Polarographic Reducibility of Polyribouridylic Acid

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Abstract. Electrochemical behaviour of polyribouridylic acid (poly (U)) in dimethylformamide containing 0.1 mol/l tetrabutylammonium perchlorate was studied by means of direct current, differential-pulse, and phase-sensitive alternating current polarography, and cyclic voltammetry at a hanging mercury drop electrode. It was found that poly(U) yields reduction current around the potential of -2.0 V (vs. saturated calomel electrode). It results from electroreduction of uracil residues. The electrode process corresponding to this electroreduction has characteristics of a diffusion-controlled process.

Key words: Polyribouridylic acid — Polarography — Electroreducibility — Non-aqueous medium

Introduction

In recent years, much interest has developed in the electrochemical behaviour of biopolymers. As a result of the concern with nucleic acids, considerable work has also been done on synthetic homopolynucleotides, since they represent model compounds for naturally occurring nucleic acids. On the basis of electrochemical studies of polynucleotides so far carried out in aqueous media usually used in polarographic experiments, the conclusion has become established that among polynucleotides containing bases usually occurring in natural nucleic acids, only those containing adenine and cytosine are polarographically reducible (Paleček 1969a). The others, among them even poly(U), have been so far considered as polarographically non-reducible (Paleček 1969b).

Recently polarographic reduction of 2-hydroxypyrimidine (Wasa and Elving 1978), uracil (Cummings and Elving 1978), thymine (Cummings and Elving 1979) and uridine (Bresnahan et al. 1981) in anhydrous solvents containing tetra-n-butylammonium perchlorate has been described. The latter salt used as a component of the supporting electrolyte shifts the cathodic potential limit of the

DME to markedly more negative values making electroanalysis of hardly reducible compounds possible.

This paper shows that even poly(U) is polarographically reducible if the polarographic experiments are performed in a medium which allows measurement at sufficiently negative potentials of the DME.

Material and Methods

Uracil, uridine, UMP (dipotassium salt), and D-ribose were obtained from Calbiochem. Poly (U), potassium salt, was obtained form Serva. Polydeoxyribosephosphate was prepared as described earlier (Brabec and Paleček 1972). TBAP (Fluka) was purified according to the method of House et al. (House et al. 1971). A 30% aqueous solution of TEAH (Reachim, USSR) served as the source of hydroxide. DMF (reagent grade, Reachim, USSR) was purified by the method published earlier (Thomas and Rochov 1957). The solvent system was 0.1 mol/l TBAP in DMF.

Poly(U) did not dissolve spontaneously in 0.1 mol/l TBAP/DMF. Therefore, the sample of poly(U) in this medium, was prepared as follows. 5.0 mg of poly(U) were dissolved in 2.5 ml of 0.1 mol/l TBAP/DMF containing 10 per cent of water. This solution was then dialysed at 25°C against the same solvent system (120 ml) for 48 hours, with regular substitution of fresh solvent system in 6—8 h intervals. After the completion of the dialysis the solution containing poly(U) was diluted 1:20 with anhydrous TBAP/DMF (0.1 mol/l) and subsequently centrifuged (20 minutes, 5000 g). The resulting solution contained only about 0.5 per cent of water; electrochemical measurements were performed within the next 24 hours following the preparation. The concentration of poly(U) was determined by means of the phosphorus content determination in the sample; the phosphorus content was determined polarographically using the technique, described in "Application Brief P-1", (EG and G Princeton Applied Research Corporation, 1979). Thus for the purpose of the present study, the concentration of poly(U) was expressed in terms of the monomer content. The concentration of poly(U) was adjusted to 2.2×10^{-4} mol/l. The concentration of potassium ions in poly(U) samples was determined following combustion of the samples in a muffle furnace at 500°C using both the polarographic method (0.1 mol/l TBAP/DMF), and ion selective electrodes. The test solutions were deoxygenated by purging with argon for ca. 10 minutes; an argon atmosphere was maintained in the cell during the measurements.

