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The Electrochemical Oxidation of DNA

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O mecanismo da eletroxidação de ADN nativo ou desnaturado foi estudado usando eletrodos de trabalho de carbono vítreo e de dióxido de estanho em soluções de eletrólitos de suporte tamponizadas e para diferentes valores de pH. As técnicas electroquímicas empreguadas foram a voltametria cíclica e de impulso diferencial. Foram observadas diferenças significativas em relação ao ADN nativo e desnaturado devido à rigidez da estrutura de dupla hélice que impede a transferência de elétrons das bases guanina e adenina dentro da dupla hélice no primeiro caso. Os picos são maiores no ADN desnaturado do que no ADN nativo, pois a estrutura simples de ADN adsorve na superfície do elterodo. A oxidação da guanina é mais fácil e ocorre para potenciais mais baixos do que a oxidação da adenina. A oxidação da guanosina e adenosina foi usada de modo a comparar os resultados e auxiliar na clarificação do mecanismo de oxidação do ADN.

The electrooxidation mechanism of native and denatured DNA was studied using glassy carbon or tin oxide working electrodes in buffered supporting electrolytes at different pHs. The electrochemical techniques employed were cyclic and differential pulse voltammetry. Significant differences were observed for native and denatured DNA due to the rigidity of the double-stranded structure which impedes the electron transfer from the bases guanine and adenine inside the helix in the former case. For denatured DNA the voltammetric peaks are bigger owing to the adsorption of the single-stranded DNA on the electrode surface. The oxidation of guanine is easier and occurs at lower potentials than the oxidation of adenine. The oxidation of guanosine and adenosine was investigated in order to compare the results and to help clarify the oxidation mechanism of DNA.

Keywords: *DNA, electrochemical oxidation, guanosine, adenosine*

Introduction

Deoxyribonucleic acid (DNA) is the molecule of heredity because it contains and transmits genetic information. This molecule has a tridimensional double-helix structure constituted by two chains, running in opposite directions, of polydeoxyribonucleotides which are coiled around a common axis. The structure of the chain is formed by phosphate groups and pentoses alternately linked by phosphodiester bridges (Fig. 1) the chains of the double helix being held together by hydrogen bonds between the purine bases, adenine and guanine, and pyrimidine bases, cytosine and thymine. These bases, adenine and thymine and guanine and cytosine, through their sequencing and pairing carry all genetic information, sugar and phosphate being the structural backbone. Although the two chains in DNA are complementary, they are not identical and are anti-parallel. However, the purine and pyrimidine bases are on the inside of the helix and the planes of the bases are perpendicular to the axis of the helix. The diameter of the helix is 20 Å, and adjacent bases are separated by 3.4 Å along the axis and related by a rotation of 36 degrees.

The first electrochemical studies of DNA and its derivatives are not recent[1]. These studies investigated the oxidation or reduction of native or denatured DNA using glassy carbon[2], graphite[3] or mercury[4,5] electrodes, the electrochemical reacting groups being adenine and guanine. Significant differences were observed due to the adsorption of

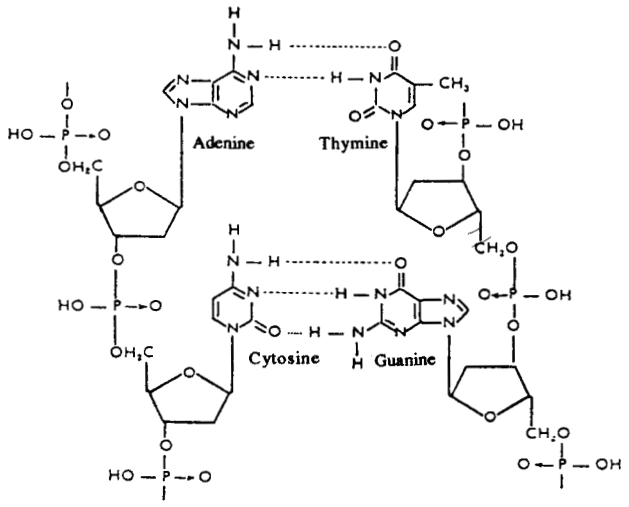

Figure 1. Hydrogen bonds between complementary bases in a fragment of a DNA molecule.

