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The Electrochemical Behaviour of DNA at Electrically Charged Interfaces * **

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Abstract. The adsorption and the reduction of native and denatured DNA has been studied at the hanging mercury drop electrode (HMDE) by triangular sweep polarography. It has been established from the dependence of the reduction peak of denatured DNA (Fig. 2) on the waiting time at the starting potential E_s of the sweep (Fig. 3) where the adsorption takes place and on pH (Fig. 4) that a protonated form of denatured DNA is reduced from the adsorbed state in a totally irreversible electrode process at a pH lower than 7.5. From the logarithmic analysis of the beginning part of the reduction peak the charge transfer rate constant and the charge transfer coefficient were determined (Fig. 5).

The reduction products are interconnected to a high molecular network strongly adhering to the surface of the electrode and blocking it for a repeated reduction on the same drop. In neutral media the protonation of DNA becomes rate-determining. The adsorption is diffusion controlled and from the time dependence of the coverage the diffusion coefficient of DNA could be estimated (Fig. 6) to 5.10^{-7} cm^2 s^{-1} in 1 M KCl at 25 °C and at pH 6.7 for denatured DNA of the mean molecular weight 10^6 g. The protonated molecules of DNA are adsorbed via strong induced dipole interactions of the π-electron system in the heteroaromatic ring of the bases with electrons of the metal while the other parts of the DNA molecule including the solvated sugar and phosphate groups are orientated to the solution.

From the time integral of the reduction peak (Fig. 6) the maximal surface concentration of the electrochemically active monomeric units in DNA was estimated to $1.4 \cdot 10^{-10}$ mol cm^{-2} with an average area per mononucleotide of about 60 Å^2. Both values vary only within 10 % in the temperature range 5 to 25 °C, in the concentration range of DNA 10 to 360 µg/ml and for starting potentials E_s — 0.2 to — 1.3 V vs. SCE.

Native DNA is also adsorbed at the electrode and reduced in acid solutions in the same irreversible electrode reaction as denatured DNA. However, before a partial opening of the helix is induced by the strong electric field at the interface leading with respect to helix-coil transition to an effect equivalent to partial melting which has to precede the electron transfer step. The extent of unscreened reducible groups and their orientation with the electrochemically reactive sites towards the electrode depends on pH (Fig. 8), on electrical parameters of the interface as the starting potential E_s (Fig. 7) and on the portion of the DNA molecule which comes into the region where the electric field is acting. The maximal extents of unscreened reducible groups are 85 % for native DNA of the mean molecular weight of $2.5 \cdot 10^6$ g and 46 % for native DNA of the mean molecular weight 10^7 g (Fig. 6). The results have with respect to the physicochemical properties of DNA general significance also for the behaviour of nucleic acids at charged biological interfaces as for instance the membrane of living cells.

Key words: Native DNA — Denatured DNA — DNA Adsorption — DNA Reduction — Helix-Coil Transition.

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** Dedicated to Prof. Dr. L. Holleck on his 70th birthday.

The interface metal electrode/aqueous electrolyte is a well adjustable electrochemical system. Although from the biochemical point of view there are obviously significant differences between the membrane of a living cell and the surface of a metal electrode both interfaces bear close resemblances from the physico-chemical point of view. Biological surfaces as well as metal electrode surfaces are usually electrically charged and consequently an electric double layer is formed at the interface. The electric field acting in this double layer influences significantly all the interfacial phenomena as the adsorption of substances, conformational and chemical changes of the adsorbate, and the course and rate of chemical reactions at the interface and in its immediate environment as well as of the electrode reaction [6]. The properties and the structure of the double layer at a metal/electrolyte interface can be altered continously by the change of the applied potential. Thus a charged metal surface represents a valuable model for the study of general physico-chemical properties and the behaviour of biomolecules at charged biological surfaces and at the same time enables one to elucidate the electrode process of the studied biomolecules.

