

Interaction of Polyadenylic Acid with the Mercury Electrode

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(Z. Naturforsch. **28 c**, 685–692 [1973]; received April 30, 1973)

Polyadenylic acid, electrode process, conformation, diffusion coefficient

Interactions of polyadenylic acid with the mercury electrode were followed by means of direct current (d.c.), alternating current (a.c.), and pulse polarography. It was ascertained that the **electrode reaction of polyadenylic acid did not differ significantly** from the reaction of its monomeric components. In contrary to the latter reaction, the polarographic reduction of polyadenylic acid takes place only in the adsorbed state. Desorption of polyadenylic acid from the surface of the mercury electrode at negative potentials inhibits the reduction current. Due to this inhibition the d.c. polarographic curves of polyadenylic acid can have the shape of a single maximum or of a "double-wave". A method was suggested for a rapid determination of diffusion coefficients of single-stranded polyadenylic acid by means of d.c. polarography. A.c. polarographic measurements yielded data on the adsorption/desorption behaviour of polyadenylic acid, on the basis of which conclusions were made concerning the conformation of polyadenylic acid in solution. While relatively concentrated solutions of polyadenylic acid must be used in the d.c. and a.c. polarographic studies, a little as 10–15 ng of polyadenylic acid can be determined by means of pulse polarography.

The use of polarographic methods in the analysis of nucleic acids is based on the one hand on the fact that the polynucleotides which contain reducible bases (adenine and cytosine) yield polarographic reduction currents as far as the reduction sites are not prevented sterically from interaction with the electrode^{1–10}, on the other hand on following polynucleotide adsorption on the mercury electrode^{11–13}.

Polyriboadenylic acid is a homopolynucleotide formed by adenosine monophosphate units. Neutral single-stranded polyadenylic acid (further poly(A)) has a helical structure with partially stacked bases¹⁴. It is supposed that the majority of reduction sites in poly(A) is accessible for the electrode process⁹. The protonation of the bases at acid pH causes formation of the double-helical structure of polyadenylic acid (further poly(A.A)⁺). The polynucleotide chains in poly(A.A)⁺ are parallel with adenine residues protonated at N-1, and are stabilized by two hydrogen bonds (between the 6-amino group and N-7, eventually an oxygen atom of the phosphate group) and by electrostatic forces between the protonated N-1 and the phosphate group¹⁵. At acid pH poly(A.A)⁺ can exist in several forms differing in their polarographic behaviour⁹. Our recent results¹⁶ indicate that the

existence of the different forms depends on the length of the molecule of single-stranded poly(A) and that the properties of poly(A.A)⁺ are strongly influenced by aggregation.

Polarographic methods were used for following the transition $\text{poly(A)} \rightleftharpoons \text{poly(A.A)}^+$ ^{7, 9, 16, 17} as well as for the determination of molecular weight of single-stranded poly(A)⁸. The present paper brings information which characterizes in a greater detail electrode processes to which both single-stranded and double-stranded polyadenylic acids are exposed.

Materials and Methods

Two samples of polyadenylic acid (samples S and L) were products of the firm Schwarz, Orangeburg, N.Y. They were used for polarographic and spectrophotometric measurements in dependence on pH, which showed¹⁶ that the length of the molecules influences the polarographic behaviour of acid forms of polyadenylic acid. The third sample (sample M) was purchased from Miles Laboratories, Elkhart, Indiana. Sedimentation coefficients $s_{20,w}$ of polyadenylic acid were kindly estimated by Dr. J. Šponar. $s_{20,w}$ were determined in 0.1 M NaCl with citrate buffer containing $1 \cdot 10^{-3}$ M ethylenediaminetetraacetic acid (EDTA) at pH 6.5 for the single-stranded form and at pH 4.9 for the protonated double-helical form; concentration of polyadenylic acid was $1 \cdot 10^{-4}$ M. $s_{20,w}$ and diffusion coefficients D for all samples of polyadenylic acid used in the present study are listed in Table I. Adenosine was

