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RELATION BETWEEN DIFFERENTIAL PULSE VOLTAMMETRIC OXIDATION OF POLYRIBOINOSINIC ACID AND ITS STRUCTURE

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Electrooxidation of poly(I) at a paraffin wax-impregnated spectroscopic graphite electrode was studied by means of differential pulse voltammetry. It was found that the transition of single-stranded poly(I) to its multistranded form, induced by increasing the ionic strength of neutral medium, is accompanied by a lowering of the oxidation current of poly(I). The marked lowering of the oxidation current is also observable as a consequence of the formation of double-stranded complex of poly(I)•poly(C). The voltammetry at carbon electrodes provides for the study of poly(I) structure in principle identical information as optical methods.

1. Introduction

The study of oxidation-reduction properties of natural and synthetic polynucleotides with the aid of methods of electrochemical analysis is an important area of nucleic acid research [1–5]. This study gave important results both from the point of view of structure and conformation of nucleic acids in the bulk of solution [1,5] and for understanding the properties of nucleic acids on their interaction with electrically charged surface [6–8].

Quite recently the electrooxidation of polyriboinosinic acid (poly(I)) at a pyrolytic graphite electrode was described by Karber and Dryhurst [9]. They found that poly(I) gives a pH dependent oxidation current and proposed an electrochemical method for the detection and determination of trace amounts of hypoxanthine in the poly(I) samples. They stated in their paper [9] that oxidation peak current of poly(I) increases quite steeply with ionic strength up to a value of about 0.4 and the peak current then becomes essentially independent of ionic strength (μ). It is, however, known (e.g. [10–13]) that poly(I) at low ionic strength (below μ = ca. 0.06) at 3–20°C exists as a single-stranded (ss) poorly-stacked helix, while at ionic strengths of above ca. 0.6 it is thought to be largely in a multi-stranded (ms) form with regularly stacked structure, probably a four-stranded helix. A differential pulse voltammetry (DPV) of other polynucleotides (e.g. DNA [4,5], RNA [14], poly(A) [15]) at carbon electrodes showed that the transition of ss structure (characterized by low extent of stacking) to an organized (double-stranded) one was always accompanied by a marked lowering of the oxidation current of the polynucleotide in question. This lowering was explained by the decrease of flexibility of the polynucleotide, which caused a lowering of the concentration of the oxidizable polynucleotide segments at the electrode surface [4,5]. For these reasons the recent report [9] that oxidation current of poly(I) is independent of ionic strength for ionic strengths higher than 0.4 seemed to us surprising.

The aim of this paper was therefore to find out whether the oxidizability of poly(I) at graphite electrode is also influenced by the structure of the polynucleotide in the bulk of solution similarly, as has been shown for other polynucleotides.

2. Materials and methods

Poly(I) was purchased from Reanal (Hungary), polyribocytidylic acid (poly(C)) and polyribouridylic acid (poly(U)) from Miles Laboratories, Inc. (U.S.A.)

and inosine-5′-monophosphate (IMP) from Calbiochem (U.S.A.). Polynucleotide concentration (related to the phosphorus content) and IMP concentration were estimated spectrophotometrically, using the following molar extinction coefficients ϵ: Poly(I) $\epsilon_{248} = 10000$, poly(C) $\epsilon_{268} = 6300$, poly(U) $\epsilon_{260} = 9100$ (in 0.1 M NaCl, pH 7) [16] and IMP $\epsilon_{254} = 12300$ (at pH 11) [17]. The paraffin wax-impregnated spectroscopic graphite electrode (WISGE) was used and prepared in the same manner as described earlier [4,5].

