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CHEMICALLY REVERSIBLE ELECTROREDUCTION OF GUANINE IN A POLYNUCLEOTIDE CHAIN

František JELEN and Emil PALEČEK *

Institute of Biophysics, Czechoslovak Academy of Sciences, Královopolská 135, 612 65 Brno, Czechoslovakia

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It was shown that synthetic polynucleotides containing guanine display in cyclic voltammetry (CV) an anodic peak close to −0.3 V (against a saturated calomel electrode). A condition for the appearance of this peak is the previous polarization of the mercury electrode to sufficiently negative potentials (around −1.8 V). The results of CV measurements with electrode polarization by repeated cycles indicate that in negative potentials there is a reduction of guanine residues and in the anodic process reoxidation of the reduction product to guanine. This chemically reversible process takes place even when a polynucleotide contains adenine and/or cytosine residues in addition to guanine, where reduction leads to the formation of products blocking the electrode surface.

1. Introduction

Modern methods of electrochemical analysis [1,2] are a useful tool in research into nucleic acids (for reviews, see refs. 1 and 3–5). Of the nucleic acid bases only adenine and cytosine produce (in a suitable aqueous environment) polarographic reduction currents. Guanine displays an anodic peak in cyclic voltammetry (CV) [6], this appearing only when the mercury electrode is previously polarized to a sufficiently negative potential. The cathodic signal of adenine and cytosine and the anodic signal of guanine are produced even by single-strand polynucleotides containing these bases [4,5]. In double-helical DNA and DNA-like polynucleotides these signals are much weaker. The cathodic signals of polynucleotides have particularly been exploited in the study of the properties and structure of DNA [3–5]. Little attention was paid to the anodic signal of guanine, probably because it was assumed that it could only be investigated using CV at scan rates higher than 3.5 V/s [7] (such high scan rates cannot be applied using ordinary polarographic analysers) or using oscillographic polarography with a controlled alternating current [8,10], which is an outdated method.

We recently showed [11,12] that the anodic signal of guanine can be studied in both monomeric units and polynucleotides using CV at the usual scan rates (around 100 mV/s) and that this method is capable of detecting minor damage to the double-helical DNA molecule induced, e.g., by ionizing radiation. The mechanism of the electrode process responsible for the occurrence of the anodic peak of guanine has not been studied as well as that of the electroreduction of adenine and cytosine [13]. For the time being it has been shown [6,8,12,14] that in the region of strongly negative potentials, around −1.9 V (against a saturated calomel electrode), monomeric guanine is reduced to dihydroguanine. The reduction product, which remains in the vicinity of the electrode surface,

* To whom correspondence should be addressed

exhibits an oxidation peak around −0.3 V during the reverse scan.

This work was devoted to CV of polynucleotides containing guanine, and it has been shown that the reduction of guanine residues at a mercury electrode is chemically reversible.

2. Material and methods

Poly(G), poly(A,G,U) and poly(C,G) were supplied by Sigma. The concentration of polyribopolynucleotides was estimated spectrophotometrically using a Zeiss VSU-2P apparatus. Other chemicals were of analytical grade.

CV measurements were carried out with an EG and G Princeton Applied Research (PAR) polarographic analyser (model 174A) connected with a PAR 175 universal programmer. Cyclic voltammograms were obtained at a scan rate (v) of 200 mV/s and were recorded on a PAR X-Y recorder (model 0074). A three-electrode system was used, including a working hanging mercury drop electrode (HMDE), platinum counter electrode, and saturated calomel electrode. The working electrode was a Metrohm HMDE, type E410, with a surface area of 2.2 mm^2.

The method of HMDE polarization is indicated in fig. 1. The electrode was first held at a potential U_a for time t_a and then a triangular voltage sweep was applied. Reduction of the guanine moiety occurred at the potential U_{Gr}. The switching potential (U_s) was usually −1.85 V. Oxidation of the guanine reduction product took place during scanning in the anodic direction and an anodic peak appeared close to −0.3 V (U_p). Accumulation of the polynucleotide at the HMDE was carried out at $U_a = -0.1$ V. At lower concentrations of polynucleotide, accumulation during time t_a was usually enhanced by stirring (using a magnetic stirrer) (150 rpm), followed by a quiescent period of 15 s. All measurements were made in 0.6 M ammonium formate with 0.1 M sodium phosphate (pH 6.8) at 25°C in a volume of 1.5 ml. Solutions were deoxygenated by passing a slow stream of argon through them (6 min) and, during measurements, over their surface.

