ABSENCE OF UNWINDING OF DOUBLE-HELICAL DNA IN THE SURFACE OF MERCURY ELECTRODE CHARGED TO DNA REDUCTION POTENTIALS AT NEUTRAL pH*

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The influence of adsorption of DNA on its double-stranded (ds) structure was studied with the aid of hanging mercury drop electrode (HMDE) in neutral media. Changes in DNA reducibility followed by linear sweep voltammetry served as an indication of the DNA conformational changes in the electrode surface. It has been shown that, due to contact of ds DNA with the electrode for periods many times longer than those which can be reached at the dropping mercury electrode, extensive surface denaturation of DNA can be detected only in a narrow potential range around −1·2 V. At potentials more negative than this region neither surface denaturation nor blocking of the electrode by the reduction product were observed. On the other hand, interaction of the denatured DNA with the mercury surface under the same conditions, resulted in blocking of the electrode surface by the reduction product. In agreement with the previous studies in acid media similar blocking of the surface was observed at pH 5 both with thermally denatured and ds DNAs.

On the basis of the experimental data the following conclusions have been drawn: *i*) in neutral media the course of interfacial events of DNA is strongly dependent on DNA conformation and differs from the course suggested for acid media; *ii*) the deduction concerning the behaviour of ds DNA on the electrode in neutral media made by other authors on the basis of their measurements in acid media is not justified.

We have shown in our previous papers[1–4] that there is a correlation between the conformation of DNA in solution and its polarographic reducibility, if measurements are performed by means of methods working with small excursions of potential during the drop life (*e.g.*, d.c. polarography or derivative pulse polarography in connection with the mercury dropping electrode (DME). Recently it has been shown in our own[4–6] and Nürnberg's[7] laboratories that double-stranded (ds) DNA undergoes structural changes due to its adsorption on the mercury electrode at potentials more positive than the DNA reduction potentials. These changes have been detected by means of methods working with large potential excursions (single sweep voltammetry or normal pulse polarography). It follows from our measurements[4–6] (performed in neutral media at the DME) that changes in DNA conformation depend on the potential of the electrode. An extensive disturbance (surface denaturation) of the double-helical structure of DNA (which can approach

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100%) occurs in a narrow potential range around $-1{\cdot}2$ V (region U) (Fig. 1). In a wide range of more positive potentials (region T) only a small extent of changes in DNA conformation was detected[6,7]. These changes probably took place in the vicinity of labile parts of the DNA molecule and represented only several per cent of the whole molecule. Nürnberg and coworkers[8,9] measured at acid pH, using a hanging mercury drop electrode (HMDE), and did not distinguish between these two regions. We have demonstrated[10] that around pH 5 both regions can merge even if measurements are performed with the aid of the DME.

However, so far it has not been established whether well-distinguished regions U and T can be observed at neutral pH even in connection with the HMDE, where much longer periods of interaction of DNA with the electrode surface can be reached (compared with the DME). The results of our studies of the behaviour of ds and thermally denatured DNAs in neutral media by voltammetry, presented in this paper, show a good qualitative agreement between the measurements at the DME and the HMDE. It follows from our data that some generalizing conclusions drawn by Nürnberg and coworkers[8,9,11] were not justified.

EXPERIMENTAL

Calf thymus DNA was isolated and characterized as described earlier[6,12]. Denaturation was performed by heating DNA at a concentration of 100 μg/ml in 0·015M-NaCl with 0·0015M sodium citrate (pH 7) at 100°C for 6 minutes.

Voltammetric measurements were performed with the Polarographic Analyzer PAR 174 in conjunction with the Universal Programmer PAR 175 (Princeton Applied Res. Corp.). A three-electrode system was used including HMDE, Ag-counter electrode and a reference Ag/AgCl electrode, Metrohm EA 428. The working electrode was a Metrohm hanging drop electrode E 410 with a surface of 1·40 mm^2. Voltammetric curves were recorded either by means of an $x - y$ recorder, Watanabe, WX 441 or by an OG2—21 Speicheroszilloskop, Musselektronik Berlin, GDR, in the $x - y$ mode. Other conditions of voltammetric measurements were described in our previous paper[6]. Full surface coverage of the HMDE with thermally denaturated DNA has been reached in less than 10 s. The measurements were performed either in 0·3M ammonium formate with 0·05M sodium phosphate pH 6·8 or in 0·3M-CsCl with 0·1M sodium phosphate 6, 9 or 7·0, *i.e.*, in the background electrolytes used in our previous work[1,2,7] with DME.

