REDUCTION AND TENSAMMETRIC PULSE-POLAROGRAPHIC CURRENTS OF POLYNUCLEOTIDES

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The behaviour of electrochemically reducible single-strand polynucleotides (poly(adenylic acid)) and poly(cytidylic acid)) was studied by the differential (derivative) pulse polarography (DPP) and by other methods. Measurements were performed with the help of the dropping mercury electrode under various conditions specified by the pulse width, pulse amplitude, drop time *etc.* For the faradaic and tensammetric DPP peaks the diagnostic criteria were proposed which make it possible to classify even very small DPP peaks of double helical polynucleotides.

Modern polarographic methods[1,2], particularly the differential (derivative) pulse polarography (DPP) have been successfully exploited in the study of biological macromolecules, notably in the analysis of changes in conformation of nucleic acids[3-5]. Two of the four bases usually found in DNA, namely adenine and cytosine, are polarographically reducible. Single-strand polynucleotides containing these bases, *e.g.* poly(adenylic acid) (poly(A)), poly(cytidylic acid) (poly(C)) or the denatured DNA, give rise to cathodic polarographic waves[3]. On the other hand, the behaviour of double helical polynucleotides is remarkably different[3]. While the native DNA gives two DPP peaks denoted[3] as peaks I (at about $-1{\cdot}0$ V *vs* SCE) and II (at about $-1{\cdot}3$ V), the denatured (*i.e.* single-strand) DNA gives instead of peak II a further peak denoted[3] as peak III. As compared with the peak II, the peak III of the denatured DNA, when measured under the same conditions, appears at a potential which is about 70 mV more negative and, for the DNA isolated from calf thymus, it is a hundred times higher. The difference in the polarographic behaviour of the single-strand DNA and the double helical DNA served as a basis of the DNA structure analysis contributing to the discovery of the DNA premelting and the polymorphy of the double helix[4]. At present the mercury electrode is used as the probe of the DNA structure[6]. In cyclic voltammetry, the use can be made of the anodic signal of guanine[6-8] (besides the reduction of adenine and cytosine), as well as of the introduction of electroactive markers[6,9,10] being simultaneously the structural probes. The strong adsorption of nucleic acids at electrodes[6,11,12] underlies the application of stripping voltammetry. In spite of the fact that the electrochemical analysis has become a powerful tool in the study of nucleic acids, the nature of some electrochemical signals provided by these substances has not been clarified yet.

Already in 1966 we identified[6] the peak I of DNA as the capacitive peak. In fact, this peak was rather low even at high DNA concentrations and did not correspond to any DC polarographic wave, whereas its potential was close to that of the AC polarographic (tensammetric) peak I. On the other hand, the peak III of the denatured DNA is faradaic. For a long time, the existence of capacitive (tensammetric) peaks on DP polarograms (besides peaks of reduction) has not been considered in literature. Only recently, Jacobson and Lindseth[13] and Canterford and Taylor[14] have pioneered the treatment of tensammetric peaks in DPP analysis of surface active substances (*e.g.* n-octanol and poly(ethylene glycol)).

The purpose of this work was to compare faradaic DPP peaks of single-strand polynucleotides containing reducible adenine and cytosine residues with non-faradaic peaks of a single-strand polynucleotide, which does not contain any reducible base, such as poly(uridylic acid) (poly(U)). Our measurements indicated, that some of the properties of reduction and tensammetric peaks are so different, that they can be used as diagnostic tests for classification of even very small DPP peaks (<1 μA), which are characteristic for samples of double helical DNA.

EXPERIMENTAL

Poly(A) was the product of Calbiochem, poly(C) the product of Serva and poly(U) the product of Schwarz. All the other chemicals were of reagent grade purity. Polarographic measurements were performed with the help of the polarographic analyzer PAR 174A at the static mercury drop electrode (SMDE), model 303 or at a classical dropping mercury electrode (DME), as described previously[15]. Effects of the pulse width and the pulse direction (Fig. 1) were followed by using the pulse polarograph A 3 100 Southern-Harwell under the conditions described elsewhere[16]. The rate of flow of the classical DME was 1·92 mg s^{-1} at the height of the mercury column of 42 cm.