In the present paper the behaviour of native and denatured DNA at the mercury/aqueous electrolyte interface has been studied by advanced electrochemical techniques. At first essentials of the electrode process of the reduction of denatured DNA are discussed. Then the adsorption of native and denatured DNA at mercury is considered. Finally the helix-coil transition of native DNA induced by the electric field in the double layer is described. After this helix-coil-transition native DNA becomes reducible in the same electrode process which has been elucidated for denatured DNA. The details of the experimental techniques applied by us are described elsewhere [11 to 13].

Experimental

The studied calf thymus DNA supplied by Miles, USA, had a molecular weight of $2.5 \cdot 10^6$ and contained 6.58% P; proteins and RNA were present in traces only. Denaturation was performed by heating the DNA in 0.015 M NaCl with 0.0015 M sodium citrate (pH 7.00) for 10 min followed by rapid cooling in an ice bath. The constant ionic strength McIlvaine buffer served as supporting electrolyte. The DNA content in the solution was measured by its absorbance at 258 nm. All measurements have been performed in a thermostated cell with a three electrode system with the aid of a PAR polarographic analyzer model 170. The built-in potentiostat and the electronic integrating circuit made the potentiostatic recording of current-potential curves and charge-potential curves possible. The working electrode was the Metrohm hanging mercury drop electrode E 410 with a surface area of 3.50 mm^2. All potentials refer to the saturated calomel electrode. The solution was deoxygenated by passing a slow stream of pure nitrogen through it.

Results and Discussion

1. Reduction of Denatured DNA

The reducible groups in the DNA are cytosine and adenine [5]. The reduction is accomplished by the uptake of four electrons and four protons by each base and is accompanied by a release of an ammonia molecule. In native DNA however the two strands are joined by horizontal interactions between the base pairs (especially

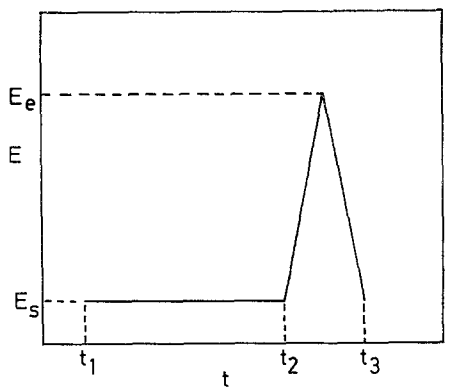

Fig. 1. Time dependence of the voltage sweep applied on the hanging mercury drop electrode (HMDE). Description in the text

hydrogen bonding) and by vertical interactions between stacked bases. The hydrogen bonding is located just at the reaction sites, i.e. the C(6)-N(1) double bond in adenine and the C(4)-N(3) double bond in cytosine so that native DNA with intact double helix structure is not reducible. On the other hand denatured DNA with its open monostranded structure can be reduced at the mercury electrodes as the reaction sites in adenine and cytosine are accessible for the electrode process [1, 7].

The reduction of denatured DNA has been studied with a stationary mercury electrode polarised by a triangular voltage sweep (Fig. 1) in the potentiostatic regime. The electrode is held at first at the constant potential E_s with respect to the reference electrode over the time interval t_1—t_2 (10 to 200 s). During this time the adsorption of the DNA at the given potential E_s takes place. Thereafter in the interval t_2—t_3 (1 to 10 s) a triangular voltage sweep is applied from the starting potential E_s to the end potential E_e and backwards to E_s. The electrode is polarised cathodically going from E_s to E_e and anodically going into the reverse direction.

The reduction current of denatured DNA at a stationary mercury electrode has the form of a peak (Fig. 2) which is followed by a response due to catalytic action of the reduction products on hydrogen ion reduction. If the direction of the potential change is reversed no anodic current (i.e. positive current) is flowing indicating a totally irreversible reduction. If one tries to record repeatedly the current-potential curve at the same mercury drop for the second and further scans only a residual current can be observed. It can be deduced thereof that the reduction products are interconnected to a high molecular network which is firmly bound to the surface of the electrode and blocks it completely. The other details of the electrode process can be found from the dependence of the reduction peak on the waiting time t_1—t_2 at the starting potential E_s, on pH, on the scan rate $v = dE/dt$ and from the logarithmic analysis of the current-potential curve. A saturation value of the surface concentration of DNA is attained (Fig. 3) after waiting sufficiently long at the starting potential E_s. At early stages of adsorption the reduction peak rises linearly with the bulk concentration of DNA. From the

