CROATICA CHEMICA ACTA CCACAA 48 (4) 623—641 (1976)

YU ISSN 0011-1643
CCA-963 541.138
Conference Paper

Voltammetric Studies of the Electrochemical and Interfacial Behaviour of DNA at Charged Interfaces*

H. W. Nürnberg and P. Valenta

Institute of Chemistry, Institute for Applied Physical Chemistry, Nuclear Research Centre (KFA), Juelich, Federal Republic of Germany

A survey of the essentials of the experimental evidence assembled in systematic extended voltammetric studies of denatured and native DNA is presented. Applying an advanced version of single triangle sweep voltammetry at the HMDE and by supporting measurements with various other polarographic methods, such as phase sensitive a.c.-voltammetry, the adsorption parameters, the sequence of interfacial events as a function of adsorption time and adsorption potential and the kinetics of the electrode process have been elucidated. While for pH $\leqslant$ 7 in adsorbed DNA the adenine and cytosine moieties are immediately reducible in a totally irreversible electrode reaction yielding a strongly adsorbed compact film of reduction products, they have to become accessible to electron and proton transfer in adsorbed native DNA in a sequence of prior deconformation steps involving opening and unwinding of the double helix under the constraint exerted by the adsorption interactions and by the interfacial electric field. In a fundamental biophysicochemical sense the results enable general conclusions on the behaviour of DNA when it interacts with charged interfaces in the living cell.

INTRODUCTION

Nucleic acids play, as carriers of the genetic code, a key role in biology. Thus, the study and clarification of their electrochemical behaviour has become one of the important present topics in bioelectrochemistry.

There has been reported evidence that in several of the most significant biological effects of DNA in the living cell, such as transcription and replication, interactions of the nucleic acid with electrically charged biological interfaces (nuclear and intracellular membranes, protein envelopes of chromosomes) are involved[1-4]. These interfacial interactions, particularly the influences exerted by the interfacial electric field, will cause certain conformational and structural rearrangements of the adsorbed nucleic acid proceeding at a rate which is to an extent depending on the magnitude of the relevant parameters of the charged interface.

However, the interfacial situation in the living cell is rather complex and not easily manipulable. In this context it is favourable that the interface metal electrode/aqueous electrolyte and the charged biological interfaces

---

* Presented in part at the 4th Internat. Summer Conference »Chemistry of Solid/Liquid Interfaces« and the Internat. Symposium »Electrochemistry of Interfacial Phenomena«, June 24th—July 3rd, 1975, Cavtat, Yugoslavia.

as membranes in the living cell have common fundamental physical properties despite their obvious biochemical differences, while the liquid phase is in both cases a rather similar aqueous electrolyte. The biological surfaces as well as the metal electrode surface usually carry electric charges. Consequently, an electric double layer is formed at the interface. The electric field gradient operating there[5] and the accumulation of the adsorbate will influence significantly all sorts of interfacial effects, such as adsorption, conformational, structural and eventually chemical changes and lateral interactions of the adsorbed material as well as the course and rate of chemical reactions (for instance of protonation)[6] in the interfacial region.

Thus, an inert metal electrode, such as the mercury electrode, represents a versatile and easily adjustable model interface for the study of the general physicochemical aspects of the interfacial behaviour of biopolymers at a charged interface[7].

Furthermore, these studies reveal the characteristics of a possibly occuring electrode process contributing also in this direction to the completion of the physicochemical characterization of nucleic acids. The resulting information is in turn, a prerequisite for the application of relevant polarographic or voltammetric methods in the analysis[8] of nucleic acids, *i. e.* for the determination of their quantity in solution, the measurement of the kinetics of conformational changes in solution induced by temperature elevations, drastic pH-changes or radiation damages and for studies of the formation of complexes with metals or of the effects of a bacterial enzymatic attack[46].

In 1970, when we started our systematic studies of the electrochemical and interfacial behaviour of DNA, this biopolymer was insufficiently known, while, due to the pioneering work of Vetterl *et al.*[9–11] and the extended and critical investigations of Janik and Elving[12–14] the electrochemical behaviour of the isolated bases (adenine, cytosine, guanine, thymine) implemented in DNA was already well understood.

The same could be said about the corresponding nucleosides[10,11] and to a certain extent particularly with respect to the electrode process, even about mononucleotides[12–14]*. The main conclusion was that only adenine and cytosine are reducible and the other bases are not.

In McIlvaine buffer at pH-values below 8 adenine undergoes a 4-electron reduction with a half wave potential *vs.* SCE of $E_{1/2} = -0.975 - 0.084$ pH, while for the mononucleotide deoxyadenylic acid a similar behaviour with $E_{1/2} = -0.985 - 0.080$ pH is reported[12]. For cytosine this value is $E_{1/2} = -1.125 - 0.075$ pH and for the mononucleotide deoxycytidylic acid contained in DNA $E_{1/2} = -0.908 - 0.110$ pH[14]. It can be concluded that the pH-dependence of the reduction responses are rather similar for the reducible bases and the corresponding mononucleotides and it is to be expected that in the polynucleotide DNA a common reduction response for the reducible bases adenine and cytosine will be observed. The electrode reactions are preceded by a protonation of the $N(1)$ in adenine and the $N(3)$ in cytosine. In the following rate determining step of the electrode reaction the same double bond in the pyrimidine ring, *i. e.* $N(1) = C(6)$ in adenine and $N(3) = C(4)$ in cytosine is reduced by the uptake of 2 electrons and a further proton. Afterwards adenine

* Meanwhile also the interfacial behaviour of the adenine mononucleotides and of the cytosine mononucleotides has been elucidated to a considerable extent[15,16].

takes up 2 further electrons and 2 further protons while cytosine takes up, after a relatively fast deamination at C(4) one further proton and electron, thus bringing the total n to 3 (viz. Figure 1)*.

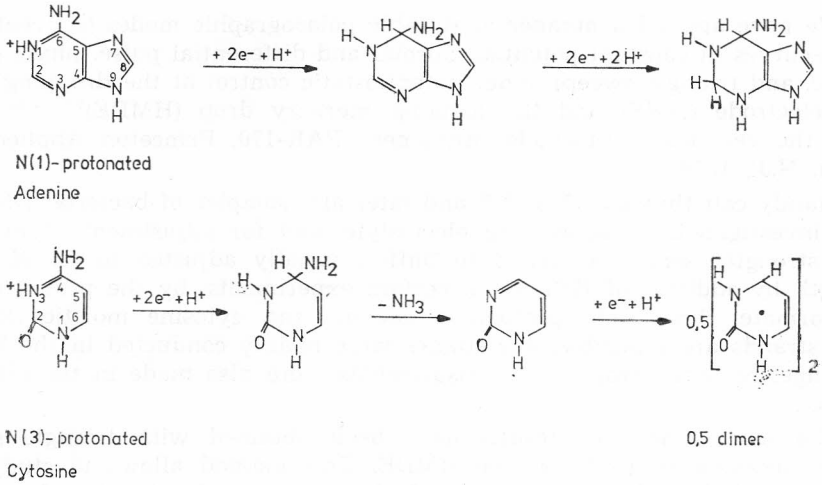

Figure 1. Mechanism of reduction of protonated adenine and cytosine.

These reduction stages are relevant to polarographic conditions (*i. e.* short term electrolysis) while for the long term macro scale electrolysis of adenine, an even further reduction after a relatively slow deamination step, resulting in a total electron uptake of n = 6, has been reported[12,14].

Much more sporadic and contradictory was the knowledge about DNA, particularly native double helical DNA, although some important singular informations had been provided by the extended investigations of Paleček, Brabes *et al.*[17–22], Berg, Flemming *et al.*[23–26] and by the early studies of Miller[27]. The adsorption of denatured, *i. e.* single stranded DNA, and also of native DNA characterized by its double helical structure had been identified while native DNA was reported as nonreducible[28]. The existing unsettled situation, typical for the exploratory phase in research on a complicated topic, was partly due to the nature of the methods applied and also to the general necessity to explore the scene before systematic investigations could be performed.