The programming of the electrode potential by the potentiostatic double step-sweep method applied in some experiments agreed in principle with the procedure described by Malfoy and coworkers[9]. The potential corresponding to the first step is in this paper indicated by E_{i1} (instead of E_c) and the potential of the second step, E_{i2} (instead of E_s). In some cases we have applied also a third step, the potential of which is denominated by E_{i3}. Accordingly, the times for which the respective potentials were applied to the HMDE are specified as t_1, t_2 and t_3. Measurements were performed in a Metrohm cell. Chemicals used for the preparation of the background electrolytes were of analytical grade.

RESULTS

We studied the dependence of voltammetric curves of DNA on the initial potential E_i which was applied to the electrode (HMDE) for a period $t = 60$ s or 120 s. In the medium of 0·3M-CsCl with 0·05M sodium phosphate pH 6·9 ds and denatured DNA

produced similar peaks as those obtained by means of DME. (If DME is used denatured DNA produces a capacitive peak I at about $-1{\cdot}2$ V and a faradaic peak III at about $-1{\cdot}5$ V (against S.C.E.); the height of peak III is almost independent of E_i in the regions T and U (ref.[6,7]). Ds DNA yields, besides these two peaks, peak II which is about 150 mV more positive than peak III; heights of peaks II and III of ds DNA are strongly dependent on E_i). Also the shape of dependence of the height of peak III of ds DNA on E_i qualitatively agreed with that of the dependences obtained with the aid of the DME (ref.[5-7]): the peak heights were very small if the applied E_i was in the region T (Fig. 1), while relatively high peaks were produced due to interaction of ds DNA with the electrode charged to E_i around $-1{\cdot}2$ V (region U). The maximum peak height at $E_i = -1{\cdot}20$ V and $t = 120$ s reached about 85% of the height of the peak produced by thermally denatured DNA under the same conditions. At E_i of the region T a small more positive peak II was observed. This peak disappeared completely at E_i of the region U (Fig. 1). The thermally denatured DNA yielded under the same conditions only peak III whose height was almost independent of E_i.

Potentistatic double step-sweep method. In our previous paper[6] we have used a DME with a drop life of 10 s, which was first kept for 5 s at a certain potential of the region U and then a different potential was applied (*e.g.* that of region T) for another 5 s. (The purpose of our measurements was to find out whether the changes in ds DNA caused by its adsorption on the DME were reversible.) This method has recently been used by Malfoy and coworkers[9] in connection with a suitable commercially available apparatus for studies of the DNA behaviour at the HMDE in weakly acid media. The method has been called by these authors the potentiostatic double step-sweep method. We have used this method in our studies of the behaviour of ds and denatured DNA at the HMDE in neutral media. DNA was first in contact with the electrode charged to E_{i1} for a period $t_1 = 60$ s and then for another 60 s (t_2) with the same drop of mercury charged to E_{i2}. The dependence of the peak heights of denatured and ds DNA on E_{i1} (at $E_{i2} = -0{\cdot}4$ V) in a neutral medium is shown in Fig. 2*a*.

Thermally denatured DNA. For the thermally denatured DNA the course of this dependence is in a good agreement with the measurements in weakly acidic media[9]. A steep decrease in the peak height around $E_{i1} = -1{\cdot}4$ V suggests that the electrode surface is blocked by the reduction product. An increase in the peak height that follows around $E_{i1} = -1{\cdot}6$ V corresponds with the idea of Malfoy and coworkers assuming that no blocking of the electrode surface occurs at more negative potentials (around $-1{\cdot}6$ V). A decrease of the peak III at potentials more negative than $-1{\cdot}6$ V may be connected with changes at the electrode surface due to background discharge.