Before use poly(I) was deproteinized with chloroform according to the method of Marmur [18]. After deproteinization the polymer stock solution was extensively dialyzed against sodium phosphate ($\mu = 0.01$ and pH = 7) with 0.01 M EDTA for 48 hours; for a further 24 hours it was dialyzed only against sodium phosphate. The samples of poly(I) and IMP in media differing in μ were prepared by successively adding small quantities of 5 M sodium acetate of appropriate pH to achieve a final pH of 6.8 in all poly(I) samples. Corrections for the dilution by 5 M sodium acetate were applied only to the absorbances shown in figs. 2b and 3b; no such correction for dilution was necessary in the DPV measurements shown in figs. 1–3, because they were carried out under conditions when peak current of poly(I) was practically independent of poly(I) concentration. Sodium chloride, usually used for the control of μ in poly(I) studies [10,11], could not be used in this study because its presence in the background electrolyte solution significantly lowers the anodic potential limit of carbon electrodes [19]. The DPV and absorbance measurements with poly(I) samples thus prepared were performed 4–5 minutes after adjusting μ with 5 M sodium acetate, unless stated otherwise. Poly(I) used for the preparation of the complex of poly(I)• poly(C) was diluted with sodium phosphate to obtain $\mu = 0.1$ and pH 7.

Differential pulse voltammetric curves at the WISGE were obtained with a prototype of the Polarographic Analyzer PA 3 (Laboratory Instruments, Prague, Czechoslovakia) with a pulse amplitude of 50 mV and a sweep rate of 20 mV s^{-1}. Current sampling for DPV was set with the drop time control of the PA 3 set at 0.2 s. The basic procedure for DPV has been described earlier [3–5], but was slightly modified for the experiments reported here. Thus, once the WISGE was inserted into the test solution contained in the electrochemical cell, it was allowed to stand for 10 s without an applied potential. Then an initial or presweep potential of 0.8 V was applied for 240 s (unless stated otherwise) after which time the voltammetric sweep was commenced. Spectra were measured on a Unicam SP 700 recording spectrophotometer with thermostated cuvette holder.

3. Results and discussion

DPV curves of poly(I) at the WISGE at 3°C and μ values of 0.1 and 1.0 (pH 6.8) are shown in fig. 1a,b; for purposes of comparison a DPV curve of IMP is presented in fig. 1c. Poly(I) at the higher μ, i.e. its ms form [10,11] (fig. 1b), yielded a markedly lower oxidation current than ss poly(I) at the lower μ [10,11] (fig. 1a). If dependence of the DPV peak of poly(I) and its absorbance at 247 nm on μ are investigated at 3°C (fig. 2a,b), it is possible to observe changes corresponding to the transition ss poly(I) → ms poly(I) upon increasing μ to 1.0 [11]. For comparison, fig. 2c illustrates also results of Thiele and Guschlbauer [11], who investigated the transition of ss poly(I) → ms poly(I) by means of changes in circular dichroism (CD) spectra under nearly the same conditions. Fig. 2 demonstrates that the transition towards ms poly(I), characterized by a decrease of absorbance at 247 nm and an increase of circular dichroism of the positive band at 253 nm (figs. 2b,c), is accompanied by a parallel decrease of the DPV peak of poly(I) (fig. 2a). The DPV peak of IMP (occuring in the vicinity of the potential of the poly(I) peak (fig. 1c)) was not influenced by changes of μ if the measurement was carried out

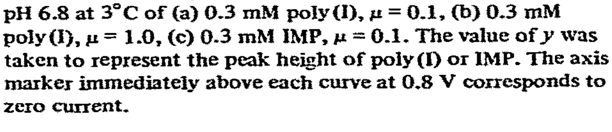

Fig. 1. Differential pulse voltammograms in sodium acetate, pH 6.8 at 3°C of (a) 0.3 mM poly(I), $\mu = 0.1$, (b) 0.3 mM poly(I), $\mu = 1.0$, (c) 0.3 mM IMP, $\mu = 0.1$. The value of y was taken to represent the peak height of poly(I) or IMP. The axis marker immediately above each curve at 0.8 V corresponds to zero current.