Meanwhile the situation has become significantly clearer. From our systematic and extended studies the main contours of the electrochemical and interfacial behaviour of both denatured[7,29–32] and native DNA[7,29,33–35] have emerged. Moreover, all our essential experimental findings have been recently comfirmed by Paleček and Brabec[36,37], Barker *et al.*[38], Parsons *et al.*[39] and Malfoy and Reynaud[40]. Although in our opinion this also applies to the experimental results of Berg, Flemming *et al.*[41,42], these authors still prefer a controversial interpretation, particularly with regard to important conclusions on the interfacial behaviour and the reduction of native DNA. Nevertheless, now they also accept[41], contrary to their earlier views[28], that native DNA is

* It has to be noted, however, that according to Webb, Janik and Elving[14] the total uptake of electrons by cytosine moieties implemented in oligo- or polynucleotide strands remains restricted to n=2 as then the deamination step occurs rather slowly.

also reducible. This fact was first established and clarified by us[7,29,33] and later confirmed by other authors,[36-38].

METHODOLOGY

We have applied a number of suitable polarographic modes (conventional dc, i-t-curves at constant potential, normal and differential pulse, phase sensitive ac, and triangle sweep) under potentiostatic control at the dropping mercury electrode (DME) and the hanging mercury drop (HMDE)[7,15,16,30-35] by using the versatile multi-mode instrument PAR-170, Princeton Applied Research, N.J., USA.

Mainly calf thymus DNA[7,30-35] and later also samples of bacterial DNA[10,35] were investigated. As supporting electrolyte, and for adjustment of pH and ionic strength, served a McIlvaine buffer, usually adjusted to 0.5 M ionic strength by addition of KCl or, in certain experiments, by the proton donor NH_4-formate. Since only protonated adenine and cytosine moieties in the DNA strands are reducible, our studies were mainly conducted in the 7 to 4 pH-range, however supporting measurements were also made in the alkaline region.

The most conclusive results have been obtained with triangle single sweep voltammetry (SSV) at the HMDE. This method allows to study the reduction of the adsorbed amount of the rather surface active denatured and native DNA as function of the adsorption potential E_s and the adsorption time t_s elapsed at this potential. E_s corresponds to the sweep starting potential. After a preselected time t_s has elapsed at E_s the electrode potential is driven rapidly by a cathodic sweep to and through the potential range of reduction. At a sufficiently cathodic potential the sweep reverses and returns, at the same sweep rate the anodic direction to the sweep starting potential E_s (viz. Figure 2). The resulting current, or preferably the electric charge Q corresponding to its time integral, is recorded using a Mod. 1090 Nicolet digital storage oscilloscope, (for details see ref.[15,16,30-35]).

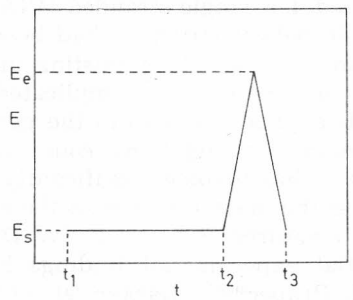

Figure 2. Time dependence of the voltage applied to the hanging mercury drop electrode (HMDE). E_s-starting potential of the sweep, E_e- end potential, $t_1 - t_2$ waiting time t_s at the starting potential E_s, $t_2 - t_3$ duration of the triangle sweep.

The other polarographic modes mentioned above yielded results[31] that in full agreement with SSV, which will be the main topic of discussion in this paper. Particularly useful evidence of the interfacial behaviour of DNA was also provided by the out-of-phase component of the ac-current measured by phase sensitive ac-polarography[31,34,35].

RESULTS AND DISCUSSION

The following sections will present a survey of the main results and of the most significant conclusions of our extended studies.

a) *Denaturated DNA*

We have determined the adsorption parameters[30] of this single stranded polynucleotide, the kinetics of the electrode process of its reduction[31] and the characteristics of its diffusion[30].

With SSV at the HMDE (area $3.5 \cdot 10^{-2}$ cm^2) in acid to neutral solutions a totally irreversible *reduction peak* is observed, which is followed by a response due to catalytic hydrogen evolution (Figure 3). During the reversed anodic part of the sweep no response in the potential range of reduction is obtained, indicating that the electrode process is totally irreversible. The reduction response is only obtained for the 1st sweep at a fresh drop. Even if the waiting time at the sweep starting potential E_s is extended to 15 or more minutes no reduction response is obtained by subsequent sweeps. Only catalytic hydrogen evolution prevails. Obviously the even more strongly adsorbed reduction product blocks completely the electrode surface by the formation of a high molecular network inpenetrable for further DNA diffusing towards the electrode. This reduction product film inhibits the adsorption of DNA, which is obviously a prerequisite for the reduction of its reducible adenine and cytosine moieties. On the other hand, the reduction product film has

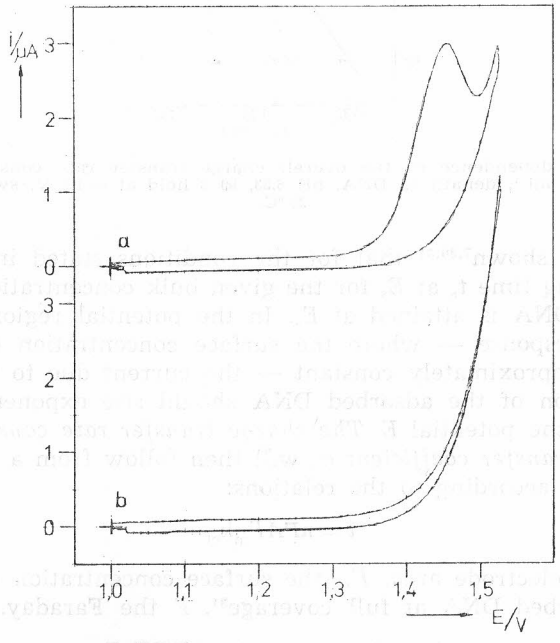

Figure 3. Current potential curve of denatured DNA on the HMDE polarized with a single triangular voltage sweep. 0.5 M McIlvaine buffer, pH 5.8, 240 μg ml^{-1} denatured DNA; sweep rate 100 mVs^{-1}, waiting time 20 s at — 1.0 V (SCE), area of HMDE 3.50 mm^2; (a) First curve recorded at a fresh mercury surface, (b) second curve at the same drop after 15 min. hold at — 1.0 V.

still sufficient electron mediating properties via the π-electrons of the remaining conjugated double bonds to act as an efficient catalyst of hydrogen evolution*.

Because of the total blocking of the electrode surface by the reduction product film is also the reason why with dc- or normal pulse polarography at a DME no diffusion controlled limiting currents are obtainable but due to progressive inhibition of the electrode process peak shaped polarograms[31,32].

The reduction response obtained during the 1st SSV-sweep at a fresh drop of the HMDE occurs at about the same potential as that of the free bases adenine and cytosine and their corresponding mononucleotides[12,14] under the same pH- and ionic strength conditions. From this it is concluded, in accordance with earlier suggestions[44,45], that the reduction response of adsorbed denatured DNA is due to the reduction of the adenine and cytosine moieties in the adsorbed regions of the single stranded DNA.

