Electrochemistry of Double-Stranded Complexes of Synthetic Polyribonucleotides Having Interferonogenic and Antiviral Activity

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Abstract. Double-stranded (ds) complexes of poly(C) with poly(G) and poly(G, I) were studied using differential pulse polarography (DPP) and differential pulse voltammetry at a pyrolytic graphite electrode (DPV). The complex formed by copolymer was found to be DPP inactive. On the other hand, poly(G).poly(C) yielded a small DPP peak corresponding to single-stranded (ss) poly(C). It was suggested that ss poly(C) present in the solutions of poly(G).poly(C) appeared due to the existence of segments in poly(G) during the complex-forming process in which guanine residues were unable to be hydrogen-bonded with bases in poly(C). Polynucleotide complexes investigated in this report yielded a DPV peak corresponding to electrooxidation of guanine residues, which was markedly lower than that yielded by ss polymers. Moreover, this DPV peak yielded by the complex prepared from an equimolar mixture of poly(G) and poly(C) was still markedly higher than that yielded by poly(G, I).poly(C), or by poly(G).poly(C) prepared in the excess of poly(C). The lowering of the DPV peak was explained as being particularly due to the presence of the polynucleotide segments with an intact and regular secondary structure. The results of our electrochemical analysis of the complexes investigated were compared with their biological activity reported earlier. This comparison calls attention to the fact that biological effectiveness of these biopolymers may be dependent on details of their secondary structure which may be monitored using the methods of electrochemical analysis.

Key words: Synthetic polynucleotides — Electrochemical analysis — Structure-biological activity correlation

Introduction

Synthetic polynucleotides are studied in particular as simplified models of natural
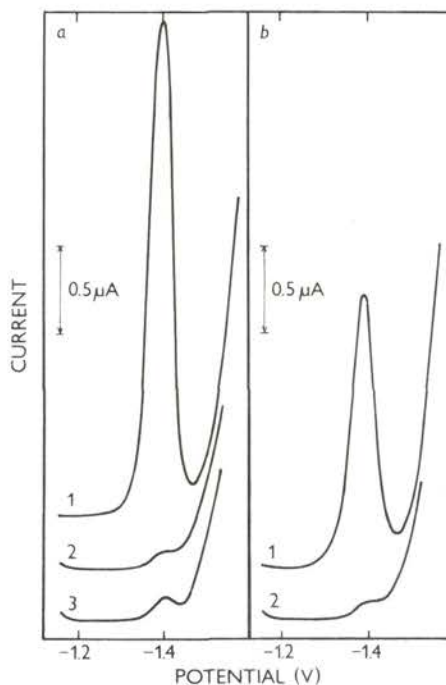
Fig. 1. Differential pulse polarograms of poly(C) and of its double-helical complexes with poly(G) in a medium containing 0.3 mol/l cesium chloride plus 0.025 mol/l sodium chloride with 0.05 mol/l sodium phosphate, pH 6.3. (a) curve 1 — 2×10^{-4} mol/l poly(C), curve 2 — 4×10^{-4} mol/l poly(G).poly(C), curve 3 — 4×10^{-4} mol/l poly(G).poly(C) plus 4×10^{-6} mol/l poly(C) (this sample was prepared by adding the surplus of poly(C) to the complex of poly (G).poly(C) only after its formation in the way described in the experimental part of this paper). (b) curve 1 — 4×10^{-4} mol/l poly(G).poly(C); the complex was formed in a medium of 0.05 mol/l sodium chloride with 0.001 mol/l sodium phosphate, pH 6.8 incubated at 20 °C for 15 hours, curve 2 — the sample which gave curve 1 in Fig. 1b, incubated at 100 °C for 5 mins and then slowly cooled to 25 °C.

leotides (containing polarographically reducible bases), with the bases in opposite chains being hydrogen-bonded in a way similar to ds DNA, results in a suppression of the polarographic reduction current.