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supplied by Calbiochem, Los Angeles, California. The supporting electrolyte used for the polarographic measurements was 0.5 M NaCl with citrate buffer or McIlvaine buffer (M.I.b.); EDTA was always present in $1 \cdot 10^{-3}$ M concentration. The chemicals were of analytical grade. In the medium of 0.5 M NaCl with citrate buffer polyadenylic acid exhibited single-stranded structure at $\text{pH} > 5.7$; whereas at $\text{pH} < 5.3$ it assumed double-stranded conformation¹⁶. The samples of polyadenylic acid for the polarographic measurements were prepared in the following way: The polynucleotide was dissolved in distilled water in concentration twice as high as was used in the experiments. To this solution equal volume of suitable background electrolyte (again in concentration twice as high as requested in the experiments) was added dropwise. pH was measured after the mixing. The solutions of polyadenylic acid of different concentration (for the determination of dependence of the d.c. polarographic step * height on polynucleotide concentration (Fig. 2)) were obtained from a sample of 2.0 mM polyadenylic acid prepared in the above described way by stepwise dilution by background electrolyte to the concentrations given in the graph. The concentration of polyadenylic acid related to the mononucleotide content was estimated spectrophotometrically.

The macroscale electrolysis at a controlled potential was performed on a mercury pool electrode bubbled with a stream of N_2 using a potentiostat (SVUOM, Prague). The area of the mercury electrode was approximately 2 cm^2 . During the electrolysis d.c. polarograms and ultraviolet absorption spectra were recorded. The macroscale electrolysis was carried out at 25°C at the potential of a third of the d.c. polarographic step height. pH of the electrolysed solution was controlled.

D.c. and a.c. polarographic measurements were carried out with a GWP 563 Polarograph (Akademie Werkstätten für Forschungsbedarf der Deutschen Akademie der Wissenschaften zu Berlin) in nitrogen atmosphere. A.c. voltage amplitude was 20 mV at 78 c.s.^{-1} . A.c. polarograms were obtained at a sensitivity of $6 \mu\text{A}/\text{scale}$; other details were already published^{19, 20}. The variation of d.c. with time during the lifetime of a single drop (i/t curve) was recorded for sequential drops from a vertical capillary. Even though the shape of i/t curves thus

recorded could be influenced by depletion of the solution in the vicinity of the drop²¹, the depression on i/t curves could be used as a criterion for the adsorption process²¹⁻²³. i/t curves were recorded by means of a Tesla oscilloscope BM 62 equipped with a d.c. preamplifier containing an operational amplifier Tesla MAA 501. Traces on oscilloscope screen were photographed with an Exacta Varex camera using NP 20 Orwo film. The dropping mercury electrodes (DME) used in d.c. and a.c. polarographic measurements had the following constants: At the mercury column height $h = 50 \text{ cm}$, capillary A had the mercury flow rate $m = 2.38 \text{ mg s}^{-1}$ and the drop time $t = 3.2 \text{ s}$; at $h = 40 \text{ cm}$, capillary A had $t = 4.1 \text{ s}$. At $h = 55 \text{ cm}$, capillary B had $m = 2.81 \text{ mg s}^{-1}$ and $t = 4.8 \text{ s}$ (measured in distilled water with an open current circuit and at 25°C). The mercury column height data used for the estimation of current dependence on h were corrected for backpressure. In d.c. and a.c. polarographic measurements potentials were measured against saturated calomel electrode.

The pulse-polarographic measurements were carried out with an A3100 Southern-Harwell Pulse Polarograph (Southern Analytical Ltd.) using the DME; other details were already published⁷. In pulse-polarographic measurements the mercury pool on the bottom of the polarographic cell served as a reference electrode. The capillary used in pulse polarography had $t = 5.5 \text{ s}$.

Spectra were measured on a Unicam SP 700 recording spectrophotometer; pH values were measured with a Radelkis OP 205 pH-meter. All measurements were carried out at 25°C .

Results

1. Macroscale electrolysis

The course of electrolysis of adenosine and poly(A.A)⁺ (sample M) was followed in solutions of concentration $2 \cdot 10^{-4} \text{ M}$. During the electrolysis both the d.c. polarographic step and the typical spectral maximum in the vicinity of 260 nm decreased (Figs 1 a, b). The electrolyses were brought to an end after approximately five hours, when the d.c. polarographic step had completely disappeared. The products of the electrolysis of both substances did not yield any more the spectral maximum in the vicinity of 260 nm, but exhibited a new maximum at approximately 240 nm (Figs 1 c, d). The appearance of a precipitate was not observed during the electrolysis of poly(A.A)⁺, in contrary to *e.g.* the electrolysis of polycytidylic acid (poly(C))²⁰ or denatured DNA²⁴.