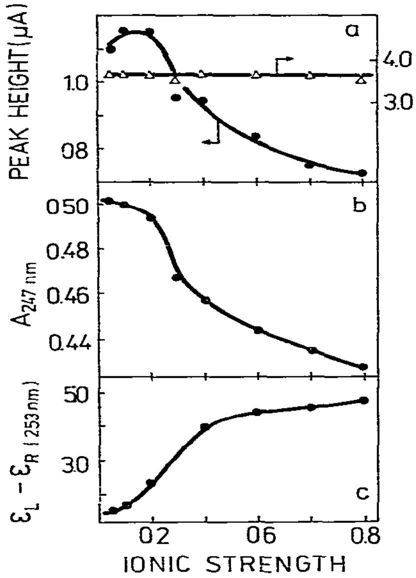

Fig. 2. (a) Variation of the differential pulse voltammetric peak height with sodium acetate concentration at pH 6.8 and 3°C for 0.3 mM poly(I) (●) and 0.3 mM IMP (△). (b) Dependence of absorbance at 247 nm ($A_{247\,nm}$) of 0.05 mM poly(I) at pH 6.8 and 3°C on sodium acetate concentration. (c) Dependence of circular dichroism at 253 nm of poly(I) on sodium chloride concentration at neutral pH and 3°C (taken from the paper of Thiele and Guschlbauer [11]).

under identical conditions (fig. 2a). A small peak yielded by the IMP solution in the vicinity of 0.97 V (fig. 1c) arises from a trace impurity in the IMP sample [9].

The same measurements were also carried out at 20°C, i.e. at a temperature close to that at which the experiments of Karber and Dryhurst [9] were performed. It follows from fig. 3a that in those cases where the DPV curve was recorded relatively rapidly, within 5 minutes after adjusting μ by adding 5 M sodium acetate, the oxidation current of poly(I) was independent of μ approximately in the region of μ = 0.2–0.6. Increasing μ above 0.6 led, however, to a decrease in the oxidation current of poly(I) even at this temperature. If the DPV measurement was performed at μ = 1.0 one week after adding 5 M sodium acetate to a poly(I) solution (the sample was stored at room temperature during this period), the decrease of the oxidation current of poly(I) reached even at 20°C a level (fig. 3a) that was approximately identical with that reached at 3°C (fig. 2a). It is also apparent from fig. 3 that the decrease in the DPV peak of poly(I) proceeded at 20°C in parallel with the changes in optical properties of poly(I) solution (fig. 3b,c) which monitored the transition ss poly(I) → ms poly(I) [10,11].

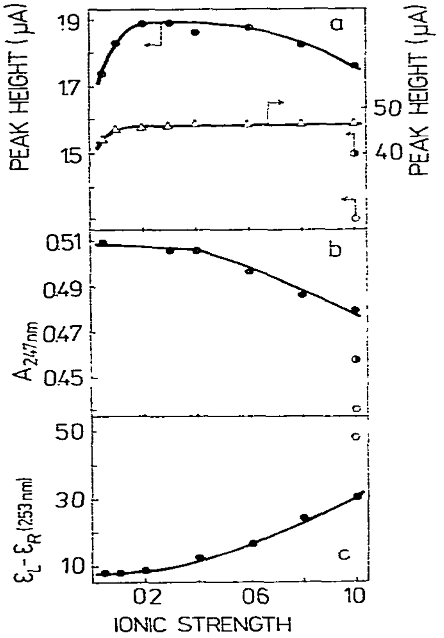

Fig. 3. (a) Variation of differential pulse voltammetric peak height with sodium acetate concentration at pH 6.8 and 20°C for 0.3 mM poly(I) (●) and 0.3 mM IMP (△); poly(I) sample incubated at room temperature for two days (◑) and seven days (○). (b) Dependence of absorbance at 247 nm ($A_{247\,nm}$) of 0.05 mM poly(I) at pH 6.8 and 20°C on sodium acetate concentration; poly(I) sample incubated at room temperature for two days (◑) and seven days (○). (c) Dependence of circular dichroism at 253 nm of poly(I) on sodium chloride concentration at neutral pH and 20°C; poly(I) sample incubated at room temperature for seven days (○) (taken from the paper of Thiele and Guschlbauer [11]).