In several more recent works (e. g. Novokhatsky et al. 1975; Timkovsky et al. 1973) the procedure used for the preparation of the poly(G).poly(C) complex differed from that described in the experimental part of this paper. The difference consisted in the equimolar mixture of poly(G) and poly(C) being incubated only at 20 °C for 12—16 hours. The complex formed in this way yielded a high peak on DPP curve at the same potential as ss poly(C) (Fig. 1b). If it is taken into account that the DPP activity of a ds DNA-like complex is markedly lowered (in comparison with the parent ss polynucleotide), it is apparent that the peak yielded
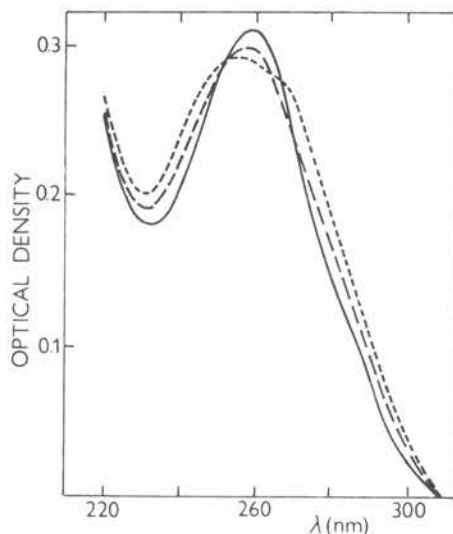
Fig. 2. Ultraviolet absorption spectra of 4×10^{-4} mol/l poly(G).poly(C) and the sum of the spectra of 2×10^{-4} mol/l poly(G) and 2×10^{-4} mol/l poly(C). The complex was formed in 0.1 mol/l sodium chloride with 1×10^{-3} mol/l sodium phosphate, pH 7.4 at 100 °C for 5 mins (——), at 20 °C for 15 hours (– – – –); (······) the sum of the spectra of homopolynucleotides. 0.1 cm light-path cell.

by the equimolar mixture of poly(G) (which is not polarographically reducible) and poly(C) incubated only at 20 °C corresponded to the polarographic reduction of ss poly(C). The presence of ss poly(C) in this preparation was apparently associated with the fact that the ds complex was formed under these conditions only partially. The height of the DPP peak of ss poly(C) increased linearly with the growing polynucleotide concentration. The DPP peak height (Fig. 1b, curve 1) thus shows that, in this case, approximately 53 per cent of cytosine residues in poly(C) were not hydrogen-bonded with those of guanine in poly(G).

If, however, equimolar concentrations of poly(G) and poly(C) were mixed and incubated in the way described in the experimental part of this paper (i. e. incubated at 100 °C for 5 minutes and then slowly cooled) the DPP peak corresponding to electroreduction of ss poly(C) was markedly lowered (Figs. 1a, b, curves 2). The height of this small peak, whose faradaic nature was checked by macroscale electrolysis, corresponded to ca. 3×10^{-6} mol/l ss poly(C) (Fig. 1a, curves 2 and 3); it means that, in this case only ca. 1.5 per cent of cytosine residues in poly(C) were not paired with those of guanine in poly(G).

These results thus indicate that incubation of equimolar mixture of poly(G) and poly(C) at 100 °C followed by slow cooling to 20 °C results in a markedly more complete formation of ds complex as compared with a 12—16-hour incubation of the mixture at 20 °C. Spectrophotometry in the uv region is often used to follow the

formation of polynucleotide complexes. It is, however, obvious (cf. Figs. 1 and 2) that the use of DPP for this purpose makes a significantly more sensitive and accurate detection possible.

DPP activity of ds complex poly(G).poly(C) was earlier studied by Jelen and Paleček (1979). These authors studied this complex formed under conditions that were almost identical to those described in the experimental part of the present report. From the results of their paper it follows that ds complex poly(G).poly(C) yielded no DPP peak. The different behaviour of this ds complex described in the present paper (Fig. 1a, curve 2) may be associated with different characteristics of polynucleotide samples used for the preparation of the complexes (molecular weight of polynucleotides, the presence of low-molecular weight admixtures etc.). Jelen and Paleček used commercial preparations, and they did not characterize them further so that a comparison aimed at explaining the difference in DPP activity of poly(G).poly(C) is impossible.

Our further experiments were aimed at studying DPP behaviour of the copolymer poly(G, I) and its complex with poly(C). It should be mentioned here that inosinic acid residues can be hydrogen-bonded with cytidylic acid residues of the opposite chain, but these pairs are less thermostable than similar pairs formed by guanylic and cytidylic acid residues. The ss copolymer $(2 \times 10^{-4}$ mol/l)did not yield any peak in the potential range $-1.2 - -1.5$ V. Its complex with poly(C) obtained at 100 °C and at a concentration of 4×10^{-4} mol/l did not yield any peak in this potential region either. It means, therefore, that contrary to the sample of poly(G).poly(C) studied in the present work, the complex of poly(C) with the copolymer did not contain any ss poly(C) detectable with DPP.