In some experiments the mercury drop with adsorbed polynucleotide was transferred to empty electrolyte. Adsorption of polynucleotides took place at $U_a = -0.1$ V in 2×10^{-4} M polynucleotide solution. After time t_a the electrode was removed from the solution, carefully rinsed with distilled water and immersed in another polarographic vessel containing a deoxygenated solution of the background electrolyte (volume 6 ml). After 60 s stirring (plus 15 s quiescent period) the cyclic voltammogram was recorded.

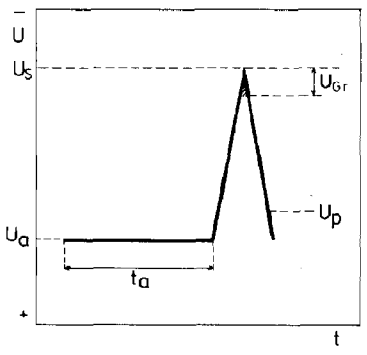

Fig. 1. Schematic representation of the voltage applied to the mercury electrode in CV experiments. t_a, time during which a polynucleotide is adsorbed and accumulated at the electrode surface (usually consisting of a stirring time and a short (15 s) quiescent period); U_a, potential of adsorption; U_s, switching potential; U_{Gr}, potential at which guanine reduction occurs.

3. Results and discussion

3.1. Single-cycle CV

We investigated the synthetic polyribonucleotides poly(G) and poly(A,G,U) in 0.6 M ammonium formate, 0.1 M sodium phosphate (pH 6.8), which has already been established in polarography [4] and CV [6,12]. We performed CV measurements with a fresh drop of mercury and $t_a = 140$ s at $E_a = -0.1$ V and $U_s = -1.85$ V. Under these conditions poly(G) and poly(A,G,U) gave an anodic peak (fig. 2a, b) at a potential close to −0.3 V. Poly(A,G,U) gave an additional cathodic peak at a potential of −1.4 V (fig. 2b) which is due to the reduction of the adenine moiety [4,5,13]. If U_s was changed to a potential

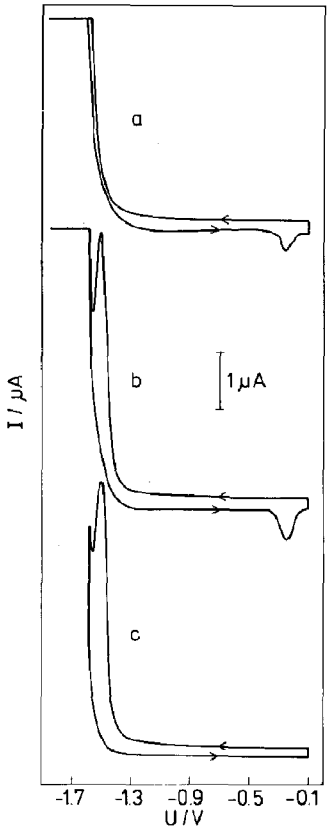

Fig. 2. Cyclic voltammograms of polyribopolynucleotides. (a) Poly(G), $U_s = -1.85$ V; (b) poly(A,G,U), $U_s = -1.85$ V; (c) poly(A,G,U), $U_s = -1.55$ V. All polynucleotides were measured at 2×10^{-4} M (related to monomer content). CV measurements were performed with an HMDE in 0.6 M ammonium formate, 0.1 M sodium phosphate (pH 6.8). $v = 0.2$ V/s, $U_a = -0.1$ V, $t_a = 140$ s, without stirring.

less negative than -1.6 V the anodic peak of both polynucleotides disappeared, while the cathodic peak of poly(A,G,U) remained unchanged (fig. 2c). The dependence of the height of the anodic peak of poly(A,G,U) on U_s is shown in fig. 3; a similar dependence was obtained with poly(G) [12].

