NORMAL PULSE POLAROGRAPHY OF DOUBLE-HELICAL DNA: DEPENDENCE OF THE WAVE HEIGHT ON STARTING POTENTIAL

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Solutions of double-helical and thermally denatured DNA were studied by means of normal and derivative (differential) pulse polarography, a.c. polarography and current sampled d.c. polarography. It was found that the height of the normal pulse-polarographic wave of double-helical DNA was dependent on starting potential; in a narrow potential range around −1·2 V (S.C.E.) the wave is, under the given experimental conditions, more than doubled. The potentials at which the increase of the wave took place corresponded with the potential of the a.c. polarographic peak I of the double-helical DNA. The height of the normal pulse-polarographic wave of denatured DNA was almost independent of starting potential. It was concluded that as a result of the interaction of the double-helical DNA with the electrode surface in the vicinity of −1·2 V, DNA conformation is disturbed, and some reduction sites, previously hidden in the interior of the molecule, are released for the electrode process.

It has been shown that single-stranded nucleic acids containing adenine and/or cytosine produce reduction polarographic currents[1-3]. Double-helical nucleic acids in which the adenine and cytosine reduction sites are hidden in the interior of the molecule (and form a part of the hydrogen-bond system) are polarographically nonreducible or produce relatively low currents at more positive potentials[1-4] compared with the corresponding single-stranded polynucleotides. For analytical purposes the derivative (differential) pulse polarography has mostly been used, due to its high sensitivity for the estimation of single-stranded polynucleotides as well as for the detection of small conformational changes in double-helical polynucleotides[1-8]. In spite of the fact that the normal variant of this method, similarly to the derivative method, yields good results in the analysis of low-molecular weight substance, the former has been used in the study of nucleic acids to a considerably smaller extent[9,10].

According to our experience normal pulse polarography reflects rather poorly differences in conformation of single- and double-stranded polynucleotides. In this paper we have attempted to find limitations of this method in nucleic acid analysis and we have ascertained that during the electrode polarization used in this technique the DNA double-helical structure may be disturbed as a result of DNA interaction with the mercury electrode surface.

EXPERIMENTAL

Calf thymus DNA was isolated and characterized as described earlier[11]. 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. Polarographic measurements were carried out in buffered ammonium formate solutions suitable for nucleic acid analysis[2,3]. The current-sampled d.c. polarographic and pulse-polarographic measurements were performed with the polarographic analyzer PAR 174 (further PAR 174), Princeton Applied Res. Corp., with a dropping mercury electrode (DME) in a nitrogen atmosphere. The capillary had a drop time 4·4 s at a mercury column height of 42 cm. The drop time was controlled mechanically. If not otherwise stated the following instrument setting was used: potential scan rate 1 mV/s, potential scan range 0·75 V, drop time 2 s. A three-electrode system was used including DME, a Pt-counter electrode and a saturated calomel electrode (S.C.E.). Some pulse-polarographic measurements were carried out on an A 3100 Southern-Harwell pulse polarograph (further A 3100), Southern Analytical Ltd. All potentials were reported *vs* S.C.E. DNA concentration was estimated spectrophotometrically on a Zeiss VSU 2-P apparatus; pH values were measured with a Pye Universal instrument. A.c. polarographic measurements were performed with a GWP 563 Polarograph; the a.c. voltage amplitude was 20 mV at 78 cycles per second; other details were given earlier[11].

RESULTS AND DISCUSSION

Native DNA at a concentration of 500 μg/ml and thermally denatured DNA at a concentration of 50 μg/ml were measured in 0·6M ammonium formate with 0·1M sodium phosphate pH 6·8 by means of current-sampled d.c. polarography, derivative and normal pulse polarography. In agreement with our previous studies[1,3,11] native DNA yielded no d.c. polarographic wave (Fig. 1*a*), while denatured DNA produced a small d.c. polarographic wave (Fig. 1*b*). The results of derivative pulse polarography resembled to a certain extent those of d.c. polarography: denatured DNA produced a well-developed peak *III* (Fig. 2*b*) while native DNA yielded a relatively small peak *II* (Fig. 2*a*) which appeared at potentials about 50 mV more positive than peak *III*.