Double-stranded DNA. The dependence of the peak height of ds DNA on E_{i1} (Fig. 2*a*) is similar to the simple dependence on E_i (Fig. 1) which does not include

the potentiostatic double step. However, in the case of the potentiostatic double step-sweep technique, more negative potential than −1·35 V can be reached. It follows from the Fig. 2*a* that in the region of $E_{i1} = -1{\cdot}35$ V to −1·50 (region W) the height of the peak of ds DNA is almost independent of E_{i1} and the peak is only slightly smaller than that obtained at $E_i = -0{\cdot}40$ V. This fact suggests that in the case of ds DNA the electrode surface is not blocked in the region W. In an attempt to verify this suggestion we have studied the dependence of the peak height of ds DNA on E_{i2} at $E_{i1} = -1{\cdot}35$ V (*i.e.*, at E_{i1}, where blocking of the electrode surface could be expected, if the DNA reduction were independent of conformation of DNA

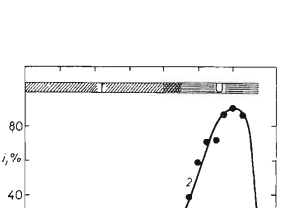

FIG. 1

Dependence of the Heights of Voltammetric Peaks II and III of ds DNA on Initial Potential E_i

100 μg of calf thymus DNA/ml in 0·3M CsCl with 0·05M sodium phosphate pH 6·9. *1* peak II; *2* peak III. Peak heights are expressed in per cent of the peak III of thermally denatured DNA; the maximum height of the peak produced by thermally denatured DNA at $E_i = -1{\cdot}20$ V was taken as 100%. HMDE, waiting time 120 s, sweep rate 1 V/s.

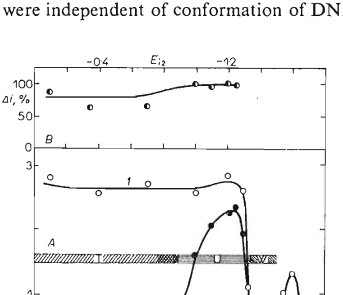

FIG. 2

Dependence of the Height of Voltammetric Peak III of ds and Thermally Denatured DNAs on Initial Potential E_i as Measured by Potentiostatic Double Step-Sweep Method

100 μg of calf thymus DNA/ml in 0·3M ammonium formate with 0·05M sodium phosphate pH 6·8. *A* Dependence on E_{i1}; $E_{i2} = -0{\cdot}40$ V, $t_1 = 60$ s, $t_2 = 60$ s. *1* thermally denaturated DNA; *2* ds DNA. *B* Influence of application of $E_{i1} = -1{\cdot}35$ V on the percentual change in the height of the peak III of ds DNA at various E_{i2}. $t_1 = 60$ s, $t_2 = 60$ s. The heights of the peak III of ds DNA obtained after prepolarization of the electrode (60 s) to the potentials indicated in the graph (as E_{i2}) without preceding application of $E_{i1} = -1{\cdot}35$ V were taken as 100%. Sweep rate 1 V/s.

in the bulk of the solution – double-helical or single-stranded – as assumed by Malfoy and coworkers[9]). The heights of the peaks were compared with those obtained at E_i corresponding to E_{i2}, without preceding application of $-1{\cdot}35$ V. The results of these measurements (Fig. 2*b*) show that the application of $E_{i1} = -1{\cdot}35$ V has in no case eliminated the peak (as it is the case with the thermally denatured DNA – Fig. 2*a*). At E_{i2} corresponding to the potentials of the region U the height of the peak was practically not influenced by the application of $E_{i1} = -1{\cdot}35$ V. At more positive E_{i2} some decrease in the peak height was observed that did not exceed 37%. We further followed the influence of the application of E_{i1} in the range of $-1{\cdot}35$ to $-1{\cdot}50$ V on the height of the peak obtained at constant $E_{i2} = -1{\cdot}20$ V. Even in this case the application of E_{i1} had only very little influence on the peak heights (Table I). If we compared the height of peak III of ds DNA at $E_{i1} = -1{\cdot}20$ V and $E_{i2} = -0{\cdot}40$ V ($t_1 = 60$ s, $t_2 = 60$ s) with the peak height obtained at $E_{i1} = -1{\cdot}20$ V ($t_1 = 60$ s) followed by immediate sweep without any further potential steps, we found that both values were practically the same. It can thus be concluded that changes observed in the region U are irreversible as suggested in our previous paper[6], on the basis of data obtained with the aid of the DME.