In measurements with PAR 174, a three-electrode arrangement was used with a counter platinum-wire electrode and the reference saturated calomel electrode K 77 connected to the cell by means of a salt bridge K 65; the bridge was filled with the base electrolyte. In measurements with A 3100, a two electrode arrangement was employed with the mercury pool at the bottom of the cell as the reference electrode. The following settings of A 3100 were used: 1 V/15 min, pulse amplitude of 25 mV, integration 3, auto trig. delay time 2 s, recorder sensitivity 1/5, and amplifier sensitivity 1/32. All measurements were carried out under the argon atmosphere.

RESULTS

Effect of the Polynucleotide Concentration

The DPP behaviour of reducible polynucleotides poly(A) and poly(C), as well as of the non-reducible poly(U) was inspected at pH 6·1 (Britton–Robinson buffer) in 0·5M-NaCl. In agreement with our previous results[3,17], both reducible polynucleo-

tides gave well-developed peaks. The peak height of poly(A) was linearly dependent on the polynucleotide concentration up to about $2 . 10^{-4}$ mol l^{-1} (Fig. 2), it reached a limiting value at $6 . 10^{-4}$ mol l^{-1} and did not vary more. Poly(C) (not shown) exhibited a similar behaviour.

The DPP peak of poly(U) was considerably lower than that of poly(A) or poly(C). At the polynucleotide concentration of $6 . 10^{-4}$ mol l^{-1}, the peak ratio (poly(A): poly(U)) was about 30 and did not vary much when the concentration was increased further more.

Effect of the Pulse Amplitude

Different types of dependences of the peak height on the pulse amplitude were found for poly(A) and poly(C) on the one hand and for poly(U) on the other hand[18]. With the conventional direction of the pulse (Fig. 1), all three polynucleotides showed a linear dependence on the pulse amplitude only up to the amplitude of 25 mV. At higher values, the peak of poly(U) exhibited a sub-linear dependence, whereas peaks of reducible polynucleotides super-linear dependences. With the pulse having the direction opposite to the potential sweep (Fig. 1), the peak height

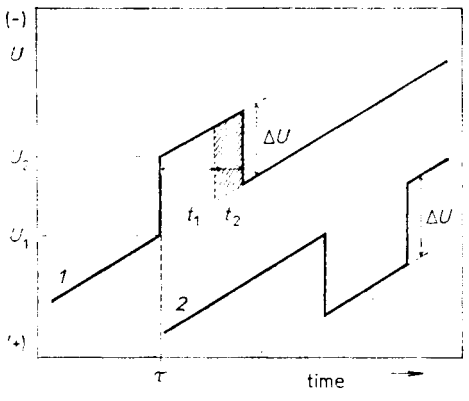

FIG. 1

Schematic representation of the signal applied in DPP: *1* conventional potential pulse (direction of the pulse identical with that of the potential sweep, *i.e.* the pulse is oriented towards more negative potentials); 2 inverted potential pulse. ΔU, pulse amplitude; $t_1 + t_2$ pulse width; t_1 delay ("integrator discharge" with A 3 100); t_2 sampling time ("integrator signal gate" with A 3 100)

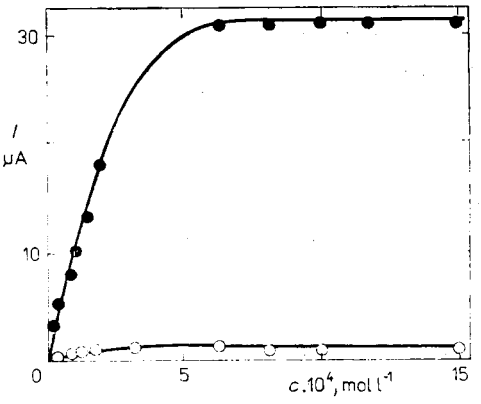

FIG. 2

The height (I) of the DPP peak as a function of the polynucleotide concentration: ● poly(A); ○ poly(U). PAR 174: pulse amplitude 50 mV, sweep rate 1 mV/s; DME: drop time 2·0 s. Base electrolyte: 0·5M-NaCl with the Britton–Robinson buffer and $1 . 10^{-3}$M--EDTA (pH 6·1)

of poly(U) was linearly dependent on the amplitude, while that of poly(A) showed a sub-linear dependence even at amplitudes as low as 10 mV and higher.