* In the present paper the term "step" refers to the curve obtained by means of d.c. polarography, while phenomena observed on the a.c. polarographic and derivative pulse-polarographic curves are called "peaks". In those cases when the course of a polarographic curve is more complex, its shape is described in detail in order to avoid any confusion.

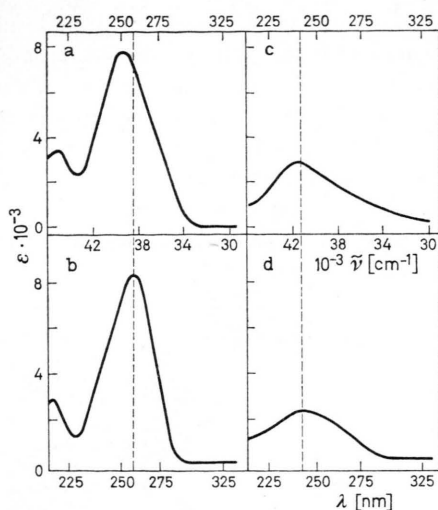


Fig. 1. Ultraviolet absorption spectra of poly(A.A)⁺, adenosine, and products of their macroscale electrolysis in 0.5 M NaCl with M.I.b. at pH 4.8; ribose concentration in all samples was $2 \cdot 10^{-4}$ M. a. poly(A.A)⁺; b. adenosine; c. product of the electrolysis of poly(A.A)⁺; d. product of the electrolysis of adenosine.

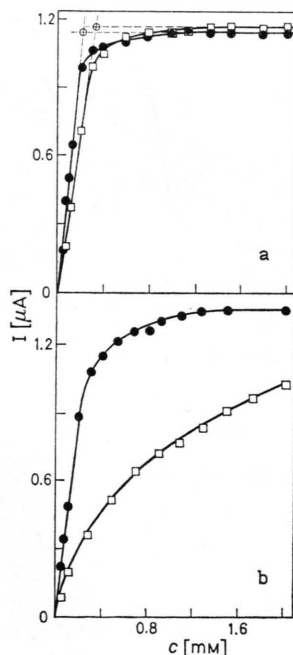


Fig. 2. The dependence of the d.c. polarographic step height of polyadenylic acid on its concentration in 0.5 M NaCl with citrate buffer. Capillary A at 50 cm. a. pH 5.8; b. pH 4.8; ● sample S; □ sample L.

** The ratio $I : h^{1/2}$ is independent of $h^{1/2}$ or decreases slightly with increasing $h^{1/2}$ if currents are diffusion controlled²⁵.

2. Classical (d.c.) polarography

The majority of measurements of poly(A) was performed at pH 5.8, *i.e.* in the region where the reduction current of poly(A) does not practically depend on pH. The dependences of the d.c. polarographic step height (I) on concentration of poly(A) or poly(A.A)⁺ (samples S and L) had a limiting character (Fig. 2). The dependences obtained for poly(A) as well as for poly(A.A)⁺ with lower $s_{20,w}$ (sample S) reached the limiting values at lower concentration than the dependences obtained for the sample L (Fig. 2). The transition poly(A) → poly(A.A)⁺ caused in both samples (S and L) a shift to higher values of polynucleotide concentration at which the limiting value was reached (Fig. 2).

The character of the dependence of I on the mercury column height h was changed with polynucleotide concentration. The ratio $I : h^{1/2}$ was independent of $h^{1/2}$ or slightly decreased with increasing $h^{1/2}$ ** for samples S and L of both poly(A) and poly(A.A)⁺ at a concentration of $8 \cdot 10^{-5}$ M (when the limiting part of the concentration dependence curve was not yet reached — Fig. 2). However, if polynucleotide concentration was so high that current did not change with increasing polynucleotide concentration, the ratio $I : h^{1/2}$ was enhanced with growing $h^{1/2}$; the step height was directly proportional to h (Fig. 3) which is typical for adsorption currents^{23, 25}.