Electrochemical measurements were carried out using a GWP 673 Multimode Polarograph (GDR), Polarographic Analyzer PA 3 (Laboratory Instruments, Prague) and Electrochemistry System, Model 170 (Princeton Applied Research Corporation, USA). Polarographic measurements were carried out with a controlled drop-time of 2.0 s using a capillary with a mercury flow rate at open circuit of 0.62 mg/s; the voltage scan rate was 2 mV/s. Differential pulse polarograms were obtained at a pulse amplitude of -50 mV and a current sampling time of 20 ms. AC polarographic curves were recorded at the alternating voltage of 10 mV, peak-to-peak, and a frequency of 40 Hz. For cyclic voltammetry, a Metrohm E-410 microfeeder (1.77 mm^2) was used as the working electrode; waiting time at the initial potential was always 30 s.

For controlled potential electrolysis, a mercury pool, about 0.5 cm thick and 2 cm in diameter, was placed into the cell. Argon was used to stir continuously the solution during electrolysis. The electrolysis was carried out at potentials corresponding to the crest of the DC polarographic step. The potential was controlled by the potentiostat of the Polarographic Analyzer PA 3.

Potentials were measured against a modified aqueous SCE as follows: SCE (saturated KCl in water-agar) 0.1 mol/l TBAP in DMF-agar/0.1 mol/l TBAP in DMF bridge with a Vycor tip. Other

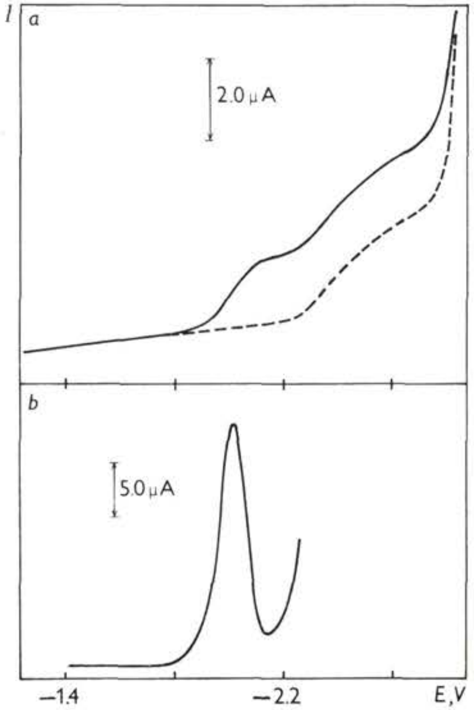

Fig. 1. Polarograms of polyribouridylic acid at the concentration of 2.2×10^{-4} mol/l in dimethylformamide (0.1 mol/l tetrabutylammonium perchlorate). (*A*) DC polarogram, (----) background electrolyte; (*B*) differential-pulse polarogram. Polarograms were recorded using the PARC Model 170 Electrochemistry System.

details concerning our electrochemical measurements have been published elsewhere (Brabec 1974; Brabec and Paleček 1976).

The ion selective electrode (Orion) was used with an Orion Model 407 A Specific Ion Meter.

Results and Discussion

So far the experiments aimed at demonstrating polarographic reduction of uracil and uridine (Cummings and Elving 1978; Bresnahan et al. 1981) have been performed using 0.1 mol/l TBAP/DMSO as medium. However, only DMF of sufficient purity was available for our experiments. To determine whether the exchange of DMSO for DMF had some influence on polarographic reduction of uracil or uridine, we first recorded a DC polarogram for the latter compounds in a medium of 0.1 mol/l TBAP/DMF. A comparison of $E_{1/2}$ and DC polarographic diffusion current constants with those obtained in DMSO (Cummings and Elving

1978 ; Bresnahan et al. 1981) showed that the exchange of DMSO for DMF had no substantial influence on polarographic behaviour of these compounds.