Effect of the Pulse Width

Plots of the peak height *vs* the pulse width (sampling time 5 ms) for poly(A) and poly(U) indicated that the peak of poly(U) decreases with time faster than that of poly(A)[18]. By adjusting the time delay (Fig. 1) and the sampling time, the capacitive current can be suppressed so that a well-developed faradaic peak is observed (Fig. 3*a*). Alternatively, the capacitive-to-faradaic current ratio is changed in favour of the capacitive current (Fig. 3*d*). The former case is usual for instruments, which generate pulses with a fixed pulse width.

Effect of the Drop Time

The effect of the drop time on the height of DPP peaks of reducible polynucleotides was followed with the help of the static drop electrode (SMDE). The remarkable feature of this electrode is that the required size of the mercury drop is reached in a very short period of time (<50 ms), and for the remaining part of the drop time (0·5 to 5·0 s) the electrode surface area remains constant. In this way, the capacitive current connected with the growth of the drop is eliminated. We tried to use this electrode for the simple characterization of electrode processes, inasmuch it makes it possible to vary independently the drop time and the electrode surface area.

On increasing the drop time (with the surface area kept constant), the height of DPP peaks of both poly(A) and poly(C) decreased (Fig. 4). However, at very low concentrations of the polynucleotide ($1 . 10^{-5}$ mol l^{-1}), an increase in the peak height was observed first, and only then the peak decreased (Fig. 4*a*). At high polynucleotide concentrations, under the conditions of full coverage of the electrode

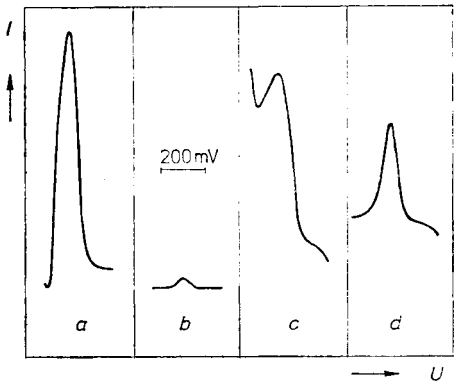

FIG. 3

DP polarograms of polynucleotides at various values of the pulse width: *a*, *c* poly(A); *b*, *d* poly(U). Pulse width: *a*, *b* 50 ms; *c*, *d* 10 ms; sampling time t_2: *a*, *b* 10 ms; *c*, *d* 5 ms. Base electrolyte see legend to Fig. 2; concentration of polynucleotides $1·5 . . 10^{-4}$ mol l^{-1}. Polarograph A 3 100: starting potential −1·35 V, sweep rate 1 V/15 min, drop time 2·0 s

surface, the peak height decreased with time from the very short drop times and its decrease was much more pronounced (Fig. 4*b*). The character of the time dependence of the peak height was influenced by the pulse amplitude (Fig. 5). For the amplitude of 100 mV, the peak height of poly(A) increaed with the drop time. Analogously, in the normal pulse polarography (NPP) the height of the NPP wave increased with the drop time at low polynucleotide concentrations (Fig. 4*a*), whereas rather small effect of the drop time was observed at high concentrations (Fig. 4*b*). On the other hand, the height of the DC polarographic wave of poly(C) decreased with the drop time like that the height of the DPP peak at low pulse amplitudes (Fig. 4*b*). The behaviour of both reducible polynucleotides was practically identical.

In contrast, the peak height of the non-reducible poly(U) measured under the conditions of the partially covered electrode surface increased with the drop time (Fig. 4*a*) up to its maximum length (5·0 s). When the state of the full coverage of the electrode was reached, the peak height of poly(U) was practically independent of the drop time (Fig. 4*b*). Poly(U) did not show any DC polarographic wave, and its NPP wave was poorly developed.