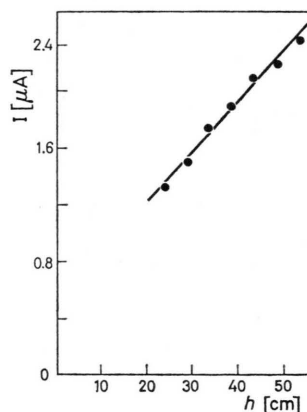


Fig. 3. The dependence of the d.c. polarographic step height of poly(A) on the mercury column height. 1.2 mM poly(A) in 0.5 M NaCl with M.I.b. at pH 5.8; capillary B.

Also the shape of i/t curves of poly(A) (sample L and S) recorded at various potentials along the rising part of the d.c. polarographic step (Fig. 4 a)

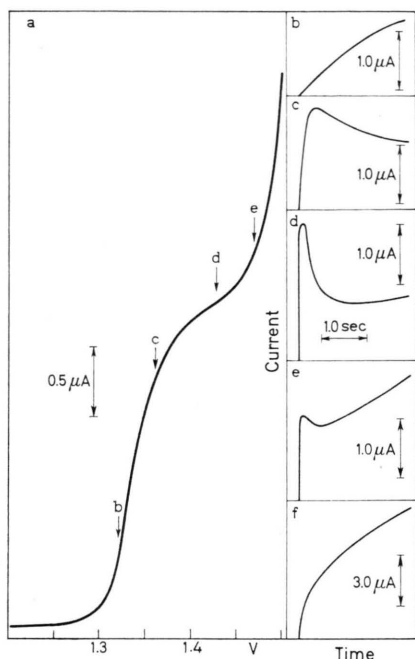


Fig. 4. D.c. polarogram (a) and current time curves (b–f) of 1 mM poly(A) in 0.5 M NaCl with M.I.b. at pH 5.8. Current-time curves were taken at potentials: b. -1.32 V; c. -1.36 V; d. -1.43 V; e. -1.47 V; f. -1.52 V. Capillary B.

depended on the poly(A) concentration. For $8 \cdot 10^{-5}$ M poly(A) i/t curves had a shape of parabolas without any depression. Increasing concentration to the value which corresponded to the limiting part of the concentration dependence (Fig. 2 a) caused an appearance of a depression on i/t curves in the vicinity of potentials corresponding to the limiting current of poly(A) reduction (Figs 4 c–e). This depression is characteristic for adsorption currents^{21–23}.

In the region of pH 4–5 half-step potentials ($E_{1/2}$) of samples S and L of poly(A.A)⁺ were shifted linearly to more negative values with increasing pH. The slope of this linear dependence was approximately 0.04 V/pH. A break could be observed in these dependences in a pH region which corresponded to the transition $\text{poly(A)} \rightleftharpoons \text{poly(A.A)}^+$.

At pH 6.0, $1 \cdot 10^{-4}$ M poly(A) with the lower $s_{20,w}$ (sample S) yielded a d.c. polarographic curve having a shape of a single maximum (Fig. 5 a). On the contrary poly(A) with the higher $s_{20,w}$ (sample L) yielded under identical conditions a

“double-wave” composed of a step and a more negative maximum (Fig. 5 b). The decrease of current, due to which a maximum arose on the d.c. polarograms of both samples, appeared for the sample L at potentials by approximately 40 mV more negative than for the sample S (Fig. 5). Similar dependence of the shape of the d.c. polarographic curve of poly(A) on $s_{20,w}$ was also observed by Janík and Sommer⁸.

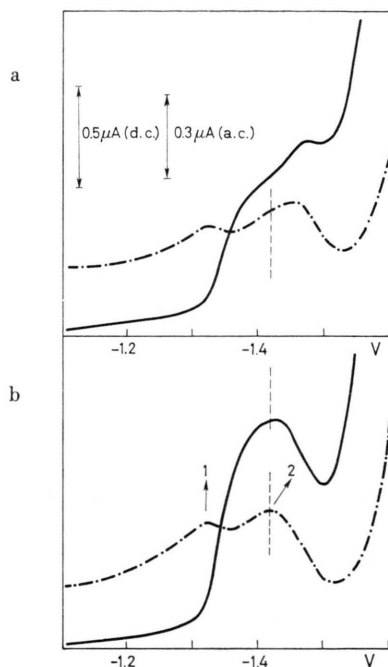


Fig. 5. D.c. — and a.c. - - - polarograms of 10^{-4} M poly(A) in 0.5 M NaCl with M.I.b. at pH 6.0. a. sample S; b. sample L. Capillary B.