The anodic peak of both polynucleotides depended linearly on their concentration in the range of low concentrations (fig. 4); at 2×10^{-4} M its height reached a limiting value in both polynucleotides which remained practically unchanged over quite a broad concentration range (suggesting that full coverage of the electrode was attained). In this range the potential of the peak U_p was independent of concentration; in the area of lower concentrations U_p shifted with increasing concentration to more positive values. This shift in U_p could result from intermolecular interactions on the electrode surface hindering the electrode process.

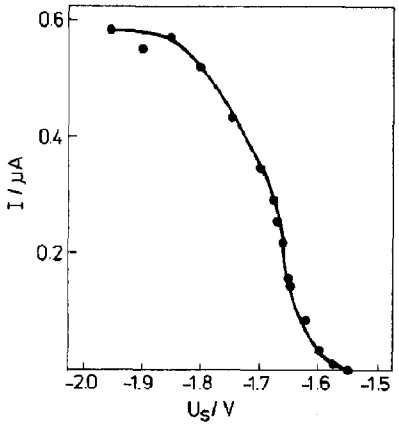

Fig. 3. Dependence of the height of the anodic voltammetric peak of 2×10^{-4} M poly(A,G,U) on switching potential. For other conditions see fig. 2.

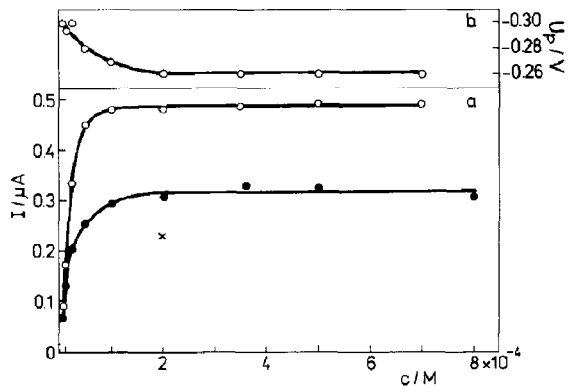

Fig. 4. Dependence of (a) height, I, and (b) potential of the anodic voltammetric peak, U_p, of poly(A,G,U) and poly(G) on polynucleotide concentration, c. (○———○) Poly(A,G,U), (●———●) poly(G), (×) poly(C,G) at 2×10^{-4} M. $U_s = -1.85$ V. For other conditions see fig. 2.

If during t_a the solution was stirred and t_a was sufficiently long, it was possible to work with considerably lower concentrations of polynucleotides (fig. 5). The peak height first increased linearly with time, then reached the limiting value, which was lower than that obtained in fig. 4 (which depicts the concentration dependence) suggesting that the adsorption equilibrium reached at lower concentrations (fig. 5) does not correspond to full coverage of the electrode.

Our measurements show that the height of the anodic peak of poly(A,G,U) is about 80% higher at full coverage of the electrode (fig. 4) than that of the poly(G) peak, in spite of the fact that the content of guanine residues in poly(G) is higher than in poly(A,G,U). The lower height of the poly(G) peak could be caused by a higher content of ordered structure in poly(G) (four-stranded helical structure) than in poly(A,G,U), similarly to the case of native (double-helical) and denatured DNAs.

3.2. *Repeated cycles*

When poly(G) (not shown) and poly(A,G,U) were measured in the repeated-cycle mode the anodic peak appeared even on the application of a second and further cycles (fig. 6) and did not disappear even after the application of 25 cycles. This result for poly(A,G,U) (obtained even at pH 5.8, not shown) is surprising, since, as was shown by Valenta et al. [15], on reduction of poly(A) at an HMDE the strongly adsorbed reduction product blocks the electrode surface and hinders the adsorption of further poly(A) on the mercury surface and consequently also the polynucleotide reduction. Due to this the cathodic peak did not appear on the CV curve in the second cycle. Our measurements show that the same process occurs owing to reduction of adenine residues in poly(A,G,U), since the cathodic peak (corresponding to adenine reduction) also disappeared after the application of a second cycle (fig. 6). The presence of an anodic peak whose height is only slightly decreased following the application of a second cycle can be explained by either (a) the electrode process (i.e., the reduction of the guanine residues at negative potentials and oxidation