DNA onto the electrode surface. The adsorption of native DNA is weaker due to the rigidity of the double helix which makes the transfer of electrons from the bases in the interior of the DNA to the electrode surface more difficult. The use of denatured DNA facilitates the electron transfer flow, due to an unravelling of the double helix which becomes more like a ribbon, this way enabling it to follow the contours of the electrode more easily.

In this work the electrochemical oxidation of DNA, GMP and AMP was studied using different electrochemical techniques with the objective of understanding these complicated mechanisms.

Experimental

Calf thymus DNA (sodium salt, Type I), guanosine 5'-monophosphate (GMP, disodium sigma grade) and adenosine 5'-monophosphate (AMP, sodium 99%) from yeast were obtained from Sigma Chemical Co., and were used without further purification. The denaturation of DNA, GMP and AMP was done by the following procedure. An accurately weighed sample of approximately 3 mg of DNA, GMP or AMP was treated with 0.5 cm^3 of pure perchloric acid and stirred for 10 min until dissolved. Then 0.5 cm^3 of 9 M NaOH was added to neutralize the solution, followed by ~ 9 cm^3 of the appropriate buffer, such that the final volume was 10 cm^3. Buffer solutions of 0.2 ionic strength were always used and were prepared using analytical reagents and purified water from a Millipore Milli-Q system. The pH of each solution was measured immediately before use.

The working electrode was glassy carbon (Tokai GC-20, area 0.070 cm^2), the counter electrode was a Pt gauze, and the reference electrode was a SCE, which were used in a one-compartment cell. The voltammograms were recorded using a μAutolab potentiostat, Eco-Chemie, Netherlands. The potential range studied was from 0 V to +1.6 V *vs.* SCE.

Results and Discussion

The electrochemical oxidation of DNA involves a complex process which occurs at the specific groups, adenine and guanine, with different electron transfer reaction rates. In this study the electrochemical oxidation of native DNA (Fig. 2a) clearly showed the difficulty of the electron transfer because of the rigidity of the double helix which contains the purine groups folded inside the DNA structure and therefore distant from the electrode surface. It should be remembered that the thickness of the inner Helmholtz layer at an electrode is ~ 1 Å and compare that with the dimensions of the helix diameter. The peaks corresponding to the oxidation of guanine (E_p = +0.95 V) and adenine (E_p = +1.22 V) can only be seen if the native DNA is adsorbed electrostatically at 0.0 V for half an hour.

During the denaturation of DNA no covalent bonds are broken, and the double-helix structure only unwinds and separates. This means that the forces responsible for maintaining the double-helix, hydrogen bonds between pairs and the stacking interactions between successive bases, are no longer effective, leading to single-stranded or denatured DNA. There are two stages in the denaturation of the DNA helix. In the first stage the two strands are partially unwound, but remain united by at least a short segment of the double-helix structure, this being responsible for the reversibility of DNA denaturation. At this stage, by reversing the denaturation conditions DNA goes back to the native helix conformation. In the second stage, the two strands become completely separated from each other and renaturation is not possible.

If DNA is denatured, according to the procedure mentioned above, the electrochemical oxidation of the groups on an unfolded helix is much easier, as the DNA structure can become almost like a ribbon and is able to follow the contours of the electrode surface. As such, the oxidation peaks of this single strand DNA can be observed much