On the contrary the normal pulse-polarographic wave of native DNA (at a concentration of 500 μg/ml) was higher than that of denatured DNA (50 μg/ml) (Fig. 1 *c*, *d*), and $E_{1/2}$ of both waves was almost the same. When the pulse direction was reversed (with an A 3100 apparatus) the wave of denatured DNA was almost 1/8 of that corresponding to the usual pulse direction, while native DNA produced no wave. This fact suggests an irreversible reduction of DNA, in agreement with our previous oscillopolarographic studies of DNA (ref.[1]), as well as with the pulse-polarographic behaviour of poly(C) (ref.[5]) and poly(A) (ref.[6]). At equal DNA concentrations (and the usual pulse direction) the wave of native DNA was only about five times smaller than the wave of denatured DNA (Fig. 3). This small difference in the wave heights of native and denatured DNA (as compared with d.c. polarography and derivative pulse polarography) is rather surprising, because normal pulse polarography had so far given results which agreed well with that of d.c. polarography and with polarographic theory (*e.g.*[12]). However, with few exceptions only relatively simple systems

were studied by means of normal pulse polarography. In normal pulse polarography the chosen starting potential is applied to each drop of mercury during the time preceding the application of the voltage pulse. If the depolarizer is adsorbed on the electrode at potentials corresponding to the starting potential, and besides if some properties of the depolarizer might be changed as a result of its adsorption, it can be expected that normal pulse polarography will produce data differing from d.c. polarographic measurements.

Our study of the dependence of the normal pulse-polarographic wave height of native DNA on the starting potential showed a steep increase of the wave in the potential region of $-1{\cdot}0$ to $-1{\cdot}2$ V (Fig. 4); further shift of the starting potential to more negative values caused a decrease of the wave height. The height of the wave of denatured DNA was only slightly dependent on starting potential. Further, the ratio between the wave heights of native and denatured DNAs was studied in dependence on drop times at various starting potentials (Fig. 5). The measurements showed that this ratio grows with increasing drop time (in the range 0·5 to 5·0 s) at a starting potential of $-1{\cdot}2$ V, while at $-0{\cdot}8$ and $-1{\cdot}37$ V it is almost independent of the time of drop.

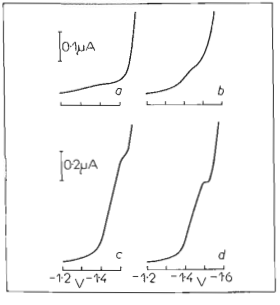

FIG. 1

Polarograms of Native and Denatured DNA

Upper curves: current-sampled d.c. polarography; lower curves: normal pulse polarography. *a*, *c* native DNA 500 μg/ml; *b*, *d* denatured DNA 50 μg/ml. 0·6M ammonium formate with 0·1M sodium phosphate pH 6·8. PAR 174.

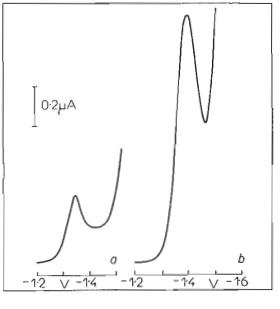

FIG. 2

Derivative Pulse Polarograms of Native and Denatured DNA

a Native DNA 500 μg/ml; *b* denatured DNA 50 μg/ml. Other conditions as in Fig. 1.

In agreement with the dependence of the DNA normal pulse-polarographic wave on starting potential (Fig. 4) was the dependence of the derivative pulse-polarographic peak on the pulse amplitude (Fig. 6). The heights of peaks of native and denatured DNA grew non-linearly with increasing pulse amplitude similarly to peaks of poly(C)[5] and poly(A)[6]. Up to 50 mV both peaks increased roughly in the same way. When the amplitude was increased to 100 mV a sudden change in the relation between peak heights of both DNA forms was observed. The peak of denatured DNA increased by a factor of 2·3 (as compared with the peak height at 50 mV) while the peak of native DNA increased about five-times. The anomalous behaviour of native DNA can be understood if we take into consideration that if the electrode is charged to about −1·25 V before the pulse starts, the maximum potential of the 100 mV pulse corresponds to the summit potential of the peak. When the potential of −1·25 V was used as a starting potential in normal pulse-polarographic measurements, the normal pulse-polarographic wave was roughly twice as high (see arrow in Fig. 4) as compared with the wave height at starting potential of −0·2 V.