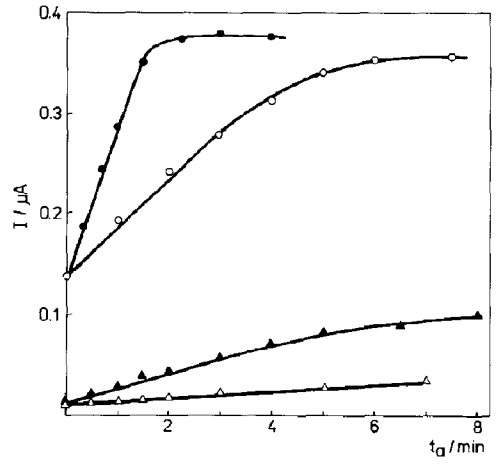

Fig. 5. Dependence of the height of the anodic voltammetric peak of poly(A,G,U) on preconcentration time (t_a). (●———●) 6×10^{-6} M with stirring, (○———○) 6×10^{-6} M without stirring, (▲———▲) 6×10^{-7} M with stirring, (△———△) 6×10^{-7} M without stirring. For details see section 2 and fig. 2.

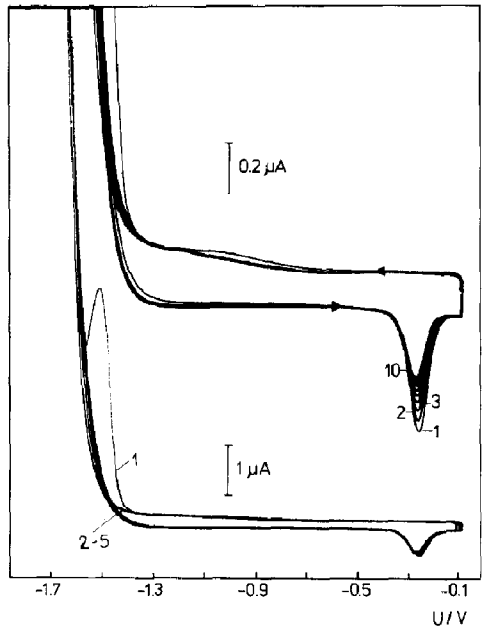

Fig. 6. Repetitive cyclic voltammograms of poly(A,G,U). U_s = 1.85 V. For other conditions see fig. 2.

around −0.3 V) taking place through the adsorbed layer, or (b) reoxidation of the reduced guanine residues (in the strongly adsorbed polynucleotide containing adenine reduction products) back to guanine.

We tried to determine which of these two mechanisms was involved in the case of poly(A,G,U). We first left the polynucleotide to adsorb at the electrode from a very dilute resting solution (6×10^{-7} M poly(A,G,U)) at t_a 20 s. Under these conditions we observed only an indistinct anodic peak (fig. 7a). We then allowed accumulation of poly(A,G,U) at the electrode from the same solution to take place but with stirring and a long waiting time ($t_a = 8$ min). CV measurements with repeated cycles showed that the height of the anodic peak in the second and subsequent cycles was in the latter case many times greater (fig. 7b) than the peak height measured at $t_a = 20$ s from the resting solution (fig. 7a). This result shows that mechanism (a) could not be the cause of the presence of the high anodic peak on the second and subsequent curves in CV measurements. If the irreversible redox process took place through the adsorbed layer the anodic peak in the second cycle could achieve, at most, the height measured under conditions of adsorption of 6×10^{-7} M poly-(A,G,U) from the resting solution with t_a 17.5 s (fig. 7a). (At the given scan rate this t_a corresponds to the period of one cycle.) It can therefore be supposed that, during the electrode process to which poly(A,G,U) is subjected, mechanism (b) is involved, i.e., reoxidation of the reduction product to guanine. This assumption was confirmed by further experiments where poly(A,G,U) adsorbed at the electrode was transferred to empty electrolyte. The height of the anodic peak measured after this transfer of the drop did not differ substantially from that of the peak obtained in the polynucleotide solution (fig. 8).

Our preliminary results indicate that the transfer of a layer of polynucleotide adsorbed at a mercury electrode will be useful for the microanalysis of nucleic acids. In this manner it will be possible to reduce considerably the volume of sample needed for analysis, which could be particularly useful in working with synthetic oligonucleotides and DNA fragments. A more detailed work is to be published elsewhere.