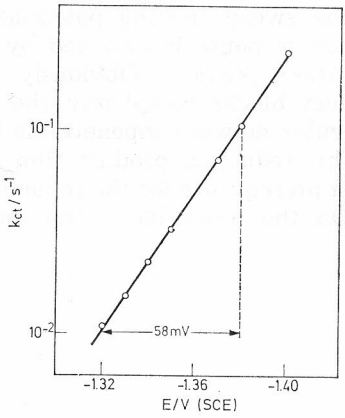

Figure 4. Potential dependence of the overall charge transfer rate constant k_{ct}. McIlvaine buffer 0.5 M, 105 μg ml^{-1}, denatured DNA, pH 6.33, 90 s hold at —1.1 V, sweep rate 100 mVs^{-1}, 25 °C.

It has been shown[7,30,31] that for the conditions stated in Figure 4 with respect to waiting time t_s at E_s for the given bulk concentration full coverage with adsorbed DNA is attained at E_s. In the potential region of the foot of the reduction response — where the surface concentration of the adsorbed DNA remains approximately constant — the current due to the totally irreversible reduction of the adsorbed DNA should rise exponentially with the negativation of the potential E. *The charge transfer rate constant* k_{ct} and the *overall charge transfer coefficient* α_a will then follow from a half logarithmic plot (Tafel plot) according to the relations:

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

where A is the electrode area, Γ_m the surface concentration of the reducible moities of adsorbed DNA at full coverage[31], F the Faraday.

$$k_{ct} = k_{E=0} \exp -(\alpha_a n_a F/RT)\, E \tag{2}$$

* For the mechanism of catalytic hydrogen evolution due to adsorbed N-heterocycles see ref. 43.

with n_a uptake of electrons in the rate determining step of the electrode process.

With $n_a = 2$ an $\alpha_a = 0.48$ results. The k_{ct}-values are obtained from eq. (1) in the dimension s^{-1}. As they are in reality heterogeneous rate constants they are usually expressed in cm s^{-1}. This transformation involves a reasonable assumption of the thickness of the adsorbed DNA-strand regions the implemented adenine and cytosine moieties of which are reducible. Assuming a thickness of 50 Å, one obtains k_{ct}-values in cm s^{-1} being a factor $2 \cdot 10^6$ smaller than those in Figure 4. These k_{ct}-values between $5 \cdot 10^{-9}$ and $5 \cdot 10^{-7}$ cm s^{-1} for the studied potential range indicate that the electrode process is very slow indeed.

The dependence of the reduction response on the *adsorption time* t_s at a given starting potential E_s (Figure 5) shows clearly that only adsorbed DNA is reducible. The t_s-values necessary to reach full coverage decrease with growing bulk concentration. As revealed by linear dependence of the reduction response on $t_s^{1/2}$ until full coverage is attained the DNA-adsorption is diffusion controlled. From this dependence, for denatured calf thymus DNA, a diffusion coefficient of about $5 \cdot 10^{-7}$ cm^2 s^{-1} is obtained for a molecular weight of $1 \cdot 16^6$, while with the Einstein-Stokes law, presuming inherently a spherical shape of the diffusing particle a value of $3.6 \cdot 10^{-7}$ cm^2 s^{-1} is computed. This leads of the conclusion that denatured DNA forms a rather compact and spherical coil in the electrolyte solution[30].

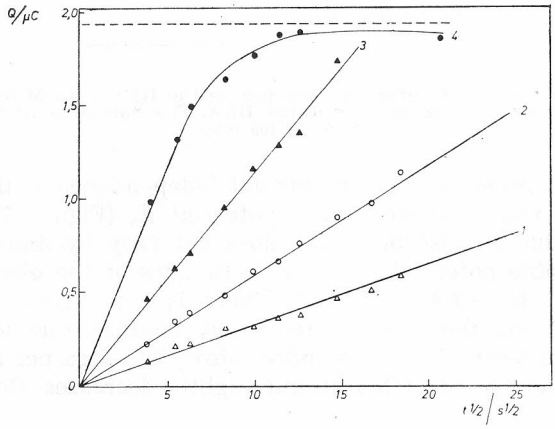

Figure 5. Dependence of charge on time t_s and on the concentration of DNA. Britton-Robinson buffer plus 1 M KCl, pH 6.69, 25 °C, sweep rate 100 mVs^{-1}, $E_s = -1.2$ V, concentration of DNA: (1) 1.72 μg ml^{-1}, (2) 3.44 μg ml^{-1}, (3) 6.88 μg ml^{-1}, (4) 13.8 μg ml^{-1}. (- - -) Average limiting charge Q.

A prerequisite of the above mentioned measurements is that the sweep rate v be rapid enough to render additional contributions to the reduction response by diffusion during the sweep virtually negligible. It has been shown[30,31] that this requirement is almost completely satisfied in the case of $v = 0.1$ V s^{-1}. After introduction of the Nicolet digital storage oscilloscope for the evaluation of the reduction response, measurements have been made at an even higher sweep rate of 1 V s^{-1} without obtaining significantly different results. Under these conditions the reduction response, solely due to the adsorbed DNA, virtually reflects the interfacial behaviour of the adsorbed material

developed during the elapsed adsorption time t_s at the sweep starting potential E_s which in this manner becomes the operative adsorption potential as well.

The *pH-dependence* of the reduction response (Figure 6) shows that prior protonation is a prerequisite and that further proton uptake is involved in the reduction process. This was shown already earlier for the free bases adenine and cytosine and their corresponding mononucleotides by other authors[12,14]. The decrease of the reduction response by analogy with an acid dissociation curve around pH 7 indicates that here kinetics of protonation contribute to the control of the overall rate of reduction[31].

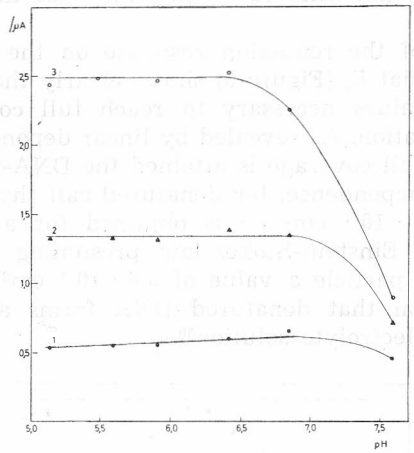

Figure 6. pH-dependence of the reduction response on the HMDE. 0.5 M McIlvaine buffer with 0.3 M ammonium formate, 107 μg ml^{-1} denatured DNA, 15 s hold at —1.1 V, sweep rate (1) 20, (2) 50, (3) 100 mVs^{-1}.

Of particular importance is the virtual independence of the coverage with denatured DNA from the *adsorption potential* E_s (Figure 7). The reduction response solely due to adsorbed DNA does not vary by more than 10% over the whole accessible potential range on both sides of the electrocapillary zero (E_{ecm}) from —0.1 to —1.3 V (SCE)[30]. There is a marginal increase of 10% between —1.0 V and the onset of reduction, because, due to a reorientation — to be discussed later — the demanded adsorption area per adsorbed adenine and cytosine moiety of the DNA strand slightly decreases. Completely similar

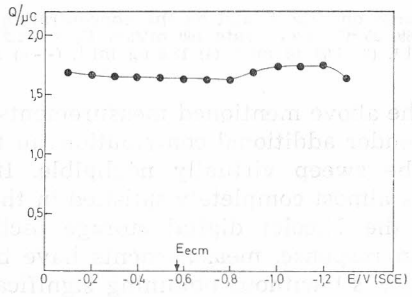

Figure 7. Dependence of reduction response (charge) on the potential of adsorption E_s. Britton-Robinson buffer plus 1 M KCl. 107 μg ml^{-1} denatured DNA, pH 6.69, 25 °C, sweep rate 100 mVs^{-1}, waiting time t_s 30 s.