Recently, a higher interferonogenic and antiviral activity has been observed in some biological systems if poly(G) in the complex with poly(C) was substituted by the copolymer poly(G, I) used in the present work (Vilner et al. 1976). The reasons for a better biological activity of poly(G,I).poly(C) have not, however, so far been clarified. The results of the present work indicate that the lower interferonogenic and antiviral activity of the complex poly(G).poly(C) could be brought in association with the presence of ss segments of poly(C) in the complex preparation. However, the presence of a small amount of ss poly(C) in the poly(G).poly(C) sample by itself may not be responsible for the lower biological effectiveness of this complex. As already shown (Novokhatsky et al. 1978) the biological activity of the complex poly(G).poly(C) prepared from the mixture of poly(G) and poly(C), in which poly(C) was in a surplus (1:1.5 mixture), was higher than that of the complex prepared from an equimolar mixture of these polynucleotides. The presence of ss segments of poly(C) in the complex samples studied here apparently depends on the completness of base pairing in the reaction during which the complex is formed. Incompletness of this reaction in the case of mixing poly(G) with poly(C) followed by heating to 100 °C demonstrated here (Fig. 1b) might also
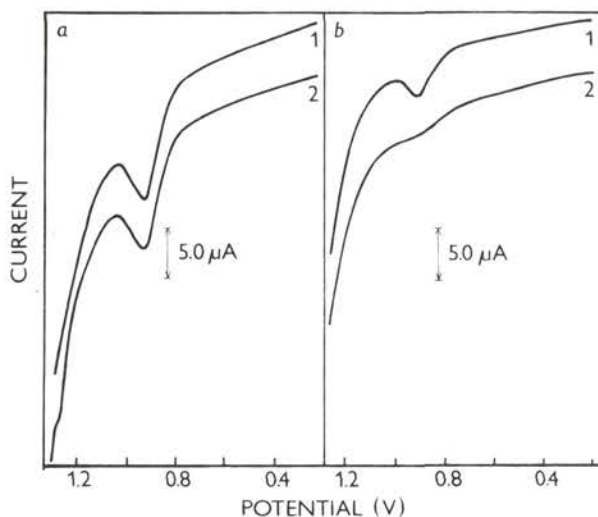
Fig. 3. Differential pulse voltammograms at the pyrolytic graphite electrode of polynucleotides in 0.2 mol/l sodium acetate, pH 6.45. (*a*) curve *1* — 3×10^{-4} mol/l poly(G), curve *2* — 3×10^{-4} mol/l poly(G, I). (*b*) curve *1* — 6×10^{-4} mol/l poly(G).poly(C), curve *2* — 6×10^{-4} mol/l poly(G, I).poly(C).

be brought into association with the tendency of poly(G) to form a stable secondary self-structure (Pochon and Michelson 1965). It has been assumed (Timkovsky et al. 1973; Subbotina et al. 1972) that this structure might be at least partially preserved during the formation of the complex with poly(C). On the other hand, the incorporation of a higher number of inosinic acid residues into the poly(G) molecule could influence its secondary structure during the complex-forming reaction. This incorporation could thus hinder the formation of segments in the purine polynucleotide chain, in which guanine residues would be unable to form hydrogen-bonded pairs with the complementary bases in poly(C).

Differential pulse voltammetry at the graphite electrode

It has been shown (Novokhatsky et al. 1978) that, in a model system, both the antiviral activity and stability of the complex poly(G).poly(C) increased if the complex was prepared in the presence of the surplus of poly(C). It is, however, apparent that DPP characterization of the quality of the preparation of complexes described in the preceeding paragraph cannot be accomplished with the complex poly(G).poly(C) prepared in the presence of a relatively high excess of poly(C). The excessive ss poly(C) itself produces a high DPP peak at -1.40 V (Fig. 1*a*) so that in this case it is difficult to determine quantitative pairing of guanine residues in the way described in the preceeding paragraph.

Quite recently it has been demonstrated (Brabec and Dryhurst 1978a) that polynucleotides containing purine bases are electrochemically oxidizable at graphite electrodes. It has been demonstrated that the oxidizability of nucleic acid bases may also be influenced by the secondary structure of polynucleotides (Brabec and Dryhurst 1978b; Brabec 1979; 1981). This is so because electrooxidizable sites in polynucleotides of a more intact and regular structure are less accessible for the reaction at the electrode than those contained in flexible randomly-coiled molecules.