When we measured very dilute solutions of polynucleotides under conditions of incomplete electrode coverage, the height of the anodic peak increased slightly in the second cycle as a result of the transport of further polynucleotide molecules to the electrode during the potential scanning by diffusion. On the other hand, under conditions of full electrode coverage we observed a decrease in

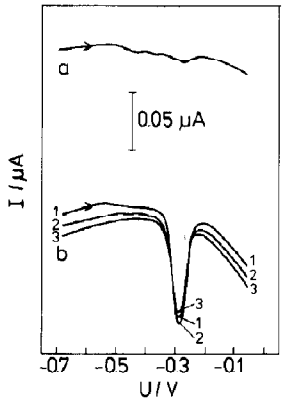

Fig. 7. Sections of cyclic voltammograms of 3×10^{-7} M poly(A,G,U). (a) $t_a = 20$ s, without stirring, (b) $t_a = 8$ min, with stirring. $U_s = -1.85$ V. For other conditions see fig. 2.

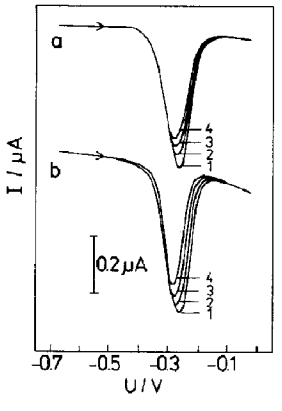

Fig. 8. Sections of repetitive cyclic voltammograms of 2×10^{-4} M poly(A,G,U). (a) In original solution, (b) after transfer of adsorbed polynucleotide to empty electrolyte. For details see section 2 and fig. 2.

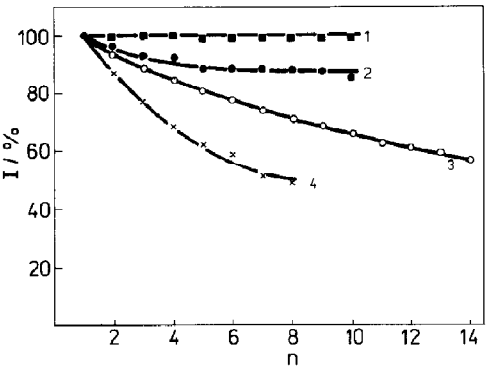

Fig. 9. Dependence of the height of the anodic voltammetric peak of 2×10^{-4} M poly(A,G,U) on the number (n) of repetitive cycles at different switching potentials, U_s. (1) −1.60 V, (2) −1.65 V, (3) −1.70 V, (4) −1.85 V. Peak height at first cycle was taken as 100%. For other conditions see fig. 2.

the peak height in the second and subsequent cycles (figs. 6 and 8). This decrease depended on U_s (fig. 9) and was greater the more negative the potential. At $U_s = -1.6$ V (which is at the heel of the S-shaped dependence of peak height on U_s) (fig. 3) we did not observe any changes in peak height as a result of repeated cycles. If the electrode with the adsorbed polynucleotide was transferred to empty background electrolyte and single-cycle CV measured repeatedly at this U_s for 30 min, no changes in the peak height were observed with respect to time (not shown).

The dependence of the peak height in the second and subsequent cycles on U_s (fig. 9) can be explained by (a) deeper reduction of guanine residues at more negative potentials which makes the process chemically irreversible and/or (b) rupture of the adsorbed polynucleotide layer at more negative potentials (perhaps due to the hydrogen evolution at the electrode) and polynucleotide transfer into the bulk of the solution.

4. Conclusions

It has been shown in this paper that a guanine residue in a polynucleotide chain represents a chemically reversible redox system; the significance of this system in biological processes has not yet been considered. It has also been shown that the polynucleotide can accumulate at the electrode surface from very dilute stirred solutions and due to this adsorptive preconcentration the sensitivity of the analysis of polynucleotides containing guanine can be greatly increased. Adsorption of polynucleotides on the electrode is so firm that the adsorbed layer can be transferred to another solution. A mercury electrode covered with polynucleotide (intact or reduced) represents a chemically modified electrode which offers new possibilities in the area of nucleic acid research, e.g., when studying the interactions of nucleic acids (immobilized on the electrode surface) with specific enzymes and/or other high- or low-molecular-weight substances.

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