results for the dependence of the adsorption of denatured DNA on E_s have been recently reported also by Brabec and Paleček[37]. These results constitute additional evidence showing that complete desorption of denatured DNA until the onset of its reduction, suggested by other authors[20–26] in the past and even recently[47] should be ruled out. Only a reorientation[31,33,35,40] of the adsorbed base units occurs before reduction if the adsorption potentials E_s for DNA are adjusted between —1.0 V and the onset of reduction. A further confirmation of the remaining adsorption is also provided by the ellipsometric measurements of Humphreys and Parsons[39]. The pronounced insensitivity of the adsorption to significant changes in potential E_s, and thus the electric field strength emphasizes the high surface activity of denatured DNA. It is to be concluded that the DNA is thus preferentially adsorbed *via* its most hydrophobic parts, *i. e.* its base units, while the hydrophilic sugar moieties and phosphate groups tend to remain hydrated and are consequently orientated towards the solution[30]. The average area per adsorbed reducible moiety of about 70 Å^0 is of the same magnitude as the area of the base units[48] implemented in DNA. Thus, it is concluded that at more positive potentials denatured DNA is anchored to the surface *via* the base units orientated rather flatly in relation to the surface due to π-electron interaction. Of course, at rather positive potentials protonation will slightly moderate this tendency. In accordance with the general interfacial behaviour of adsorbed *N*-heterocycles[49,50], at more negative potentials one expects as reorientation to a more perpendicular position of the base units[31,33]. In fact, a reorientation response has been observed for adenine[9] and all adenine containing nucleotides (mononucleotides[15,16], poly-A[51], denatured[31] and native DNA[33–35]) between —1.1 and —1.3 V, depending on the fact whether adenine belongs to a mononucleotide or is implemented in the strand of a biopolymer as DNA (Figures 15 and 16). At the same time for denatured DNA a marginal increase in coverage has been observed in this potential range (Figure 7).

b) *Native DNA*

We were first to demonstrate that[7,29,33] double helical native DNA also gives, in solutions with pH = 7 after adsorption, a similiar totally irreversible *reduction response* due to the reduction of adenine and cytosine moieties (viz. Figure 8). However, only those adenine and cytosine units will be reduced which have become accessible, by prior deconformation[34] in the interface, to the subsequent electron transfer and particularly to the proton transfer in the course of the reduction process. This makes the interfacial behaviour significantly more involved than for single stranded denatured DNA. The magnitude of the reduction response depends on many parameters, such as adsorption potential E_s and thus on the interfacial electric field, adsorption time t_s at E_s, pH, the percentage of interstrand adenine-thymine bonds via hydrogen bridging[35], and the molecular weight[35], to mention only the most important ones.

After numerous and extended studies[7,29,33–35] the following *flow chart*, consistent with all the now known experimental evidence, emerged for the sequence of the interfacial events of native DNA adsorbed at a charged interface[34] (Figure 9).

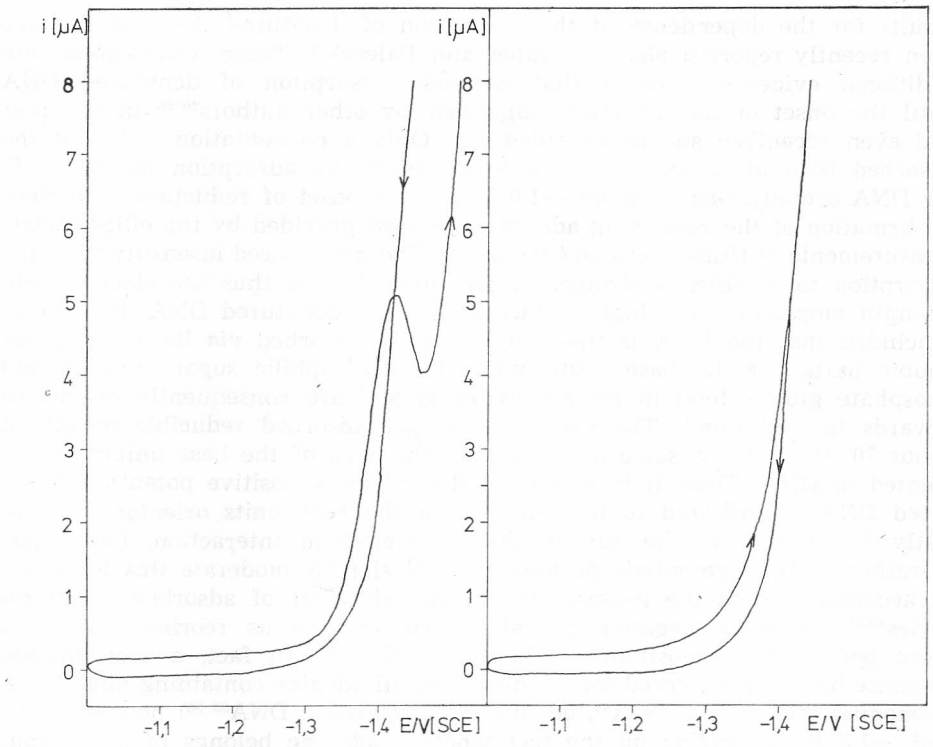

Figure 8. Current potential curve of native DNA on the HMDE. 0.5 M McIlvaine buffer, pH 6.65, 80 μg ml^{-1} native DNA, sweep rate 200 mVs^{-1}, 90 s hold at E_s — 1.0 V. (1) first curve recorded at a fresh mercury surface, (2) second curve recorded after 90 s hold at E_s — 1.0 V on the same mercury drop.

In solution the double helix of native DNA, stablished in principle by the Watson-Crick interstrand hydrogen bridges between adenine and thymine (A-T-pairs) and guanine and cytosine (G-C-pairs), and by base stacking in the vertical direction, is generally stabilized by hydration. Stabilization effects further arise from additional hydrogen bonding between the water molecules from the hydrophobic and hydrophilic regions of the rather structured primary hydration sheath. If an inert electrolyte is present in the aqueous solution there is further interstrand hydrogen bridging between phosphate groups, via the hydration sheaths of the environmental cation an effect which stabilizes the helix further[52]. Yet there is no homogeneous stability, but there are more labile and more stabile regions in the helix. Due to the differences in hydration of the respective bases and the resulting amount in the hydration water extrusion upon base pairing, regions rich in A-T-pairs are more labile than those where G-C-pairs predominate[52].

After an adsorption contact local dehydration of native DNA around its adsorption sites takes place. This is also due to the local collapse of stabilizing factors, such as the »hydrophilic/hydrophobic bridging« in the primary hydration sheath and the »hydrated cation interstrand hydrogen bridging« between phosphate groups. Now, a rather labile double helix, maintained only by the Watson-Crick interstrand base pairing and vertical base stacking