3. A.c. polarography

Parallely with the d.c. polarograms also a.c. polarograms were recorded for $1 \cdot 10^{-4}$ M poly(A) (samples S and L) at pH 6.0 (Fig. 5). The more negative a.c. polarographic peak 2 appeared always in the vicinity of the potential corresponding to the beginning of the decrease on the d.c. polarographic curve. Potential of the peak 2 of the sample L was also by about 40 mV more negative than that of the sample S. However, potential of the more positive a.c. polarographic peak 1 was for both samples, L and S, approximately the same (Fig. 5).

The a.c. polarographic behaviour of 1.0 mM polyadenylic acid (samples S and L) was followed in

the dependence on pH. Poly(A) yielded two peaks on the a.c. polarogram in the vicinity of potential -1.3 V under conditions, when poly(A) was polarographically nonreducible¹³ (Fig. 6 a). The

potential of the more negative peak 2 was more negative for the sample L than for the sample S (Fig. 6 a). A decrease of pH (conditioning the reducibility of poly(A) caused an appearance of a

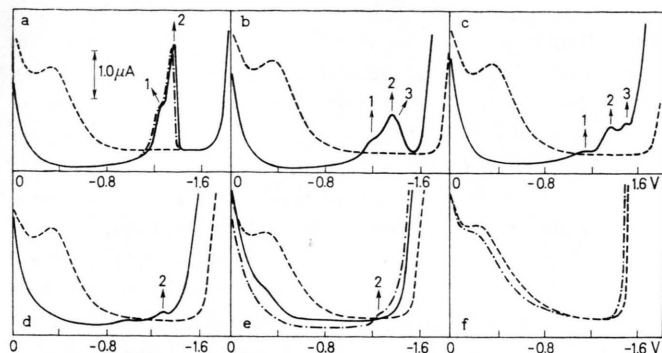


Fig. 6. A.c. polarograms of 1.0 mM polyadenylic acid in 0.5 M NaCl with M.I.b. a. pH 7.7; b. pH 6.3; c. pH 5.8; d. pH 4.8; e. pH 4.0; f. pH 3.3; sample L —; sample S ---; background electrolyte ···. Capillary A at 40 cm.

new peak 3, which was further shifted to more negative potentials with decreasing pH (Figs 6 b, c) (the peak 3 was not observed for $1 \cdot 10^{-4}$ M poly(A) — (Fig. 5) probably due to relatively low sensitivity of the a.c. polarographic method). Decreasing pH caused also a decrease of the peak 2 and a shift of the peak 1 to more positive potentials (Figs 6 a–c). A shift of the peak 2 to the more positive potentials with decreasing pH took place only in the course of the transition $\text{poly(A)} \rightarrow \text{poly(A.A)}^+$, however (Fig. 6 d). The acidification of the solution of poly(A.A)^+ to pH 4.0 caused a pronounced reduction of the difference between the alternating currents of the polynucleotide and background electrolyte in the vicinity of the potential of the electrocapillary maximum (ECM) in the case of the sample L (Fig. 6 e). For the sample S this effect was observed only at pH 3.3. At this pH value the polarogram of the sample L was already identical with the polarogram of the pure background electrolyte (Fig. 6 f).

