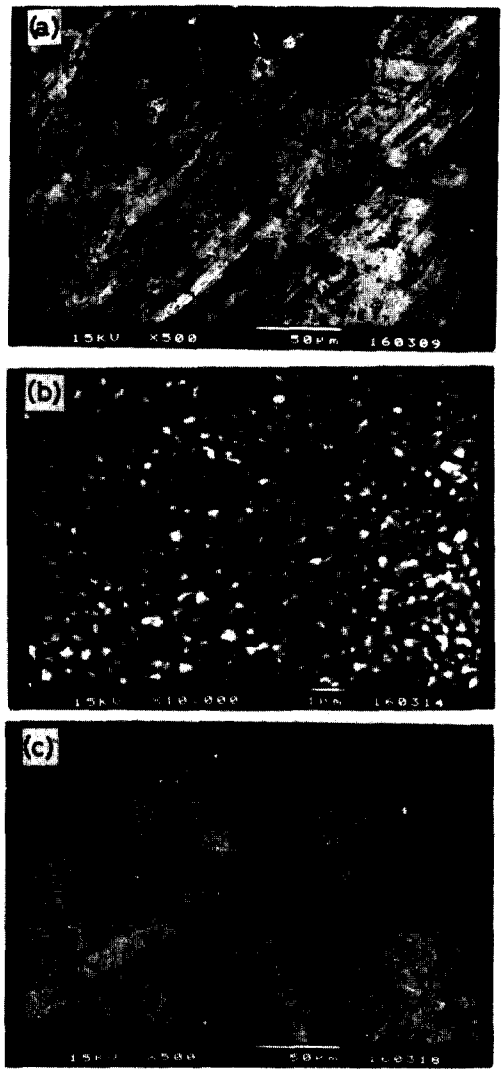


Fig. 9. Scanning electron micrographs of DNA adsorbed on a tin dioxide electrode at +0.2 V vs. SCE ([DNA] = 0.34 mg cm^{-3}) for 15 min in (a) pH 5.5 acetate buffer, (b) pH 9.3 borax buffer and (c) pH 12.3 sodium hydroxide + potassium chloride solution.

If the adsorption of DNA is multilayer, then a fairly compact coating may be formed. Indications that this is the case are given in the scanning electron micrographs of adsorption on tin dioxide electrodes in Fig. 9. At all pH values strong adsorption was seen, although the morphology varied from a strand-like appearance at low and high pH values (Figs. 9(a) and 9(c)) to a thicker coating with a completely uniform droplet-like surface morphology at intermediate pH 7–9 (Fig. 9(b)). It is interesting to compare Fig. 9(b) with scanning tunnelling microscopy results for DNA adsorption on pyrolytic graphite in phosphate buffer [22]: the texture is very similar.

It is clear that the original idea of completely irreversible electrochemical oxidation has been shown to

be incorrect, at least under our experimental conditions. Previous work did not use fixed potential adsorption before the voltammetric scans, which this work suggests leads to multilayer adsorption of DNA residues. Thus, if the oxidation of guanine and adenine residues results in products that can be desorbed but cannot easily escape from the surface, remaining partially trapped beneath an assembly of DNA strand residues, some only partly adsorbed (see Fig. 9), then reduction peaks can appear and successive potential scans will result in lower currents. This is what was observed. Finally, it turns out that the currents obtained after adsorption at +1.3 V are greater than after adsorption at lower potentials, which suggests that the attraction of the electrode to DNA adsorbate is so great that it outweighs any diffusion away of the products of the oxidation, which will occur at all pHs at such potentials.

4. Conclusions

We have shown, by using several voltammetric techniques, that the oxidation of denatured DNA adsorbed on GC electrodes is not a simple irreversible oxidation as previously suggested. The reason for the lack of appearance of reverse peaks in previous work may be due to insufficient adsorption time which, according to the explanation advanced by us, would allow desorbed products to diffuse away from the electrode surface, whereas after multilayer adsorption this would be physically impeded. We have also shown the advantages of using fast voltammetric techniques, such as SW voltammetry, in probing this type of electrode process, which opens up new perspectives in investigating this type of electrochemical problem.

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