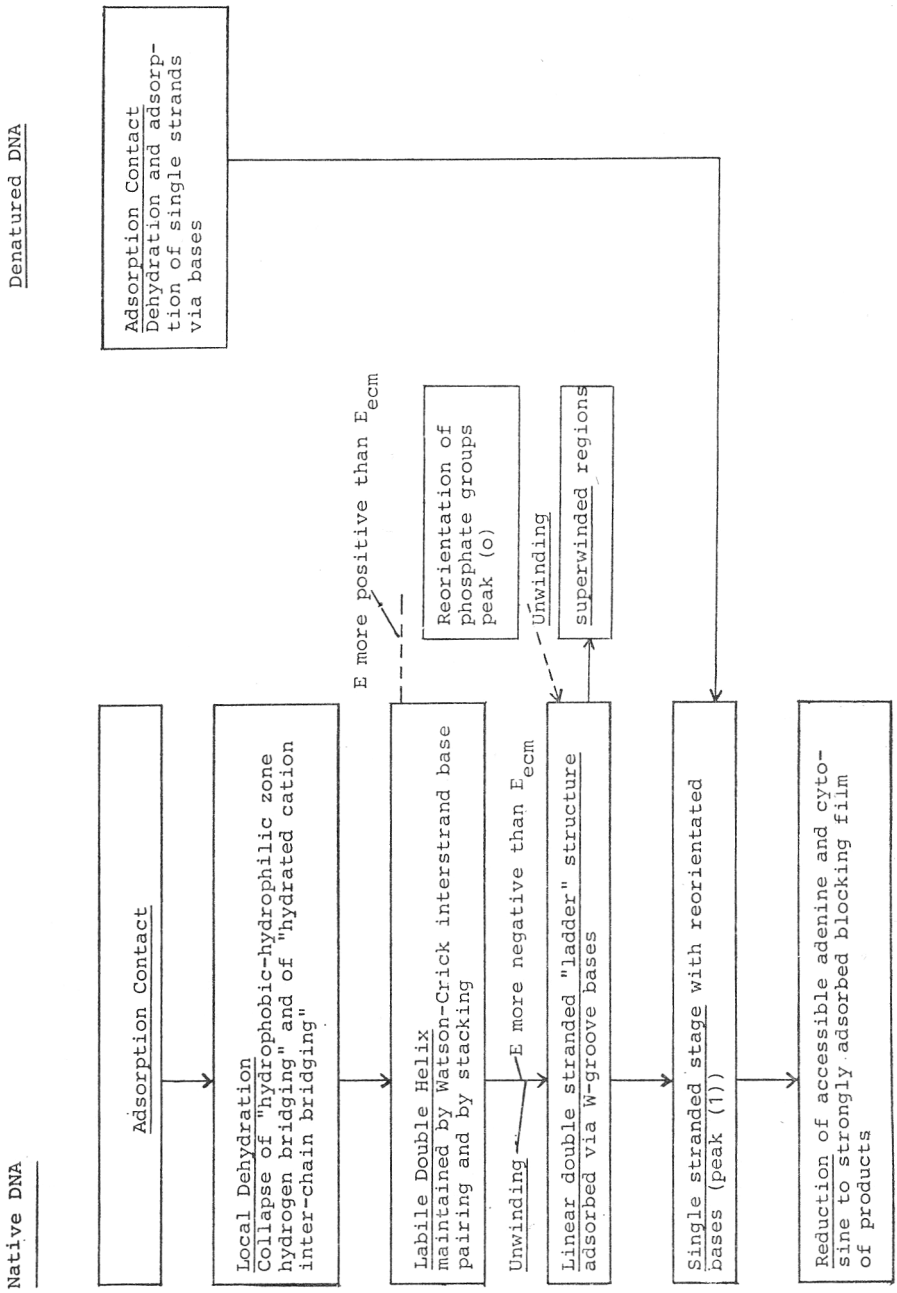

Figure 9. Flow chart of the sequence of interfacial events of native and denatured DNA adsorbed at the mercury electrode.

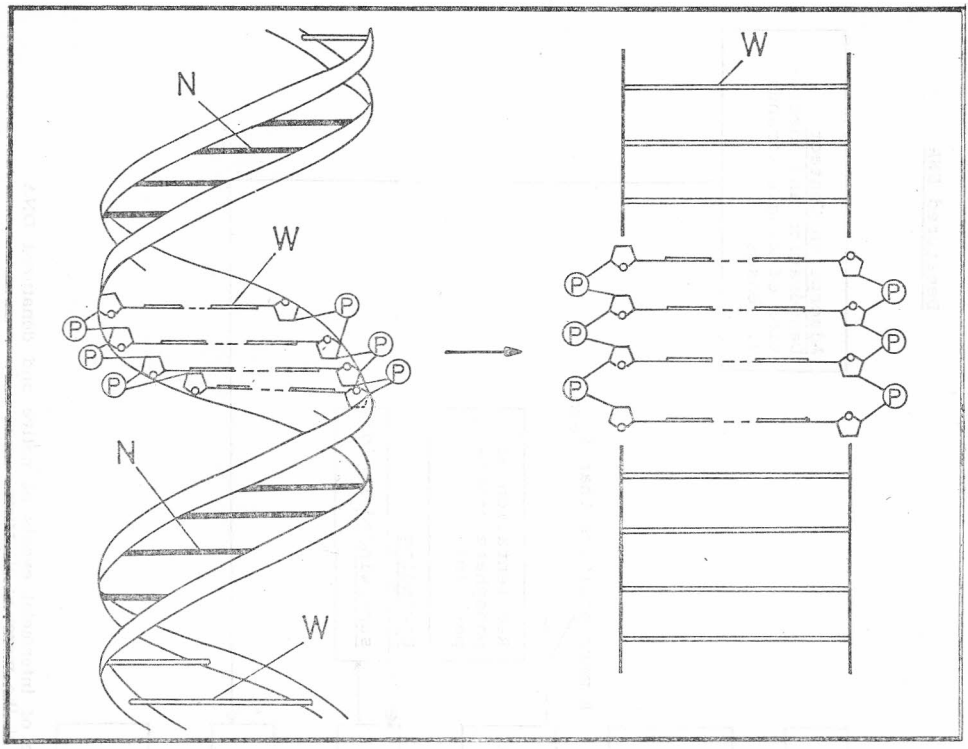

Figure 10. Scheme of the linear double stranded »ladder« structure according to Lewin[52].

results in the adsorption zones. Under the strain exerted by the adsorption forces and the interfacial electric field, depending in strength on the respective adsorption potential E_s unwinding of the helix sets in. It is to be assumed that the first intermediate unwinding stage is the linear double stranded »ladder« structure — described by Lewin[52] as an intermediate denaturation stage (Figure 10). The two strands are still bonded by the Watson-Crick interstrand base pairing and vertical stacking remains operative*. Adsorption should be preferential in the »ladder« structure *via* the W-groove bases, since then the more hydrophobic side of the »ladder« can interact with the electrode surface, while the hydrophilic 3',5'-sugar-phosphate linked chains remain

* Recently published[58] calculations based on X-ray data emphasize further the probability of an unwinded conformation of DNA, with still intact Watson-Crick--interstrand base pairing and vertical base stackin, particularly if stabilizing interaction with charged interfaces (protein structures) occurs.

directed towards the solution. The unwinding to the »ladder« structure also creates necessarily some superwinded regions, where the reducible bases remain screened. The next deconformation stage is the single stranded stage, after the rupture of the Watson-Crick interstrand base pairing hydrogen bridges. The rupture of these hydrogen bonds is a necessary prerequisite of protonation and proton uptake in the reduction of the adenine and cytosine moieties. The now mobile bases can reorientate to a more perpendicular position with respect to the electrode surface. A corresponding nonfaradaic reorientation response has been observed (viz. Figure 15 and 16) for all adenine containing substances — adenine[9], adenosine[10], mononucleotides[15,16], poly-A[51], denatured[31] and native DNA[33–35,40] — between —1.1 and —1.3 V. In an even more negative potential range the accessible adenine and cytosine moieties are reduced in a totally irreversible electrode process which has been clarified by Janik and Elving[12,14] in respect to these adsorbed bases and their corresponding mononucleotides and later studied quantitatively by us[30] in connection with adsorbed denatured DNA. Again, as in the case of denatured DNA the reduction products form a compact film which completely blocks the electrode surface and prevents the adsorption and subsequent deconformation and reduction of additional native DNA diffusing from the bulk of the solution towards the electrode. This is evidenced by the fact that a reduction response can be obtained only with the 1st sweep on a fresh drop of the HMDE. During subsequent sweeps at the same hanging drop only catalytic hydrogen evolution is maintained (Figure 8)*.