4. Pulse polarography

The sample M was used in the pulse-polarographic measurements. Normal pulse-polarographic curves of poly(A) exhibited the shape of peaks, the height of which depended on starting potential. *E.g.*, for $1 \cdot 10^{-4}$ M poly(A) in 0.5 M NaCl with citrate buffer at pH 5.8 the peak heights were 46, 45, and 48 scale divisions for the values of starting potential 0.0, -0.75 , and -1.25 V, respectively. If the measurements were performed at lower polynucleotide

concentrations, the differences in the peak heights were more pronounced. At reversed direction of pulse potential, poly(A) yielded the peak approximately 1/8 as high as observed at the usual direction of the pulse; poly(A.A)^+ did not yield any current. The height of derivative pulse-polarographic peaks of poly(A) and poly(A.A)^+ depended linearly on the pulse amplitude only in the interval 2–10 mV (Fig. 7); at 25 mV a small deviation from the linearity could be observed and the peaks obtained at the amplitude 50 and 100 mV were substantially higher than it could be expected for the

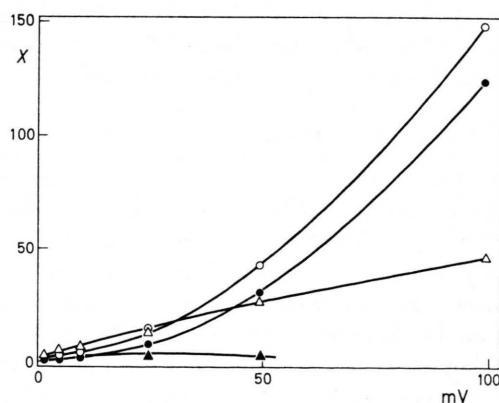


Fig. 7. The dependence of the derivative pulse-polarographic peak height X (divisions) of $5 \cdot 10^{-5}$ M polyadenylic acid (sample M) on the pulse amplitude. Medium 0.5 M NaCl with citrate buffer. Pulse of the same direction as voltage ramp: ○ pH 5.8; ● pH 5.1. Pulse of opposite direction as voltage ramp: △ pH 5.8; ▲ pH 5.1. Amplifier sensitivity 1/256 or higher; number of scale divisions recalculated to sensitivity 1/64.

linear dependence. The peak height — amplitude dependence thus differed from dependences so far observed for inorganic substances²⁶. If sufficiently high pulse amplitude is used, the determination of poly(A) by the derivative method is substantially more sensitive than by the normal pulse polarography. The dependence of the height of the derivative peak on polyadenylic acid concentration at pulse amplitude 50 mV is illustrated on Fig. 8. The lowest

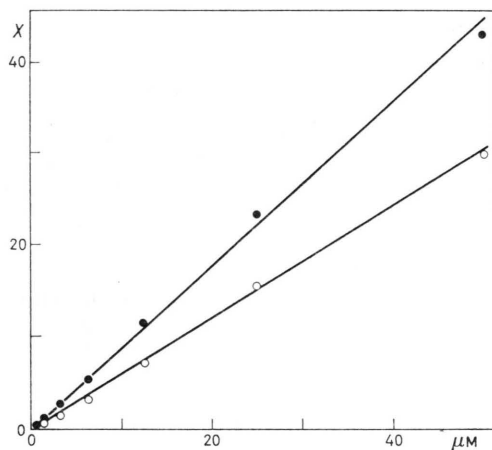


Fig. 8. The dependence of the derivative pulse-polarographic peak height X (divisions) on the concentration of polyadenylic acid (sample M) in 0.5 M NaCl with citrate buffer. ● pH 5.8; ○ pH 5.1. Pulse amplitude 50 mV, amplifier sensitivity 1/64 or higher; number of scale divisions recalculated to sensitivity 1/64.

detectable concentration of poly A was about $2 \cdot 10^{-7}$ M for pulse amplitude 100 mV; if the analysis was performed in the volume 0.2 ml, approximately 10–15 ng of poly A could be detected.

Discussion

Monomeric adenine and its derivatives are subject to an irreversible four-electron reduction on DME^{27,28}. At the reaction of these substances on DME, 1,6 and 3,2 N=C double bonds are reduced; the necessary condition for the reduction is protonation of adenine moiety on N1. Polarographic reduction current is diffusion-controlled at pH < 5, when the current is almost independent of pH. It has kinetic character at higher pH, when the current decreases distinctly with increasing pH^{27,28}.