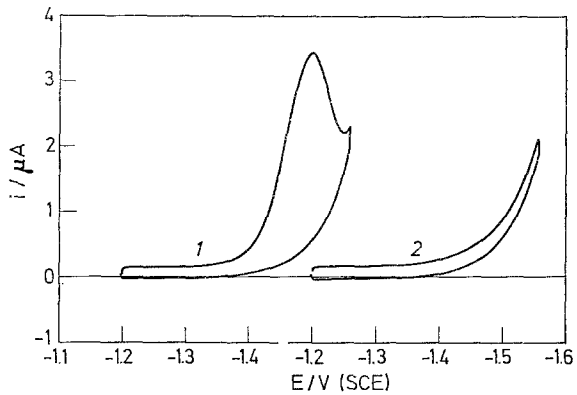

Fig. 2. Current-potential curve of denatured DNA on the HMDE. Britton-Robinson Buffer with 1 M ammonium formate, pH 6.79, 105 μg ml^{-1} denatured DNA, scan rate 100 mV s^{-1}, 15 s hold at −1.2 V, area of HMDE 3.50 mm^2. (1) First curve taken a fresh mercury surface, (2) second curve taken after 30 s hold at −1.2 V, 25 °C

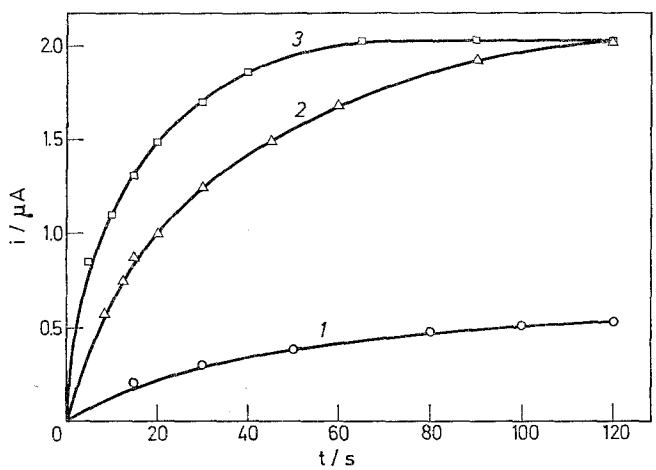

Fig. 3. Time dependence of the reduction peak of native DNA on the HMDE. 0.5 M McIlvaine buffer, pH 5.89, scan rate 100 mV s^{-1}, E_s −1.1 V, concentration of DNA: (1) 10.5 μg ml^{-1}, (2) 36.0 μg ml^{-1}, (3) 63.0 μg ml^{-1}

dependence of the reduction peak on pH (Fig. 4) it becomes evident that the reduction takes place only in acid and neutral media. The reduction peak rises linearly with scan rate in acid media and ceases to depend on scan rate in alkaline media. The conclusion is that a reduction of the protonised form of denatured DNA which is adsorbed at the electrode takes place. In neutral and alkaline solutions this protonation preceding the electron transfer step contributes to the control of the overall rate. In the case of a totally irreversible reduction [3] the current should rise exponentially with negativation of the potential at the foot of

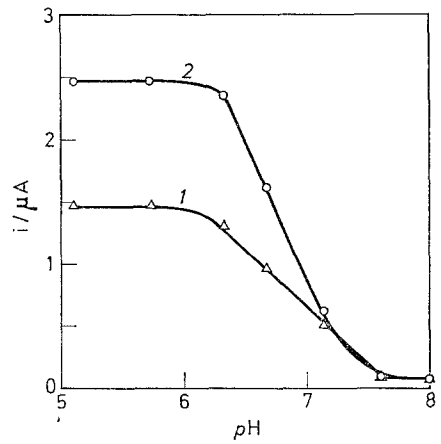

Fig. 4. pH dependence of the reduction peak of denatured DNA. 0.5 M MacIlvaine buffer, scan rate: 50 mV s^{-1} (1) 100 mV s^{-1} (2), 20 s hold at −1.1 V, 210 μg ml^{-1} denatured DNA