The completely similar reduction behaviour of the adsorbed native and denatured DNA is a further strong piece of evidence of the necessity of helix opening before the reduction of the adenine and cytosine moieties becomes possible. Recently published results by a number of other authors[36–38,40] confirm all our essential results and conclusions. Yet it is to be mentioned here that Berg *et al.*[41] have recently attempted to dispute, by a variety of unproven statements or inconsistent interpretations of experimental data, the essential conclusions on the behaviour of native DNA at charged interfaces. However, a consistent interpretation of the experimental results of Berg *et at.*[41,42] would reveal no significant contradiction to our findings.

Naturally, the extent of the above described deconformation of the adsorbed native DNA depends on various parameters. Particularly important are the adsorption potential E_s and the adsorption time t_s elapsed at E_s. In this respect triangle single sweep voltammetry at the HMDE, with sufficiently high sweep rates to overrun diffusion during the sweep, has been found to be the most efficient experimental approach for the elucidation of the complicated interfacial behaviour of DNA. Some other voltammetric methods will provide

---

* Recent measurements with a new potentiostatic double step-sweep method have furnished further confirming evidence for the outlined interfacial behaviour of adsorbed native and denatured DNA[57].

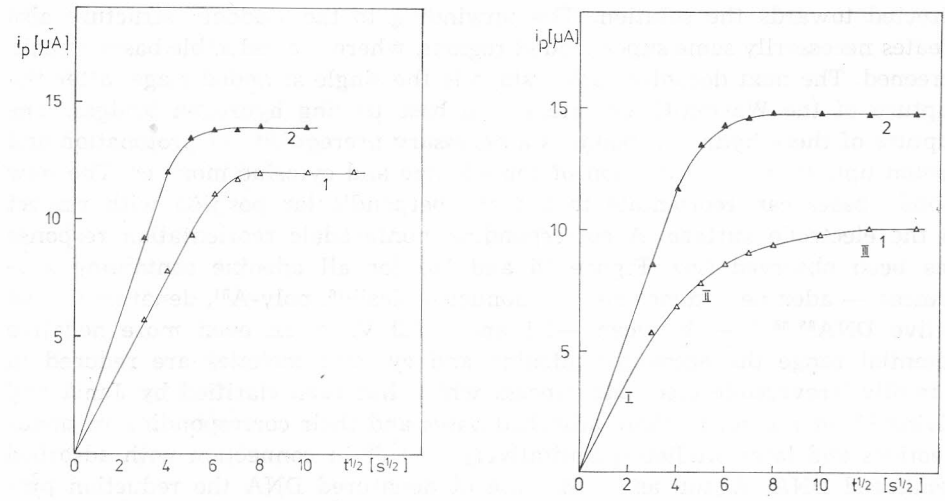

Figure 11. Time dependence of the sweep voltammetric reduction response at an adsorption potential of — 0.4 V (a) and at an adsorption potential of — 1.2 V (b) for an ionic strength of 1 M. pH 5.6, McIlvaine buffer; (1) native DNA 130 μg ml^{-1}, (2) denatured DNA 50 μg ml^{-1}. The lower bulk concentration for denatured DNA was used to exclude renaturation. The differences in bulk concentration for native and denatured DNA are unimportant since full coverage is attained during segment I.

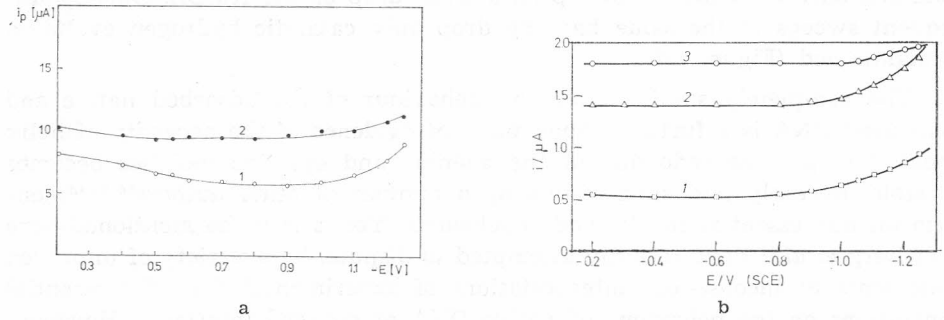

Figure 12a. Dependence of the sweep voltammetric reduction response of native DNA on sweep starting potential E_s for various adsorption times t_s. Ionic strength 0.1 M; pH 5.6, McIlvaine buffer, 130 μg/ml native DNA, sweep rate 1 V s^{-1}; (1) 9 s hold at E_s, (2) 145 s hold at E_s.
Figure 12b. Dependence of the sweep voltammetric reduction response of native DNA on sweep starting potential E_s for various adsorption times t_s. Ionic strength 0.5 M, pH 5.89, McIlvaine buffer, 100 m Vs^{-1}, (1) 13.5. μg ml^{-1}, 60 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.

additional single pieces of valuable supporting information which can contribute to the understanding of this problem.

Figure 11 shows the dependence of the reduction response of adsorbed native DNA on *adsorption time* t_s for two E_s-values. During the first section (I) the diffusion controlled full coverage of the surface occurs. Immediately after adsorption, the described sequence of interfacial deconformation begins and proceeds to an extent and at a rate depending on the respective adsorption potential E_s. Moreover, due to the superwinded regions, loop formation and general sterical hindrance in the interface, the reduction response of the adsorbed native DNA always remains lower than that of denatured a priori single stranded DNA.

The *dependence on adsorption potential* E_s at a given t_s in Figure 12 shows an increase of the reduction response at potentials differing from the electrocapillary zero (E_{ecm}), particularly at more negative E_s-values. Here the influence of the progressively increasing *interfacial electric field* on the helix deconformation of DNA is manifested. Similar results have been later reported by Paleček *et al.*[36,37]. In a general biophysicochemical sense[7] this finding seems to be of great significance for various interactions of DNA with charged biological interfaces in the living cell, *e. g.* for the nuclear and intracellular membranes in the primary stages of replication[1-4]. It should also be mentioned that Neumann, Katchalsky and Revzin[53,54] have reported rather long lasting partial helix unwinding for ribosomal RNA after application of electric field pulses of only 20 to 44 kV cm^{-1} for only 30 μs in homogeneous solutions of a very low ionic strength. These authors have shown that due to shifts in the ionic atmosphere of the helical polyion by the electric field, large dipole moments are induced in the polynucleotide causing strand repulsion and consequently partial unwinding of the helix. As has been theoretically predicted already by Hill[55], electric field strengths above 10^4 V cm^{-1} lead to such a change in the polarizibility of DNA that the single stranded form becomes thermodynamically more stable than the double helix. A similar electric field effect will be operative permanently at a charged interface on adsorbed native DNA. Under our experimental conditions the ionic strength is larger — particularly due to a static ψ-effect on the mobile counterions in the diffuse double layer — but also the interfacial electric field will be significantly larger (10^5 V cm^{-1} or even more) at adsorption potentials E_s more distant from E_{ecm} and will furthermore act permarently over a much larger adsorption time t_s. Thus, a joint effect of the constraint exerted by the adsorption forces and the additional interfacial electric field at the respective E_s is

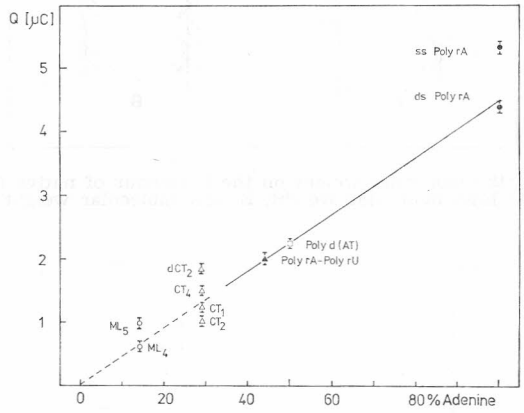

Figure 13. Variation in the reduction response Q of various native DNAs and polynucleotides as the function of percentage in adenine and of molecular weight Mw. 0.5 M McIlvaine buffer, pH 5.6, (pH 6 in the case of single strand Poly rA). Sweep rate 1 V s^{-1}; E_s — 0.4 V.