A comparison of the heights of the normal pulse-polarographic peaks of polyadenylic acid in the course of a shift of the pulse potential either to more negative or more positive values shows that,

similarly to monomer reduction, also the reduction of the polynucleotide is irreversible²⁹. Changes of $E_{1/2}$ with pH indicate that the reduction of polyadenylic acid takes place in the protonated state. Previous results together with the results of the electrolysis (Figs 1 c, d) show that the electrode reaction of polyadenylic acid probably does not very differ from reactions of its monomeric units. In contrary to the reduction of these units, adsorption of polyadenylic acid on DME represents a very important factor in its reduction. It is supposed that the reduction of polyadenylic acid at negative potentials can (considering the polyanionic character of the molecule) take place only in the adsorbed state³⁰. The adsorption character of the current of polyadenylic acid manifested at higher polynucleotide concentrations (Figs 2 and 3), the shape of the d.c. polarographic curves as well as their relation to the a.c. polarographic curves (Fig. 5) are in agreement with this assumption. If polyadenylic acid is reduced only in the adsorbed state, its desorption from the surface of negatively charged DME must inhibit the reduction current and a decrease can appear on the d.c. polarographic curve. Due to this decrease the d.c. polarographic curve can have the shape of the single maximum (Fig. 5 a) or of the "double-wave" (Fig. 5 b). The latter case takes place if the polynucleotide desorbs at potentials only a little more negative than the potentials of the beginning of the limiting portion of the d.c. polarographic curve. Under these circumstances first the more positive step arises (the beginning of the reduction limiting current), and the following current decrease has, due to superposition on the curve of background electrolyte discharge, the shape of a maximum (Fig. 5 b). If the polynucleotide desorption takes place at more negative potentials, the current decrease merges with the too high background electrolyte discharge current. On the contrary, if the polynucleotide desorption takes place at potentials more positive than are the limiting current potentials, the current decrease is manifested in the way demonstrated in Fig. 5 a. The influence of $s_{20,w}$ on the shape of the d.c. polarographic curve of poly(A) at pH 6.0 (Fig. 5) can be thus explained by an influence of the length of the molecule on its desorption potential. The more positive potential of poly(A) desorption (following from potential of the a.c. polarographic peak 2²⁰) for the sample S is probably connected with the fact

that the adsorbed polymers with lower molecular weight are, as a rule, desorbed easier than polymers of higher molecular weight³¹. D.c. polarographic curves of similar shape and nature were also yielded by denatured DNA³².

The unusual dependence of the height of the derivative pulse-polarographic peak of polyadenylic acid on the pulse amplitude (Fig. 8) is connected probably with the proximity of potentials of reduction and desorption of the polynucleotide. Similar dependence was also observed with poly(C)⁷, DNA, and RNA³³, and it can be supposed that the explanation of this dependence suggested for poly(C)⁷ will be essentially the same also for poly(A).

It has been shown for many polyelectrolytes including DNA and RNA^{11, 34-36} that under conditions when the DME surface is not fully covered by polyelectrolyte molecules, at a constant drop time t , the measured surface property (surface tension, surface charge density, and differential capacity) is directly proportional to polyelectrolyte concentration in the bulk of solution c and thus also to the amount adsorbed Γ_t . It means consequently that diffusion is the controlling process of polyelectrolyte adsorption on the DME surface¹¹. We have also shown for polyadenylic acid that the value of current (I), which is probably also a surface property of polyadenylic acid, is directly proportional to polyadenylic acid concentration under conditions, when the full coverage of the DME surface is not reached (Fig. 2 a). It can be therefore supposed that diffusion is the controlling process also in polyadenylic acid adsorption on the DME surface at potentials of its reduction. For short drop times, when the full coverage of the DME surface is not yet reached, the adsorption process controlled by diffusion can be described by the relation

$$\Gamma_t = 0.745 (D t)^{1/2} c, \quad (1)$$

where D is the diffusion coefficient of the adsorbed substance^{7, 38}. The saturated surface concentration Γ_{sat} can be reached for certain c_{sat} at each given t . Thus, if we had two samples of poly(A) differing in D and performed the measurement at the same t , then, under the assumption that Γ_{sat} is identical for both samples, S and L, of poly(A) it follows from Eqn (1)

$$\left(\frac{D_L}{D_S} \right)^{1/2} = \frac{(c_{\text{sat}})_S}{(c_{\text{sat}})_L}, \quad (2)$$

where the indexes L and S refer to the samples L and S. Critical concentrations c_{sat} , at which the surface of DME is just fully covered (at potentials of the reduction), can be estimated from the intersection of two lines on the graph of the dependence I vs c , as it is illustrated in Fig. 2 a. Substitution of the measured values c_{sat} into Eqn (2) led to $D_L/D_S = 2.3$, which agrees with the value obtained on the basis of values D calculated from the measured $s_{20,w}$ by means of Svedberg relation¹⁸. Hence it follows that Eqn (2) can be used for a rapid estimation of D of poly(A) if D of one standard sample of poly(A) is known.