TABLE I

Heights of the Voltammetric Peaks Produced by ds DNA after Prepolarization of HMDE to Various E_i

Medium	E_{i1}, V	t_1, s	E_{i2}, V	t_2, s	E_{i3}, V	t_3, s	Height of peak III %
0·3M Ammonium formate,	−1·20	60	–		–		100[a]
	−1·30	60	−1·20	60	–		114
0·05M sodium phosphate, pH 6·8	−1·35	60	−1·20	60	–		97
	−1·40	60	−1·20	60	–		91
	−1·45	60	−1·20	60	–		92
	−1·50		−1·20	60	–		87
0·3M-CsCl,	−1·20	60	–		–		100[a]
0·1M sodium phosphate, pH 7	−1·20	60	−1·35	60	–		0
	−1·20	60	−1·35	60	−1·20	60	0
0·3M-CsCl,	−1·20	60	–		–		100[a]
0·1M sodium acetate, pH 5	−1·35	60	−1·20	60	–		12

[a] Peak height taken as 100%.

Our results show that the application of E_i around $-1{\cdot}35$ V on the HMDE immersed in solution of ds DNA at neutral pH does not result in blocking of the electrode surface (Fig. 2*b*, Table I). On the other hand, if the solution contains thermally denatured DNA, the electrode surface is under the same conditions blocked by the reduction product (Fig. 2*a*). We attempted to find out whether surface blocking occurred in the case where ds DNA was subjected to surface denaturation at the electrode charged to $E_i = -1{\cdot}20$ V. We compared the height of peak III obtained with a ds DNA solution at $E_{i1} = -1{\cdot}20$ V ($t_1 = 60$ s) to those obtained by means of potentiostatic double step-sweep ($E_{i1} = -1{\cdot}20$ V, $E_{i2} = -1{\cdot}35$ V; $t_1 = 60$ s, $t_2 = 60$ s) and triple step-sweep techniques ($E_{i1} = -1{\cdot}20$ V, $E_{i2} = -1{\cdot}35$ V, $E_{i3} = -1{\cdot}20$ V; $t_1 = 60$ s, $t_2 = 60$ s, $t_3 = 60$ s). As it follows from Table I, the application of $E_{i2} = -1{\cdot}35$ V resulted in the disappearance of the peak and the application of $E_{i3} = -1{\cdot}20$ V did not cause any reappearance of the peak. It can thus be concluded that if DNA originally contained in the bulk of the solution in its double-helical form undergoes denaturation at the electrode surface (charged to potentials of the region U) it behaves similarly to that which has been denatured by heating in the bulk of solution: *i.e.*, in either case reduction products block the electrode surface.

We performed also measurements at pH 5 using the potentiostatic double step-sweep method. Our data (Table I), in agreement with conclusions of Malfoy and coworkers, suggest that the application of $E_{i1} = -1{\cdot}35$ V results (at acid pH) in the blocking of the electrode surface, both with thermally denatured and ds DNA in the bulk of the solution.