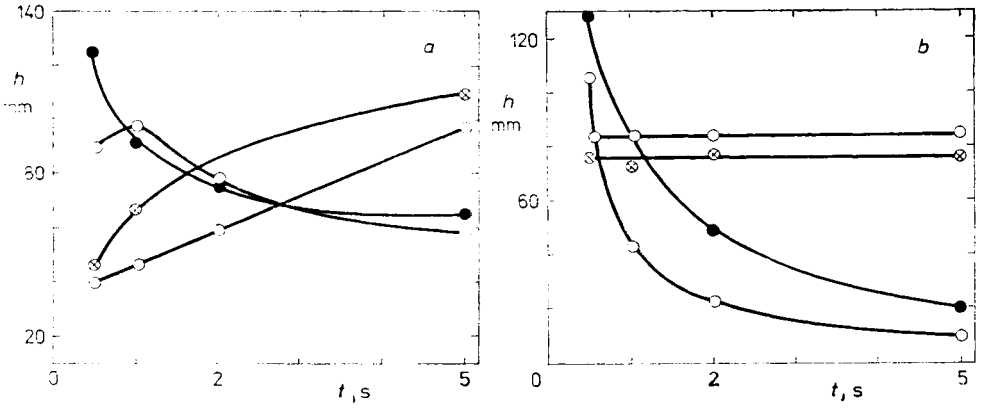

FIG. 4

The height of the DPP peak, NP or DC waves as a function of the drop time of SMDE/DME under the conditions of *a* partial, *b* full coverage of the electrode surface: *a* 1 . 10^{-5}M-poly(C), ⊙ DPP (10 μA), ○ NPP (10 μA); ● 2·5 . 10^{-5}M-poly(A), DPP (20 μA); ⊗ 5 . 10^{-5}M-poly(U), DPP (1 μA). *b* 1 . 10^{-3}M-poly(C), ● DPP (50 μA), ⊙ DC "sampled polarography" (2 μA), ○ NPP (20 μA); ⊗ 2 . 10^{-3}M-poly(U) DPP (2 μA). PAR 174, Model 303 SMDE/DME: drop size *S*, sweep rate 2 mV/s, pulse amplitude 50 mV. At the drop time of 5·0 s the sweep rate was 0·2 mV/s; at other values the sweep rate was chosen so that the product of the rate and the drop time was equal to 1·0. Numbers in parentheses give the full scale (*i.e.* 20 cm) sensitivity in μA. For electrolyte see the legend to Fig. 2

DISCUSSION

Present results reveal that single-strand polynucleotides can give tensammetric (non-faradaic) DPP peaks, besides reduction (faradaic) peaks[3]. From the chemical point of view, polynucleotides compared here are very much alike. The reducible poly(C) and the non-reducible poly(U) differ from each other only in one substituent on the C-4 pyrimidine ring. The reducibility of polynucleotides at the mercury electrode is connected essentially with the presence of the double bond —N= =C(NH_2)— in the pyrimidine ring, which appears to be the primary reduction site. In poly(U) this bond is replaced by the non-reducible bond —NH—CO—. Under the given conditions all three polynucleotides are single-stranded and the content of helical fragments can be expected to be higher in poly(A) and poly(C) than in poly(U) molecules. In alkaline media, all three polynucleotides are not reducible and exhibit almost identical AC tensammetric behaviour. It can be concluded that these polynucleotides represent suitable models for the study of tensammetric and reduction DPP peaks of nucleic acids. For this purpose, the identification of peaks provided by various samples of nucleic acids is essential. Therefore, simple diagnostic criteria, which would enable us to distinguish between tensammetric (capacitive) and faradaic DPP peaks, ar needed.

In case that the period of time t_1 prior to sampling one (Fig. 1) is long enough, as it has been usual in the pulse-polarographic analysis, small capacitive currents are predicted by the theory[14,19,20], to which only low DPP (tensammetric) peaks can correspond. Actually, it can be seen from Fig. 2 that the maximum height of the DPP peak of poly(U), which is capacitive in nature, did not exceed 1 μA (the current density about 0·4 μA/mm^2), while the faradaic peak of poly(A) was approximately thirty times higher. It is then clear that peaks of polynucleotides, the height of which reaches several microamperes under the present conditions, can hardly be classified as capacitive ones. However, this criterium cannot be applied *e.g.* to peaks of double helical polynucleotides, which are often small but in spite of this can have faradaic character.

When the degree of coverage of the electrode by the adsorbate changes during the time interval t of the pulse duration, the current can comprise a contribution to its capacitive component due to the slow establishment of adsorption equilibrium. This actually could happen in the presence of polynucleotides[20], because of their small diffusion coefficients and the possibility that the adsorption of their segments is retarded sterically.