double strand Poly rA	25 μg/ml	Mw: $0.2 \cdot 10^6$
single strand Poly rA	25 μg/ml	Mw: $0.2 \cdot 10^6$
Poly d(AT)	35 μg/ml	Mw: $0.9 \cdot 10^6$
Poly rA. Poly rU	100 μg/ml	Mw: $1 \cdot 10^6$
calf thymus DNA CT_4	870 μg/ml	Mw: $0.5 \cdot 10^6$
calf thymus DNA CT_1	85 μg/ml	Mw: $3 \cdot 10^6$
calf thymus DNA CT_2	150 μg/ml	Mw: $5.3 \cdot 10^6$
(denatured) DNA dCT_2	50 μg/ml	
Micrococcus Lysodeikticus DNA ML_4	150 μg/ml	Mw: $9 \cdot 10^6$
Micrococcus Lysodeikticus DNA ML_5	50 μg/ml	Mw: $0.8 \cdot 10^6$

certainly responsible for the observed deconformation and the helix unwinding of the adsorbed native DNA[7,29,33,34]. A similar opening of the adsorbed double helical form of the homopolynucleotide polyriboadenylic acid (poly-A) has also been observed[51].

The extent of unwinding at a given value of E_s and t_s is also significantly influenced by the strength of the Watson-Crick base pairing. In other words, one expects a significant dependence on the *content of the more labile A-T-pairs* in a particular kind of DNA. This effect is clearly reflected by the dependence of the reduction response on the adenine content of various polynucleotides[35] (Figure 13).

The existence of rather labile zones rich in A-T-pairs in native DNA also explains why a certain reduction response is observed within very short times t_s after adsorption[34,41].

As shown in Figure 13 *molecular weight* also plays a role in native DNA of various origin[35,40]. This is because of the shape of native DNA. While at molecular weights up to 10^5 a rod like shape prevails, at higher molecular weights the double helix gradually adopts a coiled shape[56]. On adsorption the rod shaped native DNA tends to be adsorbed by a small number of contact sites per biopolymer and can be subsequently unwinded without great sterical

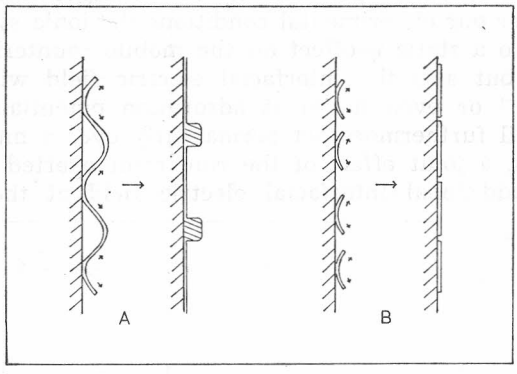

Figure 14. Influence of the molecular weight on the behaviour of native DNA at the electrode. A: high molecular weight, B: low molecular weight.

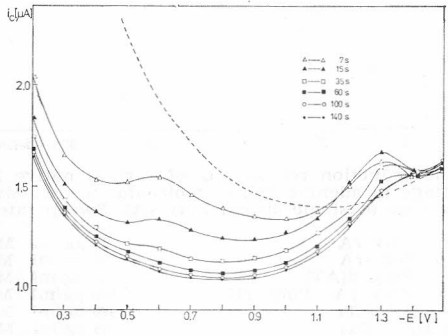

Figure 15. Capacitive a. c. component i_c as function of adsorption potential E_s and adsorption time for native calf thymus DNA. c 130 µg/ml; ionic strength 0.1 M; pH 5.6, McIlvaine buffer; 78 Hz; 5 mV_{pp}; △ 7 s; ▲ 15 s; □ 35 s; ■ 60 s; ○ 100 s; ● 145 s adsorption time t_s.

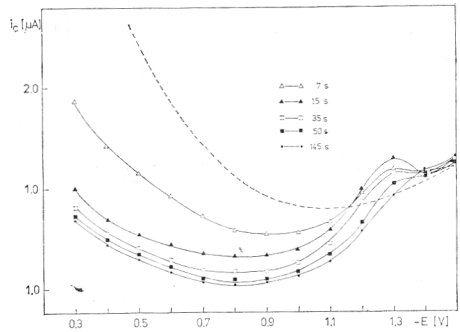

Figure 16. Capacitive a. c. component i_c as the function of adsorption potential E_s and adsorption time for denatured calf thymus DNA. c 50 μg/ml; △ 7 s; ▲ 15 s; □ 35 s; ■ 50 s; ● 145 s adsorption time. Other conditions as in Figure 15.

hindrance to a layer parallel with the electrode surface. On the other hand the coiled double helix becomes adsorbed in a looped manner. At each of its numerous contact points unwinding promotes in a convergent direction. Due to sterical constraint compact regions have to appear within a short time, and in these the reducible bases remain unaccessible to reduction[35] (schematic representation in Figure 14).

The above mentioned reorientation of the reducible bases occurring in the *a priori* single stranded denatured DNA[31,34] and in native DNA[34] after the rupture of the Watson-Crick interstrand hydrogen bonds is reflected for various adsorption times t_s by the nonfaradaic peak, which is obtained for both forms of adsorbed DNA around — 1.3 V by the out-of-phase current component of phase sensitive ac-voltammetry at the HMDE (Figures 15 and 16). For native DNA a further peak is obtained around — 0.6 V at not too extended adsorption times t_s. This nonfaradaic response is ascribed to a reorientation of the helical structure due to the electrostatic repulsion of the negatively charged phosphate groups. It can be observed only until the unwinding, progressing with the elapsed adsorption time t_s exceeds a certain limit.

CONCLUDING REMARKS

As a general conclusion results that the main contours of the complicated interfacial and electrochemical behaviur of native and denatured DNA at charged interfaces could be elucidated with the help of the large body of experimental evidence accumulated so far. Although it is possible to assess the experimental data obtained by various authors in a fully consistent manner, further experiments should be conducted to provide evidence supporting those details which are still of a speculative character.

Acknowledgement. The authors are indebted to Dr. B. Malfoy and Dr. J. M. Sequaris a Hached to our institute for many clarifying discussions and numerous inspired experiments on the subject.

REFERENCES

1. A. T. Ganesan and L. Lederberg, B.B.R.C. **18** (1965) 824.
2. D. E. Comings, *Am. J. Human Genetics* **20** (1968) 440.
3. R. L. O'Brien, A. B. Sanyal, and R. H. Stanton, *Exp. Cell Res.* **76** (1972) 106.
4. S. Gilmour, *New Scientist,* **65** (1975) 390.