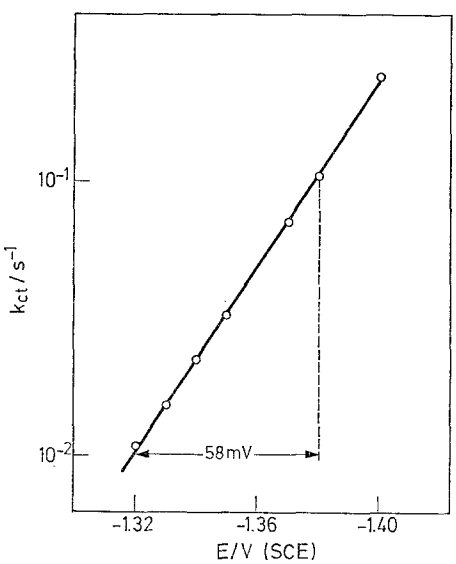

Fig. 5. Potential dependence of the overall charge transfer rate constant k_{ct}. MacIlvaine buffer 0.5 M, 105 μg ml^{-1} denatured DNA, pH 6.33, 90 s hold at −1.1 V, 100 mV s^{-1}, 25 °C

the reduction peak — where the surface concentration of DNA is approximately constant — according to the relation

$$i = nFA\Gamma_m k_{ct} \tag{1}$$

with

$$k_{ct} = k_{E=0} \exp\left[-(\alpha\, n_a F/RT)\, E\right], \tag{2}$$

where A is the electrode area in cm^2, Γ_m the surface concentration of adsorbed DNA in mole cm^{-2} at full coverage, $k_{E=0}$ the charge transfer rate constant at

$E = 0$ in s^{-1}, α the charge transfer coefficient (overall value including possible further preceding elementary steps prior to the electron transfer), n_a the number of electrons consumed in the rate determining step, and E the electrode potential vs. SCE. This relationship has been verified experimentally for the electrochemical reduction of DNA and the overall charge transfer rate constant has been determined. Its potential dependence is shown in Fig. 5.

In neutral and alkaline media also a nonfaradaic response has been observed. It is attributed to a reorientation of the reducible bases form a more or less flat orientation in parallel to the surface of the electrode to a more perpendicular orientation. For pH greater than 8 where the reduction current of DNA disappears it is the only observable process.

2. Adsorption of Denatured DNA

The irreversible reduction of denatured DNA in the adsorbed state can be utilised for the study of adsorption of denatured DNA at the electrode in the following manner. The experimental conditions have been chosen so as to eliminate in practice the component of the current due to the diffusion of DNA from the bulk of the solution. If the current is controlled only by the surface concentration of DNA then the time integral of the current peak gives the charge consumed during the reduction of adsorbed DNA. This charge is proportional to the surface concentration of DNA according to the relation

$$Q_m = nFA\,\Gamma_m, \tag{3}$$

where Γ_m is the maximal surface concentration of DNA, Q_m the corresponding limiting value of the charge consumed during the reduction, n the number of electrons consumed and A the surface area of the electrode.

The limiting value of the charge Q_m was determined in a wide range of experimental conditions varying the temperature between 5° C and 25°C, the bulk concentration of DNA between 10 to 360 μg/ml, the nature of the supporting electrolyte, the potential of adsorption from $^{-}0{,}2$ to $^{-}1{,}3$ V vs. SCE and pH between 5.0 to 6.5. The Q_m-value was the same within 10% giving for the corresponding maximal surface concentration of the electrochemically active monomeric units in DNA $\Gamma_m = 1.4 \cdot 10^{-10}$ mol cm^{-2}. From this value an average area per mononucleotide of about 60 Å^2 emerges assuming that the ratio of the reducible to the nonreducible basic groups in calf thymus DNA is one [2]. This value is about the same as the average area of purine and pyrimidine residues in cristalline mononucleotides [10]. From this value and from the remarkable degree of independence of the limiting surface concentration on physical and chemical parameters and on the electrode potential results that DNA is bound to the mercury surface through strong induced dipole interactions of the π-electron system in the heteroaromatic ring of the bases with electrons in the metal. The value of the limiting coverage and the blocking effect of the reduction products of the bases indicates a rather compact adsorption layer where the bases come close together. The other parts of the mononucleotide units including the sugar and the phosphate group are probably orientated to the solution side and are solvated. The protonation at the N(1) position in adenine and the N(3) position in cytosine leads to a lowering of the activation barrier for the electron transfer step. From the slope of the time dependence of the charge consumed during the reduction (Fig. 6) the