The measurements in which the transition ss poly(I) → ms poly(I) was investigated (figs. 1—3) were carried out at a relatively high concentration of poly(I) and a long presweep time at initial potential of 0.8 V. This means that these measurements were performed under conditions of full coverage of the WISGE by adsorbed molecules of poly(I). Therefore the decrease of the oxidation current of poly(I) at the higher ionic strength could not be connected with slower transport of ms poly(I) molecules to the electrode surface. On the basis of an analogy with the behaviour of other polynucleotides oxidizable at graphite electrodes [4,5,15] it can be supposed that the described decrease of the oxidation current of poly(I) is connected with the change of poly(I) structure. The decrease in the number of hypoxanthine residues in ms poly(I) accessible for the oxidation reaction could be a consequence of the decreased flexibility of ms poly(I) [11], or also of the fact that some oxidizable groups are hidden inside the ms structure so that they cannot participate in the electrooxidation process at the WISGE.

In neutral solutions of sodium salts of monovalent acids of $\mu = 0.1$ poly(I) associates with poly(C) (which is inactive at graphite electrodes under DPV conditions), forming a double-stranded helical complex [20,21]. Mixing of poly(I) with poly(C) caused a marked decrease in the height of the DPV peak of poly(I) (fig. 4). Mixing of poly(I) with poly(U) had a much smaller influence on the height of the peak of poly(I) (fig. 4) (poly(U) does not form a complex with poly(I) under the conditions used [20]). The decrease of the peak of poly(I) due to its complexing with poly(C) (the complex formation was checked spectrophotometrically [20,21]) can thus be explained in the same manner as its decrease accompanying the transition of poly(I) into the ms form. Moreover, this effect (fig. 4) could perhaps be caused in part also by the fact that the molecules of the complex poly(I)·poly(C) were, owing to their lower diffusion coefficient, transported to the electrode more slowly than the molecules of ss poly(I); it should be noted that the DPV mixing curve was recorded under conditions of incompletely covered electrode surface by adsorbed polynucleotide molecules. The decrease of the peak of poly(I) after its mixing with poly(U) (significantly lower than after mixing with poly(C)) was probably connected with the lowering of poly(I) concentration at the surface of the WISGE. This lowering might be caused by the competition of poly(I) molecules with poly(U) molecules for adsorption at the WISGE surface.

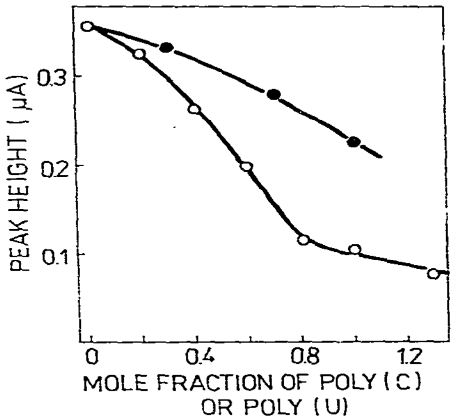

Fig. 4. Formation of the 1:1 complex of poly(I)·poly(C) followed by differential pulse voltammetry at 25°C. Homopolymers were mixed in phosphate buffer of ionic strength of 0.1 and pH 7.0. Voltammetric measurements were carried out after two hours of incubation at room temperature. Concentration of poly(I) (0.03 mM) was held constant in all samples, while the amount of poly(C) (○) or poly(U) (●) varied as indicated in the figure. The mole fraction of poly(C) or poly(U) at 1.0 corresponds to the poly(C) or poly(U) concentration of 0.03 mM. Before commencing the voltammetric sweep, initial potential of 0.8 V was applied for only 120 s.

4. Conclusions

The results of DPV investigations of polynucleotides at carbon electrodes show that this electrochemical method is a useful technique for studies of the structure of both natural nucleic acids and synthetic polynucleotides in the bulk of solution. In the present paper the influence of changes in the structure of poly(I), on its DPV behaviour at the WISGE is demonstrated. The structural changes were induced by varying ionic strength, temperature and time of incubation of the poly(I) sample or by complexing poly(I) with poly(C). It also follows from our results that in the interpretation of the electrochemical behaviour of poly(I) great attention has to be paid to the factors influencing the structure of the polynucleotide. Especially such factors as ionic strength, temperature and time should always be considered.

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