5. H. W. Nürnberg in U. Zimmermann and J. Dainty (Eds.), »*Membrane Transport in Plants*«, Springer, Berlin—Heidelberg—New York 1974, p. 48.
6. H. W. Nürnberg and G. Wolff, *J. Electroanal. Chem.* **21** (1969) 99.
7. P. Valenta and H. W. Nürnberg, *Biophys. Struct. Mechanism* **1** (1974) 17.
8. E. Paleček, in J. N. Davidson and W. E. Cohn (Eds.), *Progress in Nucleic Acid Research and Molecular Biology*, Vol. 9, Academic Press, New York 1969, p. 31.
9. V. Vetterl, *Coll. Czechoslov. Chem. Commun.* **31** (1966) 2105.
10. V. Vetterl, *J. Electroanal. Chem.* **19** (1968) 169.
11. U. Retter, H. Jehring and V. Vetterl, *J. Electroanal. Chem.* **57** (1974) 391.
12. B. Janik and P. J. Elving, *Chem. Rev.* **68** (1968) 295.
13. B. Janik and P. J. Elving, *J. Amer. Chem. Soc.* **92** (1970) 235.
14. J. W. Webb, B. Janik, and P. J. Elving, *J. Amer. Chem. Soc.* **95** (1973) 911; 8495.
15. D. Krznarić, P. Valenta, and H. W. Nürnberg, *J. Electroanal. Chem.* **65** (1975) 863.
16. P. Valenta, H. W. Nürnberg, D. Krznarić, *Preprints 3rd Int. Symp. Bioelectrochemistry* Juelich 1975, p. 16; *Bioelectrochem. Bioenerg.* **3** (1976) in press.
17. E. Paleček and B. D. Frary, *Arch. Biochem. Biophys.* **115** (1966) 431.
18. E. Paleček and V. Vetterl, *Biopolymers* **6** (1968) 917.
19. E. Paleček in L. Grossman and K. Moldave (Eds.), *Methods in Enzymology*, Vol. 21, Academic Press, New York 1917, p. 3.
20. V. Brabec and E. Paleček, *Biophysik* **6** (1970) 290.
21. V. Brabec and E. Paleček, *J. Electroanal. Chem.* **27** (1970) 145.
22. V. Brabec and E. Paleček, *Biopolymers* **11** (1972) 2577.
23. H. Berg, H. Bär, and A. Golmick, *Biopolymers* **5** (1967) 61.
24. J. Flemming, *Biopolymers* **6** (1968) 1697.
25. H. Berg, D. Tresselt, J. Flemming, H. Bär, and G. Horn, *J. Electroanal. Chem.* **21** (1969) 181.
26. J. Flemming, *J. Electroanal. Chem.* **47** (1973) 137.
27. I. R. Miller, *J. Mol. Biol.* **3** (1961) 229.
28. H. Berg, J. Flemming, E. Paleček, and V. Vetterl, *Studia Biophys.* **31** (1972) 81.
29. P. Valenta and H. W. Nürnberg, *Wiss. Ergebnisber.* KFA Juelich (1972) p. 173; (1973) p. 201
30. P. Valenta and P. Grahmann, *J. Electroanal. Chem.* **49** (1974) 41.
31. P. Valenta and H. W. Nürnberg, *J. Electroanal. Chem.* **49** (1974) 55.
32. H. W. Nürnberg and P. Valenta, *J. Electroanal. Chem.* **57** (1974) 125.
33. P. Valenta, H. W. Nürnberg, and P. Klahre, *Bioelectrochem. Bioenerg.* **1** (1974) 487.
34. B. Malfoy, J. M. Sequaris, P. Valenta, and H. W. Nürnberg, *Preprints 3rd Int. Symp. Bioelectrochem.* Juelich 1975, p. 223; *Bioelectrochem Bioenerg.* **3** (1976) in press.
35. J. M. Sequaris, B. Malfoy, P. Valenta, and H. W. Nürnberg, *Preprints 3rd Int. Symp. Bioelectrochem.* Juelich 1975, p. 255; *Bioelectrochem Bioenerg.* **3** (1976) in press.
36. E. Paleček, *Coll. Czechoslov. Chem. Comm.* **39** (1974) 3449.
37. V. Brabec and E. Paleček, *Biophys. Chem.* **4** (1976) 79.
38. G. C. Barker and D. McKweon, *Preprints 3rd Int. Symp. Bioelectrochem.* Juelich 1975, p. 199, *Bioelectrochem Bioenerg.* **3** (1976) in press.
39. M. W. Humphreys and R. Parsons, *J. Electroanal. Chem.* in press. *trochem.* p. 174, *Bioelectrochem. Bioenerg.* **3** (1976) in press.
40. B. Malfoy and J. A. Reynaud, *J. Electroanal. Chem.* **67** (1976) 359.
41. H. Berg, J. Flemming, and G. Horn, *Bioelectrochem. Bioenerg.* **2** (1975) 287.
42. H. Berg, J. Flemming, and G. Horn, *Preprints 3rd Int. Symp. Bioelectrochem.* p. 174, *Bioelectrochem. Bioenerg.* in press
43. H. W. Nürnberg in I. S. Longmuir. (Ed.), *Advances in Polarography*, Vol. 2, Pergamon Press, London 1960, p. 694.
44. D. L. Smith and P. J. Elving, *J. Amer. Chem. Soc.* **84** (1962) 2741.
45. B. Janik and E. Paleček, *Arch. Biochem. Biophys.* **105** (1964) 225.

46. B. Malfoy, J. A. Reynaud, and P. J. Sicard, *Bioelectrochem. Bioenerg.* **1** (1974) 126.
47. J. Flemming, and H. Berg, *Bioelectrochem. Bioenerg.* **1** (1974) 460.
48. P. O. P. Ts'o, in D. Fasman and S. N. Timasheff (Eds.), *Biological Macromolecules*, Vol. 4, M. Dekker, New York 1970, p. 49.
49. H. W. Nürnberg and G. Wolff, *Coll. Czechoslov. Chem. Comm.* **30** (1965) 3997.
50. N. Vanlaethen-Meurée, J. P. Lambert, and L. Gierst, *Chem. Ing. Techn.* **44** (1972) 219.
51. P. Valenta, H. W. Nürnberg, and P. Klahre, *Bioelectrochem. Bioenerg.* **2** (1975) 204.
52. S. Lewin, *Displacement of Water and its Control of Biochemical Reactions*, Academic Press, New York 1974.
53. E. Neumann and A. Katchalsky, *Proc. Nat. Acad. Sci. USA* **69** (1972) 993.
54. A. Revzin and E. Neumann, *Biophys. Chem.* **2** (1974) 144.
55. T. L. Hill, *J. Amer. Chem. Soc.* **80** (1958) 2142.
56. M. T. Record and C. P. Woodbury, *Biopolymers* **14** (1975) 393.
57. B. Malfoy, J. M. Sequaris, P. Valenta, and H. W. Nürnberg, *J. Electroanal. Chem.*, in press.
58. G. A. Rodley, R. S. Scobie, R. H. T. Bates, and R. M. Lewitt, *Proc. Nat. Acad. Sci. USA* **73** (1976) 2959.

SAŽETAK

Voltametrijska istraživanja elektrokemijskog i međufaznog ponašanja DNA na nabijenim međufazama

H. W. Nürnberg i P. Valenta

Dan je pregled opsežnog i sustavnog istraživanja denaturirane i nativne DNA primjenom nove modifikacije cikličke voltametrije na živinoj elektrodi i nekih polarografskih tehnika. Izvedeni su adsorpcijski parametri, objašnjena je kinetika elektrodnih procesa, te slijed reakcija na granici faza, kao funkcija vremena i potencijala adsorpcije.

Kod pH $\geqslant$ 7, u adsorbiranoj denaturiranoj DNA jedinice adenina i citozina ireverzibilno se reduciraju tvoreći čvrsto adsorbirani kompaktni film reakcionog produkta. Međutim, kod jedinica adenina i citozina u adsorbiranoj nativnoj DNA dolazi do prenosa protona i elektrona tek nakon slijeda procesa dekonformacije, koji uključuju otvaranje i odmotavanje dvostrukog heliksa pod utjecajem adsorpcijskih interakcija i električnog polja na granici faza.

U fundamentalnom biofizičkom smislu dobiveni rezultati omogućuju opće zaključke o ponašanju DNA pri interakciji sa nabijenim međufazama u živoj ćeliji.

INSTITUTE OF CHEMISTRY,
INSTITUTE FOR APPLIED PHYSICAL CHEMISTRY,
NUCLEAR RESEARCH CENTRE (KFA), JUELICH,
F. R. GERMANY