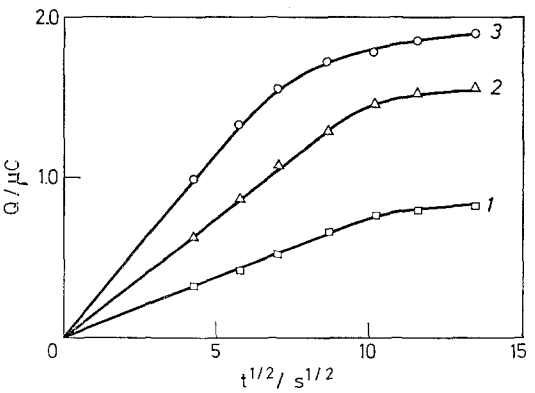

Fig. 6. Dependence of charge on the time and on the concentration of DNA. 0.5 M MacIlvaine buffer, pH 5.75, E_s — 1.1 V, scan rate 100 mV s^{-1}, 25 °C, (1) 130 μg ml^{-1} native DNA (E. coli)[1], (2) 74 μg ml^{-1} native DNA (calf thymus), (3) 74 μg ml^{-1} denatured DNA (calf thymus)

diffusion coefficient of denatured DNA in the given solution can be estimated. In the case where the adsorption coefficient of a substance is very large and the time necessary to attain full coverage is small, the spherical correction for diffusion may be neglected and thus the following relation holds [8]

$$\Gamma_m = 1.128\, c\, (Dt)_o^{1/2}, \tag{4}$$

where Γ_m is the maximum surface concentration of DNA in mole cm^{-2}, c its bulk concentration in mole cm^{-3}, D its diffusion coefficient in cm^{-2} s^{-1} and t_0 the time of the intercept of the linear portion of the dependence with the value of maximum coverage.

The value of $5 \cdot 10^{-7}$ cm^{-2} s^{-1} for the diffusion coefficient of denatured DNA with a mean molecular weight of $1 \cdot 10^6$ g in 1 M KCl at pH 6.7 and 25 °C is approximately the same as the value obtained from the Einstein-Stokes law for spherical particles. This indicates that at the high ionic strength in neutral medium denatured DNA is present in a conformation which is rather compact and spherical probably because of intrastrand base-base interactions. The same conclusions has been drawn from the measurement of the sedimentation coefficient at high salt concentration [9].

3. The Helix-Coil Transition

The reduction of native DNA can occur only if the double helix is opened and the reducible adenine and cytosine residues become accessible to the electrode process. This could be achieved by elevating the temperature up to the melting range or by enhancement of pH value to strong alkaline range. However, we have observed also in neutral and acid solutions in the temperature range 5 °C to 25 °C a reduction peak of native DNA. Contrary to denatured DNA the peak height of native DNA depends on pH and on the potential of adsorption E_s and is always lower than that of denatured DNA. We conclude thereof that under the influence of the electric field in the interface electrode/aqueous solution a partial opening of

[1] Kindly supplied from the Centre de biophysique moléculaire, Orléans, France.