It follows from a.c. polarographic measurements[13] that the strongest adsorption of DNA takes place around the potential of an electrocapillary maximum (further

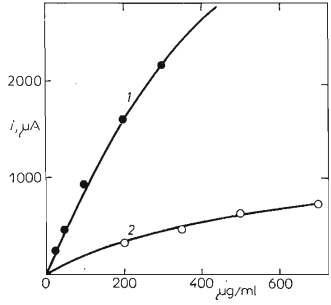

FIG. 3

Dependence of the Normal Pulse-Polarographic Wave Height on DNA Concentration

1 Denatured DNA; *2* native DNA; other conditions as in Fig. 1.

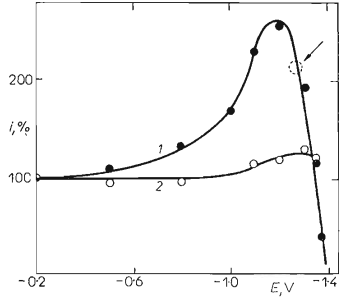

FIG. 4

Dependence of the Normal Pulse-Polarographic Wave Height of DNA on Starting Potential

1 Native DNA 500 μg/ml; *2* denatured DNA 50 μg/ml. The wave heights of native and denatured DNA at a starting potential of −0·2 V were taken as 100%. Scan range 1·5 V, other conditions as in Fig. 1.

ECM), that is under conditions of our experiments, around −0·5 V (Fig. 7) and that denatured DNA is adsorbed more strongly than native DNA. Thus the great increase of the normal pulse-polarographic wave of native DNA around −1·2 V (Fig. 4) cannot be explained by increased DNA accumulation in the electrode surface at this potential. It is more probable that in the electrode surface conformational changes occur in double-helical DNA which result in a release of the reduction sites for the electrode process. These conformational changes might be either similar to premelting of DNA[1,7] or to DNA melting which includes unstacking of bases and a collapse of the double-helical structure. The almost identical $E_{1/2}$ of normal pulse-polarographic waves of native and denatured DNA (Fig. 2) show the latter alternative to be more probable. While no quantitative calculation of the rate of the DNA conformational changes can be made on the basis of our data, certain rough estimates are possible: *1*) Conformational changes taking place in the neighbourhood of the electrode at −1·2 V are relatively slow (of the order of seconds). *2*) At starting potentials −0·8 V and −1·37 V either no conformational changes take place or they are slower than those taking place at −1·2 V. The fact that native DNA does not produce even in a high concentration a d.c. polarographic wave (Fig. 1*a*) can be understood if the decrease of the normal pulse-polarographic wave in the range of starting potentials

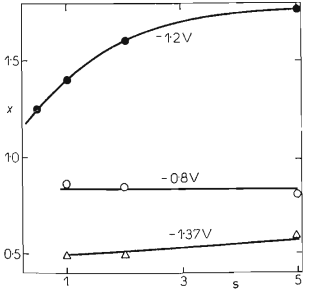

FIG. 5

Dependence of Ratio (X) of Wave Heights of Native and Denatured DNA on Drop Time at Various Starting Potentials

Mercury column height 25 cm; other conditions as in Fig. 1.

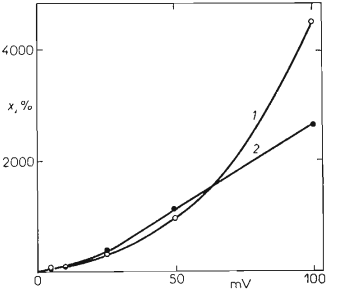

FIG. 6

Dependence of the Height of the Derivative Pulse-Polarographic Peak of Native and Denatured DNA on Pulse Amplitude

1 Native DNA; 2 denatured DNA. The heights of the peaks of native and denatured DNA at an amplitude of 10 mV were taken as 100%; x relative peak height.

of −1·3 to −1·4 V (Fig. 4) is considered. At the potentials of the denatured DNA reduction no substantial release of the reducible groups from the DNA double helix is taking place and therefore no reduction current is produced by native DNA.