DISCUSSION

This paper shows that the contact of ds DNA with the mercury electrode for periods many times longer than those which can be reached with DME, results in the extensive surface denaturation of DNA (at neutral pH) only in a narrow potential range of the region U (Fig. 1). Peak III (which serves as an indication of the surface denaturation) decreases and disappears at E_i more negative than the potentials of the region U. The disappearance of this peak may be explained either (*i*) by the absence of surface denaturation of DNA at negative potentials (region W) or (*ii*) by an assumption[9] that DNA does unwind in the electrode surface charged to potentials of region W, but peak III cannot appear due to blocking of the electrode surface by the reduction product. However, the latter possibility is excluded by our measurements by means of the double step-sweep method (Fit. 2, Table I) which unequivocally demonstrate that peak III can appear. These measurements further show that at neutral pH the electroreduction of DNA (previously denatured at the electrode charged to potentials of region U) is only very slightly effected by the history of the electrode preceding the electroreduction (*e.g.*, the application of E_{i1} in the range of $-1{\cdot}30$ to $-1{\cdot}50$ V for $t_1 = 60$ s has almost no influence on the height of peak III, which ap-

peared in connection with the surface denaturation of DNA due to application of $E_{i2} = -1{\cdot}20$ V – Table I). It can thus be concluded that no changes in the conformation of ds DNA (resulting in the release of a detectable amount of bases for the electroreduction) occur at the mercury electrode charged to potentials of the region W. This conclusion is in a good agreement with the results of our recent experiments using a large mercury pool electrode[7]. We showed that even after a 70 min contact of the solution of ds DNA with the mercury pool electrode charged to $-1{\cdot}35$ V no denatured DNA was formed. On the other hand, if this electrode was charged to more positive potentials, we found, under the same conditions, about 80% of DNA in the denatured state.

The absence of changes in DNA conformation in the region W can be either due to (*i*), no adsorption of ds DNA at potentials of the region W or (*ii*), the adsorption of certain segments of ds DNA in such a way (probably very weakly) that it does not induce changes in the DNA double helix which can be detected by linear sweep voltammetry. The results of measurements of the differential capacity of the mercury electrode by means of bridge method[13] and a.c. polarography[12] and the results of d.c. polarography are in agreement with the former possibility (*i*). On the other hand, the fact that ds DNA yields, in neutral media, the derivative (differential) pulse-polarographic peak II[2–4,15] and a phase-in a.c. polarographic peak[16] favours the latter possibility (*ii*). Case (*i*) can be excluded for degraded low-molecular weight ds DNA because the characteristic of its derivative pulse-polarographic peak indicates participation of adsorption in the electrode process[17]. (The same is true for an intact ds DNA in an acid media[3].) In the case of intact ds DNA in neutral media both alternatives should be, however, considered.

Malfoy and coworkers[11] developed a model of interfacial events during interactions of DNA with the mercury electrode. It is assumed in this model that ds DNA is adsorbed and unwound in the surface of the electrode charged to potentials of reduction of denatured DNA and that the reduction product blocks the electrode surface. While the question of adsorption of ds DNA at these potentials has not been definitely solved, our experimental data exclude the DNA unwinding and surface blocking at neutral pH. The above model[11] may be of certain significance for the behaviour of ds DNA when voltammetric methods working with large potential excursions during the life of the mercury drop are applied (*i.e.*, in cases where DNA interacted for appreciable time with the electrode charged to potentials more positive than the reduction potential). In the experiments with the methods working with small excursions of potentials in connection with the DME (at neutral pH), the scheme of Malfoy and coworkers[11] should not be considered.

It is important that even a 2 min interaction of ds DNA charged to potentials of the region T does not result (at neutral pH) in an extensive surface denaturation comparable to that occurring at E_i of the region U (the peak III at E_i of the region T is relatively very small – Fig. 1, 2*a*). It can thus be assumed that DNA adsorbed

at the electrode charged to potentials of the region T possesses an almost undisturbed double-helical structure (from the point of view of the degree of availability of bases for the electroreduction). On the other hand, the interaction of ds DNA with the electrode charged to potentials of the region U can, under certain conditions, result in unwinding of more than 90% of ds DNA[17]. It is thus possible to study at neutral pH kinetics of the helix-coil transition of DNA adsorbed in the electrode surface. Similar measurements at acid pH[9] followed only the transition of DNA from the 85% unwound form to that which reached maximum surface denaturation (*i.e.*, about 93%). It can be expected that the studies of the kinetics of the helix-coil transition of DNA adsorbed at neutral pH will bring a deeper insight into the mechanism of the interfacial events during DNA interactions with the electrode.

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