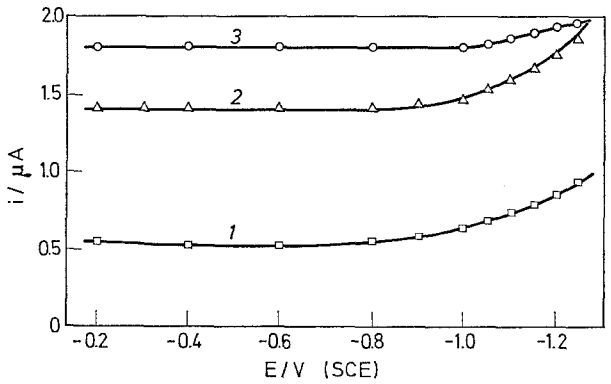

Fig. 7. Dependence of the reduction peak of native DNA on the potential of adsorption E_s. 0.5 M MacIlvaine buffer, pH 5.89, 100 mV s^{-1}, 25 °C, (1) 13.5 μg ml^{-1}, 120 s hold at E_s, (2) 23.7 μg ml^{-1}, 60 s hold at E_s, (3) 62.8 μg ml^{-1}, 120 s hold at E_s

the double helix of the adsorbed native DNA takes place, leading with respect to helix-coil transition to an effect equivalent to partial melting. Such an electric field effect was theoretically predicted by Hill [4] for electric field strengths above 10^4 V · cm^{-1}.

The DNA molecules adsorbed at the electrode are probably orientated with their helix parallel to the electrode surface. Taking the thickness of the hydrated monolayer of adsorbed DNA as 50 Å there will be acting an electric field of the order of 10^5 V cm^{-1} or more in this layer. This field can supply sufficient energy to induce the partial opening of the helix. Contrary to the thermal effect of melting there is no necessity for a complete unwinding in the adsorbed layer. The only condition for the reduction is that the bases are orientated with their electrochemically reactive sites towards the electrode. The extent of this effect in the adsorbed layer will depend on the portion of the DNA molecule which comes into the region where the electric field is acting. It can be expected that especially for the DNA with high molecular weight the helix-coil transition will not be complete. This is indicated by the observed lower value of the limiting charge in the case of native DNA and by its dependence on coverage. With calf thymus DNA having a molecular weight of $2.5 \cdot 10^6$ 75% to 90% of the value of the limiting charge for denatured DNA have been attained depending on the adjusted potential of adsorption E_s. For native DNA from *E. coli* having a higher molecular weight ($M = 10^7$) the percentage is still much lower (viz. Fig. 6, curve 1) as a just completed study has revealed.

At low coverage of the electrode a change of 100% of the peak height has been observed (Fig. 7, curve 1) if the starting potential E_s of the sweep is altered from E_{ecm} (potential of zero charge) to — 1.3 V while at potentials more positive than E_{ecm} the change of peak height remains small for native DNA (viz. Fig. 7). This indicates the growing influence on the opening of the helix of the electric field strength increasing with the negativation of the starting potential E_s beyond E_{ecm}. On the other hand no perceptible change has been observed on the reduction peak of denatured DNA as here no helix-coil transition has to occur.

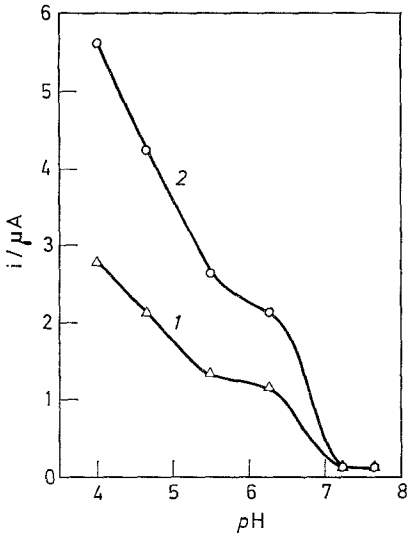

Fig. 8. pH dependence of the reduction peak of native DNA. 0.5 M MacIlvaine buffer, 76 μg ml^{-1} native DNA, 60 s hold at −1.1 V, 25 °C, scan rate: (1) 100 mV s^{-1}, (2) 200 mVs^{-1}

The change of pH of the solution towards acid media enhances the extent of protonation of native DNA leading to an accumulation of the positive charge in the interior of the molecule. The resulting electrostatic repulsion is, together with the electric field in the adsorbed layer, the cause for the separation of the two strands. In accordance with this conclusion the reduction peak of native DNA grows with decreasing pH at all scan rates (Fig. 8) in the acid medium. The peak becomes independent of scan rate in the neutral range due to control of the current by the kinetics of protonation as was also observed for denatured DNA.