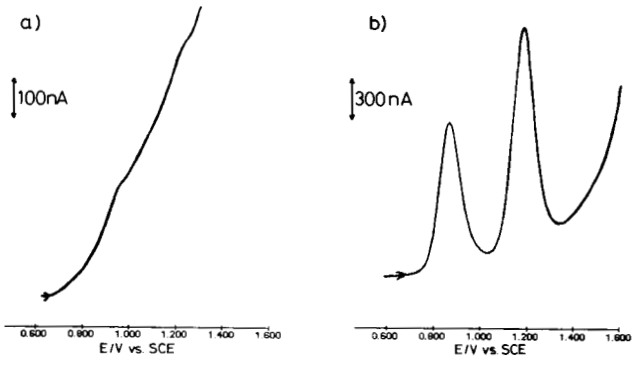

Figure 2. Differential pulse voltammetry of DNA, in acetate buffer pH = 4.5, at a glassy carbon electrode : a) native, after 30 minutes adsorption at +0.0V vs. SCE; b) denatured. [DNA] = 0.30 mg cm^{-3}. Pulse amplitude 25 mV, pulse width 50 ms, scan rate 5 mV s^{-1}.

more clearly (Fig. 2b); the first differential pulse voltammetric peak corresponds to the oxidation of guanine (E_p = +0.87 V), and the second to adenine (E_p = +1.16 V). The shift in the oxidation potentials can be explained by the proximity of the groups involved, due to the unfolding of the helix.

During our experiments it was possible to reverse the DNA denaturation, and the decrease of the peak currents as the renaturing process was taking place was observed. Cyclic voltammograms (Fig. 3) showed that the oxidation of the groups was not totally irreversible, and scanning electron microscopy of adsorbed DNA at tin dioxide electrodes, for pH 9.3, showed a very globular type of structure (Fig. 4).

A comparative study of the oxidation of the guanosine (GMP) and adenosine (AMP) ribonucleotides is a good approach to help explain this complicated mechanism. The nucleotides were evaluated using the same experimental conditions as those for DNA: first in a solution prepared in acetate buffer and secondly after using the conditions for denaturation of DNA. Differential pulse voltammograms for guanosine are shown in Fig. 5a, and for adenosine in Fig. 5b. It is interesting that both guanosine (E_p = +1.65 V) and adenosine (E_p = +1.33 V) are oxidized at more positive potentials than DNA, which means that the corresponding groups are more difficult to oxidize. Table 1 lists the potentials for the peaks observed for DNA, GMP and AMP, under all the conditions mentioned, for easy comparison.

When GMP and AMP are treated by the conditions used to denature DNA two peaks appear instead of one, the new one at a less positive potential value and with a very small current, corresponding to the free bases guanine and adenine[6-8], but not coincident with any peak of native or denatured DNA. Fig. 6a shows the differential pulse voltammograms obtained for native DNA, adenosine and guanosine, and Fig. 6b shows those obtained after the so-called denaturation treatment. From the differences between the values of the oxidation potentials one can confirm that the denaturation process only takes DNA to the first stage of denaturation, and that the observed peaks do not correspond to sections of DNA, but rather to a single strand formation.

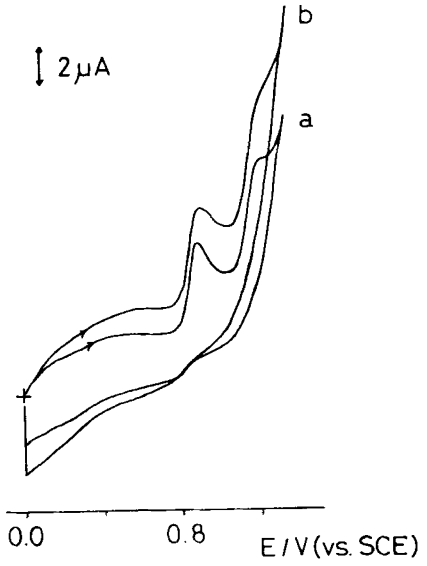

Figure 3. Cyclic voltammogram of DNA in acetate buffer, pH = 4.5, at a glassy carbon electrode after 15 min adsorption at +0.2 V *vs.* SCE: a) 10 mV/s; b) 20 mV/s. [DNA] = 0.30 mg cm^{-3}.