A typical DC polarogram of poly(U) in 0.1 mol/l TBAP/DMF is shown in Fig. 1*a*. The solution of poly(U) (2.2×10^{-4} mol/l) yielded a step with $E_{1/2}$ around -2.0 V. A more negative step with $E_{1/2} = -2.35$ V was also yielded by the solution of the supporting electrolyte, so that it can be ascribed to impurities in the TBAP/DMF solvent system.

The solutions of poly(U) used in this work were prepared from a potassium salt of this polynucleotide. Due to longer storage, some commercial preparations of polynucleotides may also contain low-molecular weight components of these biopolymers. Both potassium ions and some of these components yield polarographic currents around -2.0 V (Cummings and Elving 1978 ; Bresnahan et al. 1981 ; Heyrovský and Kůta 1965). Further experiments were, therefore, conducted to provide evidence that inorganic and possible low-molecular weight components of poly(U) had been removed from the sample of this polynucleotide by the procedure described under Material and Methods (including dialysis and centrifugation) to such an extent that they yielded no measurable polarographic current. The test solution was, therefore, prepared as follows : instead of poly(U), monomeric UMP (potassium salt) was dissolved in 0.1 mol/l TBAP/DMF containing 10 per cent water to make the same concentration as poly(U) (see under Material and Methods). The UMP solution was then processed identically as the poly(U) samples, including dialysis. The resulting solution yielded no DC polarographic step except the step with $E_{1/2} = -2.35$ V yielded by 0.1 mol/l TBAP/DMF (Fig. 1*a*). Potassium ion concentration in poly(U) samples, prepared as decribed under Material and Methods, was also determined. Poly(U) samples were combusted and the potassium ion concentration was determined using polarography and ion selective electrodes. It was shown that the concentration of potassium ions was, in these poly(U) samples, lower than ca. 1×10^{-8} mol/l ; potassium ions in this concentration could not thus yield any step on DC polarograms.

During macroscale electrolysis of the solution containing 2.2×10^{-4} mol/l poly (U) at the controlled potential, the height of the step at -2.0 V decreased. As shown on Fig. 1*b*, DPP enables a substantially better measuring of the reduction current corresponding to the DC polarographic step at -2.0 V.

Of the low-molecular components of poly(U) polarographic activity in a nonaqueous medium containing TBAP has so far been demonstrated for uracil only (Cummings and Elving 1978). As for the polarographic activity of uridine (Bresnahan et al. 1981) in this medium, the electrode process was discussed on the basis of the assumption that it was the uracil moiety that was responsible for the polarographic activity of this nucleoside. However, the polarographic inactivity of D-ribose in a nonaqueous medium containing TBAP has not yet been experimentally demonstrated. We recorded DC polarograms for both the monomeric D-ribose and polydeoxyribosephosphate (i.e. a polymer prepared from the polynucleotide by chemical splitting off the bases) in a medium containing 0.1 mol/l TBAP/DMF. At a concentration of 2.2×10^{-4} mol/l neither substances yielded any step at potentials more negative than -1.4V. This result indicates that it was the electroreduction of uracil residues that was responsible for the occurrence of the reduction current of poly(U) at about -2.0 V (Fig. 1*a*, *b*).

The addition of water to the solution of poly(U) in 0.1 mol/l TBAP/DMF (prepared as described under Material and Methods) to make a total water content of 1% did not affect either the height or $E_{1/2}$ of the DC polarographic step of this polynucleotide. This indicates that, as in the case of monomeric uracil (Cummings and Elving 1978), protonation by residual water in the solvent may unlikely play an appreciable role in the observed behaviour of poly(U). Moreover, when an equivalent molar quantity of hydroxide ion was added to 2.2×10^{-4} mol/l solution of poly(U), the DC polarographic step at -2.0 V practically disappeared. It is thus reasonable to assume that uracil residues in poly(U) are reduced at the DME in a protonized form similarly as it is the case in monomeric uracil or uracil moiety in uridine (Cummings and Elving 1978; Bresnahan et al. 1981).