A criterium, which could help us to distinguish between tensammetric and faradaic peaks, is represented by the concentration dependence of the peak potential U_p and the peak width $W_{1/2}$. For faradaic peaks of polynucleotides these quantities are practically independent of the polynucleotide concentration[17], while for tensammetric peaks U_p shifts with the concentration towards more negative values and $W_{1/2}$ decreases, just as for tensammetric maxima on AC polarograms[21].

Another criterium for the classification of DPP peaks could be the dependence of the peak height on the pulse width. As predicted by the theory[19,22], the capacitive current decreases with time (*i.e.* with the pulse width) exponentially, whereas the faradaic current of the reversible reduction is proportional to $t^{-1/2}$. Consequently, the capacitive DPP peak decreases with the pulse width much faster than the reduction peak. Since in the majority of pulse-polarographic instruments, which are available commercially, the pulse width has a fixed value, this criterium will not probably reach an extensive application for practical reasons.

This work demonstrates that there are other remarkable differences in the behaviour of tensammetric and reduction peaks of polynucleotides not arising from the diversity of the two types of peaks, but rather from specific properties of reduction peaks of polynucleotides. We have already shown[3,23,24], that the height of the DPP peak of single-strand polynucleotides (inclusive of the denatured DNA) exhibits a non-linear dependence on the pulse amplitude even when the latter is rather small. This contrasts to the theory[19,22], which predicts linear dependences both for faradaic and tensammetric peaks. We ascribed this untypical behaviour to the coincidence of potentials of reduction and desorption of the polynucleotide and to the accumulation of the polynucleotide at the electrode surface in the course of the foregoing pulse, which presumably increases with the increasing pulse amplitude. However, results of measurements of the DPP peak height as a function of the drop time of SMDE (Fig. 4) indicate that the accumulation of the polynucleotide at the electrode surface was not the only factor, which under the conditions of our experiments influenced the shape of the DPP peak height *vs* the amplitude plot for reducible polynucleotides (Fig. 5). The results obtained with SMDE/DME (Fig. 4) suggest that the electrode surface is blocked by the reduction product, which impedes the access

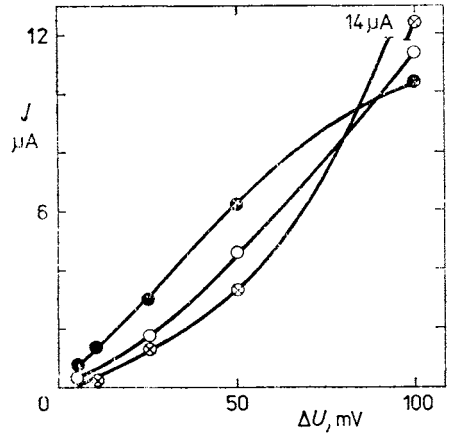

FIG. 5

The height of the DPP peak of $2{\cdot}5 \cdot 10^{-5}$M-poly(A) as a function of the pulse amplitude for various drop times of SMDE/DME. Drop time: ● 0·5 s; ○ 1·0 s; ⊗ 5·0 s. For other conditions see legend to Fig. 4

of molecules to be reduced to the surface and thereby hinders their reduction. The role of this factor becomes more pronounced when the polynucleotide concentration or the drop time increase (thus rising the surface concentration of the reduction product), or when the pulse amplitude decreases. The blocking of the surface by the product of reduction of the single-strand DNA or poly(A) in voltammetric measurements at HMDE was evidenced by Valenta and Nürnberg[25,26].

Results of this work show that also in application of techniques imposing a small potential perturbation on the mercury drop during its life, as in DPP, the blocking of the electrode surface occurs with single-strand polynucleotides, provided that measurements are carried out at a sufficiently high polynucleotide concentration and/or a sufficiently long drop time. In the adsorption-desorption process, which underlies the origin of tensammetric peaks, this mechanism does not play any role. Therefore, the characteristic shape of the peak height *vs* the pulse amplitude plot[18] and the fall in the height of the DPP peak with drop time at SMDE/DME (Fig. 5) can be evidence for the presence of reduction process of polynucleotides.

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Note added in proof: After submission of this paper G. C. Barker (J. Electronal. Chem. *214*, 373 (1986)) suggested a novel type of redox reaction responsible for the polarographic signal of the single-strand DNA at about −1·4 V at slightly alkaline pH values. This reaction involves exchange of electrons between the mercury surface and DNA loops that extend out into the solution.