DPV at the PGE was used to analyze the complex poly(G).poly(C), formed at 100 °C by mixing poly(G) and poly(C) in a ratio of 1:1.5 (complex 1:1.5). The latter complex yielded a peak at 0.93 V, apparently corresponding to electrooxidation of guanine residues (Brabec and Dryhurst 1978a). Its height was roughly three times lower than that of the peak yielded by the 1:1 mixture of poly(G) and poly(C) (complex 1:1) (also incubated at 100 °C for 5 min and slowly cooled) (Fig. 3b). This lowering of the peak at 0.93 V was significantly less pronounced if the excess of poly(C) corresponding to the 1:1.5 mixture was added at 25 °C to the sample of poly(G).poly(C) already prepared from the 1:1 mixture (incubated at 100 °C for 5 min and then slowly cooled before the addition of the excess poly(C)). The latter result thus indicates that the lowering of the oxidation peak at 0.93 V, observed upon mixing the polynucleotides in a ratio of 1:1.5 all at once, could not be associated with the competitive adsorption of the ds complex and the excessive ss poly(C) molecules at the PGE surface only. As already mentioned, the lowering of oxidation currents of polynucleotides reflects a higher content of intact and regular structure in the polynucleotide molecules. Our results of DPV at the PGE thus indicate that the preparation of poly(G).poly(C) in excess of poly(C) resulted in the formation of complex molecules of a more intact structure as compared with those of the 1:1 complex. The higher content of an irregular flexible structure in the 1:1 complex may reflect the fact that the latter complex was thought (see the preceeding paragraph) to contain also segments of poly(G) being uncapable of forming hydrogen-bonded pairs with poly(C).

Poly(G, I) and its complex with poly(C) was studied with the aid of DPV at the PGE also. Ss poly(G, I) at the PGE in a medium of 0.2 mol.l^{-1} sodium acetate, pH 6.45, also yielded a well-defined DPV peak at a potential of 0.93 V (Fig. 3a) corresponding to electrooxidation of guanine residues (Brabec and Dryhurst 1978a). Moreover, it yielded a shoulder around the potential of 1.28 V on DPV curves, probably in association with electrooxidation of inosinic acid (Karber and Dryhurst 1979; Brabec and Kleinwächter 1981). It seems somewhat surprising that the height of the guanine oxidation peak at 0.93 V yielded by poly(G, I) was in fact identical to that of poly(C) (Fig. 3a) (our sample of poly(G, I) contained only approx. 64 percent of guanine residues as compared with poly(G)). This result indicates that in the case of poly(G) a fewer base residues were in contact with the

electrode, likely due to the tendency of this polynucleotide to form a stable secondary self-structure (Pochon and Michelson 1965).

The formation of the 1:1 ds complex of this copolymer with poly(C) (poly(C) is inoxidizable at graphite electrodes (Brabec and Dryhurst 1978a)) also led to a marked lowering of the guanine oxidation peak (Fig. 3b). However, the peak of poly(G, I). poly(C) occurring at 0.93 V was almost five times lower than that yielded by the 1:1 complex containing homopolymers. This result cannot be explained by a lower content of guanine residues in poly(G, I). poly (C) only; the content of guanine residues in the latter complex was only ca. 1.6 times lower as compared with the complex containing homopolymers. The fact that the poly(G, I).poly(C) complex yielded a five times lower current than did poly(G). poly(C) seems, therefore, to be more consistent with the presence of a higher number of unpaired guanine residues in poly(G).poly(C); this could also be deduced from our DPP measurements.

Conclusions

In the present paper we could show that, in addition to polarography (used in nucleic acid research for almost three decades) voltammetry at graphite electrodes can also detect irregularities in the secondary structure of ds polynucleotide complexes. A comparison of the results of our electrochemical investigations with those of biological experiments reported in the literature indicates that further development in the methodology of analysis of polynucleotide complexes based on electroanalytical techniques, might be of value for elucidating their biological activity.

Abbreviations

DME	— dropping mercury electrode
DPP	— differential pulse polarography
DPV	— differential pulse voltammetry
ds	— double-stranded
PGE	— pyrolytic graphite electrode
poly(C)	— polyribocytidylic acid
poly(G)	— polyriboguanylic acid

poly(G, I) — poly(riboguanylic, inosinic acid), $G:I = 1.75$
ss   — single-stranded

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