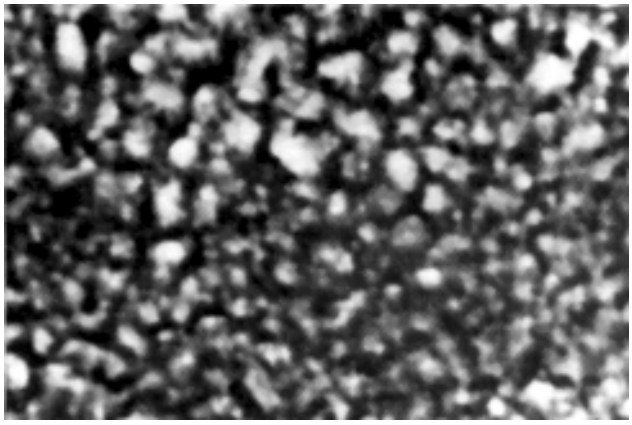

Figure 4. Scanning electron microscopy of denatured DNA in borax buffer, pH = 9.0, at a tin oxide electrode after 15 min adsorption at +0.2 V *vs.* SCE.

Table 1. Peak potentials for DNA, GMP and AMP.

	Ep /V *vs.* SCE	
DNA	0.95	1.22
'denatured' DNA	0.87	1.16
GMP		1.06
'denatured' GMP	0.76	1.06
AMP		1.29
'denatured' AMP	1.05	1.33

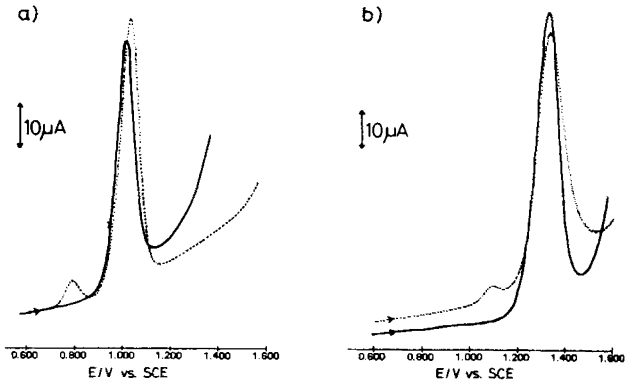

Figure 5. Differential pulse voltammetry in acetate buffer pH = 4.5, at a glassy carbon electrode of: a) (–) GMP and (···) 'Denatured' GMP; b) (–) AMP and (···) 'Denatured' AMP. [GMP] = [AMP] = 0.30 mg cm^{-3}. Pulse amplitude 25 mV, pulse width 50 ms, scan rate 5 mV s^{-1}.

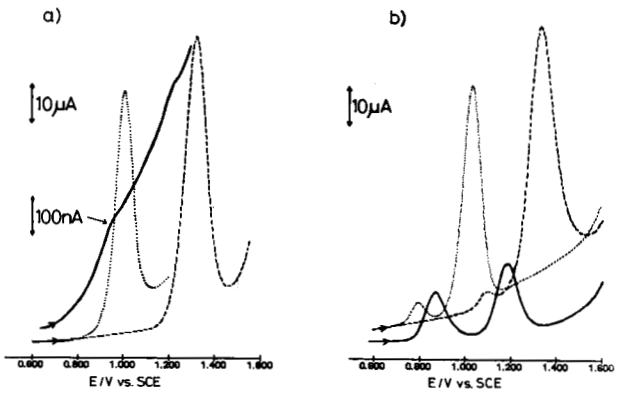

Figure 6. Differential pulse voltammetry in acetate buffer, pH = 4.5, at a glassy carbon electrode of: a) (-) Native DNA, (···) GMP and (---) AMP; b) (–) Denatured DNA, (···) 'Denatured' GMP and (---) 'Denatured' AMP. [DNA] = [GMP] = [AMP] = 0.30 mg cm^{-3}. Pulse amplitude 25 mV, pulse width 50 ms, scan rate 5 mV s^{-1}.

Conclusions

The observations concerning the mechanism of oxidation of DNA can be rationalized on the basis of surface adsorption and the distance between the electrode surface and the active groups. The results obtained show that, although the groups which undergo electrochemical oxidation are guanine and adenine, the mechanism for oxidation is greatly dependent on other groups in the DNA molecule, namely phosphate and pentoses, which in our view act as conducting bridges enabling the electron flow to reach the electrode or flow along a strand.

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