Janík *et al.*⁹ experimented with poly(A) of high molecular weight (10^6) and observed an expressive decrease of reduction current due to the transition $\text{poly(A)} \rightarrow \text{poly(A.A)}^+$. They explained the decrease of the current by a hiding of reduction sites inside the double-helix of poly(A.A)^+ . Our study of the transition $\text{poly(A)} \rightarrow \text{poly(A.A)}^+$ for samples of different molecular weight¹⁶ showed that this decrease of the current depends on the length of the poly(A) molecule and that for shorter molecules the current decrease is small or none. This result indicates that the formation of poly(A.A)^+ does not cause the hiding of reduction sites in the double-helix and that the lower currents recorded for longer molecules of polyadenylic acid as a consequence of the appearance of poly(A.A)^+ are conditioned by a change in D and perhaps by hiding a limited number of reduction sites due to aggregation. This explanation is also supported by the d.c. polarographic behaviour of both poly(A) and poly(A.A)^+ observed with the samples S and L in dependence on the polynucleotide concentration (Fig. 2): Whereas at concentration $1.5 \cdot 10^{-4}$ M (*i.e.* in the region when the current is probably diffusion controlled) the ratio of the step heights for poly(A) and poly(A.A)^+ is 1.2 for the sample S and 2.4 for the sample L, at concentration $2 \cdot 10^{-3}$ M (*i.e.* in the region when the electrode surface is fully or almost fully covered and the value of D ceases to influence the step height) this ratio is 0.9 for the sample S and 1.1 for the sample L. The estimation of D for poly(A.A)^+ by means of Eqn (2) is not reliable due to an aggregation of poly(A.A)^+ molecules, which is strongly dependent on the polynucleotide concentration and on ionic strength in the time of double-helix formation.

The a.c. polarographic behaviour of poly(A.A)⁺ at pH 4 and lower demonstrated a different adsorbability of the samples L and S. The lower adsorbability of the sample L (which follows from a decrease of the difference between the alternating currents of background electrolyte and poly(A.A)⁺ solution in the region 0.0 – (–1.2) V (Fig. 6 e)) is obviously connected with a higher tendency of longer poly(A.A)⁺ molecules to lateral aggregation in comparison with shorter molecules of the sample S under identical conditions¹⁶. The lateral aggregation leads to a decrease of *D* as well as of the number of accessible groups which have the tendency for adsorption on the DME¹³. From spectrophotometric measurements it follows¹⁶ that the total loss of adsorbability of the sample L at pH 3.3 was probably a consequence of formation of so great aggregates that they started to precipitate from solution. The decrease of adsorbability of poly(A.A)⁺ at pH 4.0 and lower thus shows that, under conditions of our a.c. polarographic measurements, not only the hiding of reduction sites inside the double-

helix took place as it had been suggested by Janík *et al.*⁹. By this mechanism the adsorbability of poly(A.A)⁺ could only be changed, but not totally disappear. Namely it was shown¹³ that all constituents of polynucleotides (*i.e.* residues of bases, sugars, and phosphoric acid) are capable of adsorption on the DME. Thus, merely the hiding of bases should not lead to the total loss of adsorbability in a broad range of potentials.

Tab. I. Sedimentation coefficients (*s*_{20,w}) and diffusion coefficients (*D*) of polyadenylic acid samples used in the present study.

Sample	<i>s</i> _{20,w} (Svedberg)		<i>D</i> · 10 ⁷ [cm ² s ^{–1}]*	
	pH 6.5	pH 4.9	pH 6.5	pH 4.9
S	2.6	4.2	3.5	6.9
L	5.9	15.1	1.5	0.6
M	5.8		1.6	

* *D* was calculated from *s*_{20,w} by means of Svedberg relation¹⁸. Details concerning this calculations are given elsewhere¹⁶.

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