The height of the DC polarographic step of poly(U) (2.2×10^{-4} mol/l) was directly proportional to the square root of the corrected reservoir height (if DME with natural drop-time was used). In the concentration range up to 2.2×10^{-4} mol/l this step was growing linearly with the polynucleotide concentration. Cyclic voltammograms of poly(U) (2.2×10^{-4} mol/l in 0.1 mol/l TBAP/DMF) were recorded at the hanging mercury drop electrode for voltage scan rates ranging from 0.01 to 1.0 V/s. The sawtooth potential function was used with the first scan initiated at -1.6 V in the negative direction; the negative switching potential was -2.2 V. Poly(U) yielded a cathodic peak at -2.04 V growing with the square root of the voltage scan rate. The peak potential was essentially independent of the voltage scan rate. The cathodic peak had an anodic counterpart of an approximately identical height occurring at a potential by about 70 mV more positive than that of the cathodic peak. The results of DC polarography described herein allow the conclusion that the electrode process responsible for the occurrence of the poly(U) step around -2.0 V is diffusion-controlled and is not totally irreversible, similarly as the process of uracil or uridine reduction under similar conditions (Cummings and Elving 1978; Bresnahan et al. 1981).

Also, phase-selective AC polarograms were recorded for poly(U) (2.2×10^{-4} mol/l in 0.1 mol/l TBAP/DMF). Quadrature and in-phase current component peaks occurred at potentials of about -2.0 V. At potentials more positive or more negative than this AC polarographic peak the quadrature current component merged with that recorded for the background electrolyte containing no poly(U). The results of AC polarography indicate that, at least within the potential range from −1.6 to −2.7 V, neither oxidized nor reduced forms of poly(U) in 0.1 mol/l TBAP/DMF adsorb at the DME surface.

The results of the present study demonstrate that poly(U) in 0.1 mol/l TBAP/DMF is polarographically reducible. However, the process of this electrode reaction has some characteristics different from those of the polarographic reduction of monomeric uracil and uridine (Cummings and Elving 1978; Bresnahan et al. 1981). For the polarographic reduction of uridine it has been proposed (Bresnahan et al. 1981) that the primary reversible electroreduction of the uracil moiety, generating a radical anion, is followed by a rapid irreversible chemical reaction, which has been thought to involve abstraction of a proton from the unreduced parent pyrimidine moiety or radical dimerization. The different voltammetric (polarographic) behaviour of poly(U) and uridine may e.g. be due to the fact that these subsequent reactions run markedly slower, or cannot occur at all, in the case of macromolecular poly(U).

A study aimed at a more detailed elucidation of the mechanism of polarographic reduction of poly(U) is in progress.

Summary

In the present communication the polarographic reducibility of poly(U) has been demonstrated for the first time. The reported electrochemical activity of poly(U) offers the possibility of following residues of uracil also in other nucleic acids; this extends the analytical exploitation of polarography in the nucleic acid research. Moreover, polarographic activity of a polynucleotide in a nonaqueous medium, where nucleic acids are devoid of secondary structure, was described. In aqueous media, where the secondary structure is preserved to a certain extent even in single-stranded polynucleotides, interpretation of results of studies of electrode processes in polynucleotides is complicated by the fact that the accessibility of the electroactive sites in segments forming this structure may be markedly different as compared with segments without secondary structure. The use of a nonaqueous medium in the electrochemical analysis of nucleic acids therefore offers a possibility of studying reactions of these substances at electrodes more exactly, without considering factors associated with the existence of any organized structure.

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Abbreviations used in the text:

AC — alternating current
DC — direct current

AC — alternating current
DC — direct current
DMSO — dimethylsulfoxide
DME — dropping mercury electrode
DMF — dimethylformamide
$E_{1/2}$ — half-wave potential
poly(U) — polyribouridylic acid
SCE — saturated calomel electrode
TBAP — tetra-n-butylammonium perchlorate
TEAH — tetraethylammonium hydroxide
UMP — uridine-5′-monophosphate

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