In the present study the substantial aspects of the electrode process of reduction of DNA have been elucidated. After this specifically electrochemical problem had been solved it was possible to draw general conclusions on the physico-chemical behaviour of DNA adsorbed at the metal/electrolyte interface which are largely independent of the chemical nature of the interface and are therefore of a general importance for electrically charged interfaces also in biological systems.

Summary

The adsorption and the reduction of native and denatured DNA has been studied at the hanging mercury drop electrode (HMDE) by triangular sweep polarography. It has been established that a protonated form of denaturated DNA is reduced from the adsorbed state in a totally irreversible electrode process. The products are interconnected to a high molecular network strongly adhering to the surface of the electrode and blocking it for a repeated reduction on the same drop. In neutral media the protonation of DNA becomes rate-determining. The adsorption is diffusion controlled and from the time dependence of the coverage the diffusion coefficient of DNA could be estimated. The protonated molecules of

DNA are adsorbed via strong induced dipole interactions of the π-electron system in the heteroaromatic ring of the bases with electrons of the metal while the other parts of the DNA molecule including the solvated sugar and phosphate groups are orientated to the solution.

Native DNA is also adsorbed at the electrode and reduced in acid solutions in the same irreversible electrode reaction as denatured DNA. However, before a partial opening of the helix is induced by the strong electric field at the interface leading with respect to helix-coil transition to an effect equivalent to partial melting which has to precede the electron transfer step. The extent of unscreened reducible groups and their orientation with the electrochemically reactive sites towards the electrode depends on electrical parameters of the interface and on the portion of the DNA molecule which comes into the region where the electric field is acting. The results have with respect to the physicochemical properties of DNA general significance also for the behaviour of nucleic acids at charged biological interfaces as for instance the membrane of living cells.

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References

1. Brabec, V., Paleček, E.: The influence of salts and pH on polarographic currents produced by denatured DNA. Biophysik **6**, 290–300 (1970)
2. Davidson, J. N.: The biochemistry of the nucleic acids, p. 57. London: Methuen 1969
3. Heyrovský, J., Kůta, J.: Principles of polarography, p. 205. New York: Interscience 1966
4. Hill, T. L.: Some possible biological effects of an electric field acting on nucleic acids or proteins. J. Am. Chem. Soc. **80**, 2142–2147 (1958)
5. Janík, B., Elving, P. J.: Polarographic behaviour of nucleosides and nucleotides of purines, pyrimidines and flavins. Chem. Rev. **68**, 295–319 (1968)
6. Nürnberg, H. W., Wolff, G.: Influences on homogeneous reactions in the diffuse double layer. J. Electroanal. Chem. **21**, 99–122 (1969)
7. Paleček, E., Vetterl, V.: Polarographic reducibility of denatured DNA. Biopolymers **6**, 917–928 (1968)
8. Phillips, S. L.: Diffusion-limited adsorption at spherical electrodes. J. Electroanal. Chem. **12**, 294–299 (1966)
9. Studier, F. W.: Sedimentation studies of the size and shape of DNA. J. Mol. Biol. **11**, 373–390 (1965)
10. Fasman, D., Timasheff, S. N.: Fine structure of proteins and nucleic acids. Chap. 2: Ts'o, P.O.P.: Monomeric units of nucleic acid-bases, nucleosides and nucleotides. New York: M. Dekker 1970
11. Valenta, P., Grahmann, P.: Electrochemical behaviour of natural and biosynthetic polynucleotides at the mercury electrode. I. The adsorption of denatured deoxyribonucleic acid at the hanging mercury drop electrode. J. Electroanal. Chem. **49**, 41–53 (1974)
12. Valenta, P., Nürnberg, H. W.: II. Reduction of denatured DNA at stationary and dropping mercury electrodes. J. Electroanal. Chem. **49**, 55–75 (1974)
13. Valenta, P., Nürnberg, H. W., Klahre, P.: III. A comparative study on the electrochemical properties of native and denatured DNA. Bioelectrochemistry and Bioenergetics (in press)

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