The disturbance of the DNA double-helical structure in the neigbourhood of the electrode might be caused by various factors, such as the electric field, changes in the ionic atmosphere and in the hydration shell of the molecule due to adsorption *etc.* There is, however, no reason to expect that any of these factors would act only in a relatively narrow potential range around −1·2 V (Fig. 4). It has been shown in our previous paper[13] that the double-helical DNA is adsorbed in the vicinity of potential of ECM by the sugar-phosphate residues and possibly by sporadic bases. At the potential of the a.c. polarographic peak *1* (Fig. 7) the sugar-phosphate residues are desorbed from the electrode. The range of starting potentials in which the conformational changes occur (Fig. 4) corresponds with the potential of the a.c. polarographic peak *1* (Fig. 7). It is highly probable that at the potential of this peak some segments of a DNA molecule (which is sequentionally and conformationally unhomogenous) are adsorbed on the electrode while other unadsorbed segments of the same molecule are strongly repulsed from the electrode surface (mainly electrostatically, due to their negatively charged phosphate groups). The DNA molecules anchored in the electrode surface at these potentials may thus be subjected to considerable strains which could result in the disruption of the DNA conformation. The explanation offered is limited to the experimetal conditions used in this paper. Miller[14,15] measured the differential capacity of the mercury electrode double layer and concluded, on the basis of his data, that double-helical DNA is unwound at positive potentials; at potentials more negative than the potential of ECM, DNA structure remained intact. Miller's experiments were performed at pH 6 in unbuffered 0·1M-NaCl. Miller's work was recently criticized by Flemming[16] (using a similar experimental technique to Miller), who believed that the DNA interaction with the electrode

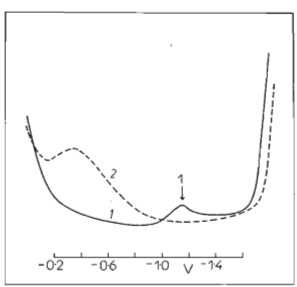

FIG. 7

A. c. Polarograms of Native DNA

1 DNA 500 μg/ml in 0·6M ammonium formate with 0·1M sodium phosphate pH 6·8; *2* supporting electrolyte.

resulted in no changes in DNA conformation. It follows from our preliminary data, that the DNA conformation may be disturbed on the electrode at various conditions. With changed conditions, however, the potentials at which these changes take place, as well as the mechanisms involved, are also changed. This involves especially a lowering of pH, which may cause protonation of some bases. There is little doubt that the DNA interaction with the electrically charged membranes play an important role in the DNA replication *in vivo*[17]. The electrode immersed in the DNA solution may become an important tool for the modelling of these interactions.

The results of this paper show that normal pulse polarography is rather inconvenient for the analysis of polynucleotide conformation in solution. Much more suitable for this purpose is derivative pulse polarography with sufficiently small pulse amplitude. On the other hand normal pulse polarography can be used for studies of conformational changes of double-helical polynucleotides which may occur as a result of longer polynucleotide contact with the electrode at a certain starting potential. For the same purposes the single sweep variant of oscillographic polarography (with both voltage and current controlled) can be used. The results of the studies of the dependence of the polarographic signal on starting potential, obtained by both techniques[18] agreed in principle with those of normal pulse polarography measurements presented in this paper.

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REFERENCES

1. Paleček E. in the book: *Progress in Nucleic Acid Research and Molecular Biology* (J. N. Davidson, W. E. Cohn, Eds), Vol. 9, p. 31. Academic Press, New York 1969.
2. Paleček E. in the book: *Methods in Enzymology* (L. Grossman, K. Moldave, Eds), Vol. 21, p. 3. Academic Press, New York 1971.
3. Paleček E., Brabec V.: Biochim. Biophys. Acta *262*, 125 (1972).
4. Bezděková A., Paleček E.: Studia Biophysica *34*, 141 (1969).
5. Paleček E.: This Journal *37*, 3198 (1972).
6. Brabec V., Paleček E.: Z. Naturforsch. *28c*, 685 (1973).
7. Paleček E., Frič I.: Biochem. Biophys. Res. Commun. *47*, 1262 (1972).
8. Paleček E., Doskočil J.: Anal. Biochem. *60*, 518 (1974).
9. Janik B., Sommer R. G.: Biochim. Biophys. Acta *269*, 15 (1972).
10. Janik B., Sommer R. G.: Biochim. Biophys. Acta *281*, 152 (1972).
11. Paleček E., Vetterl V.: Biopolymers *6*, 917 (1968).
12. Brinkman A. A. A. M.: *Theory of Pulse Polarography*. Bronder, Rotterdam 1968.
13. Brabec V., Paleček E.: Biopolymers *11*, 2577 (1972).
14. Miller I. R.: J. Mol. Biol. *3*, 229 (1961).
15. Miller I. R.: J. Mol. Biol. *3*, 357 (1961).
16. Flemming J.: Biopolymers *6*, 1697 (1968).
17. O'Sullivan M. A., Sueoka N.: J. Mol. Biol. *69*, 237 (1972).
18. Paleček E.: Unpublished results.