POLAROGRAPHY OF POLYNUCLEOTIDES

III. POLYADENYLIC ACID:

THE ELECTRODE PROCESS AND INTERACTION WITH POLYAMINES

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ABSTRACT Polyadenylic acid (poly A) was studied under various conditions using both DC polarography and phase sensitive AC polarography and by measuring the time-course of the current during the lifetime of a single drop of the dropping mercury electrode. Under certain conditions the current at potentials of the limiting portion of the DC polarographic wave does not reach its limiting value and in extreme situations peak-shaped curves are observed. This phenomenon is explained in terms of desorption and repulsion from the electrode of neutral poly A due to its polyanionic character. Consequently, the suppression of the current can be enhanced by increasing negative potential of the electrode and by exposing the negative charges of phosphate groups, e.g., by increasing pH and temperature and by decreasing ionic strength and buffer capacity; vice versa, the current suppression can be at least partially eliminated by reversing these conditions. Polyamines which seem to shield the phosphate groups through specific interactions are very effective in eliminating the current suppression. The effectiveness of a polyamine is determined by its chain length and by the density of its amino groups and the geometry of their distribution.

INTRODUCTION

Applicability of polarographic techniques for the study of natural and synthetic polynucleotides has become increasingly recognized during the past decade. Interpretation of the polarographic behavior of polynucleotides in terms of their secondary structure, conformation, and molecular parameters is mainly indirect, based, *inter alia*, on information about electron transfer processes, accessibility of electron acceptor sites, and adsorption properties (e.g., references 1–4). Consequently, a good understanding of the mechanism of electrode processes is required for correct interpretation of observed phenomena.

Under certain conditions, denatured DNA (1, 5, 6), poly A (2, 3, 5, 7),[1] and polycytidylic acid (poly C) (7) yield peak-shaped (maximum-like) DC polarographic

[1] *Abbreviations used in this paper:* poly A, polyadenylic acid; poly C, polycytidylic acid; poly U, polyuridylic acid.

curves, while adenine and cytosine as well as their nucleosides and nucleotides (8, 9), which are the reducible moieties of the above polymers, exhibit the "classically" shaped DC polarographic curve, i.e., the wave. It seems to be well documented that the peak-shaped DC polarographic curves of poly A and poly C cannot be explained by the formation of maxima of the second kind or by catalytic evolution (7). In order to explain this peak phenomenon, it was assumed (7, 10) that the polynucleotide was reducible only in the adsorbed state (because of easier protonation in this state and/or too strong repulsion of an unadsorbed polyanion by the negatively charged electrode) and that it would desorb at more negative potentials causing the current to decrease. An explanation of the effect of cations on the height and shape of the DC polarographic wave (referred to as a step) was suggested (7) in terms of the affected adsorbability of the polynucleotide by the cation screening the negative charges of the polynucleotide and being present in the electrode double layer. The greater effect of NH_4^+ compared with that of Na^+ and K^+ was connected with higher screening efficiency of NH_4^+ and/or with its proton-donor ability.

In connection with our previous studies of poly A (2, 3), we have obtained data which may help to clarify the above assumptions. We also present experimental evidence, which supports an earlier suggestion (10), that the peak shape of DC polarographic curves is caused by suppression of the current at more negative potentials on the limiting portion of the wave rather than by a current built up at the limiting portion, e.g., due to the recently criticized (11) surface catalytic wave (6). Moreover, it is demonstrated that neutral poly A interacts specifically with polyamines.

EXPERIMENTAL

Materials and methods, including the preparation of poly A fractions and the set up for polarographic measurements on the PAR 170 electrochemistry system (Princeton Applied Research Corp., Princeton, N. J.), were, unless otherwise stated, as previously described (2, 3). In a typical experiment, the examined solution was kept at 25°C, and the polarographic curves were recorded at a scan rate of 5 $mV \cdot s^{-1}$, a mercury column height h of 64 cm, and with either a naturally dropping mercury electrode or in a current-sampled mode at a drop time of 1 s and a sample time of 15 ms. AC polarograms were recorded both in phase and 90° out of phase with the applied alternating voltage (80 Hz, 10 mV peak-to-peak amplitude). The current-time, $i - t$, curves on a single, naturally falling mercury drop were also recorded on the PAR 170 electrochemistry system. The polarographic capillary had the following m values ($mg \cdot s^{-1}$) in distilled water (open circuit) at h of 64 cm: 1.707 at a natural drop time of 3.8 s and 1.734 at a controlled drop time of 1 s.

The background electrolyte was a NaCl solution buffered with phosphate or McIlvaine buffers (of an ionic strength I of 0.1 M in most cases). Polyamines obtained from the following sources were used without further purification: Eastman:[2] 1,2-diaminoethane, 1,6-diaminohexane, and bis(3-aminopropyl)amine; Aldrich: 1,4-diaminobutane(putrescine) and

[2] Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.: Aldrich Chemical Co., Inc., Milwaukee, Wis.; Sigma Chemical Co., St. Louis, Mo.; Nutritional Biochemical Corp., Cleveland, Ohio; MC&B Manufacturing Chemists, Norwood, Ohio.

1,8-diaminooctane; Sigma: 1,5-diaminopentane·2 HCl (cadaverine); Nutritional Biochemical Corp: *N*-(3-aminopropyl)-1,4-diaminobutane·3 HCl (spermidine); MCB: *N,N'*-bis(2-aminoethyl)-1,2-diaminoethane.

RESULTS AND DISCUSSION

The representative examples of DC polarographic curves of poly A shown in Fig. 1 clearly demonstrate that the peak-shaped DC polarographic curve is caused by suppression of the current. The current suppression is gradually eliminated with (*a*) decreasing pH of the medium at constant ionic strength, (*b*) increasing ionic strength (or buffer capacity) at constant pH, and (*c*) increasing concentration of a polyamine at constant pH and ionic strength. Similar changes are produced by decreasing temperature (Fig. 2) and less extensively by decreasing mercury column height and decreasing concentration of poly A. The current suppression which is built up by the reversal of the above changes in the medium is centered around −1.55 V and gradually extends to both more positive and more negative potentials finally leaving only a sharp peak[3] from the original wave, e.g., curve *d* in Fig. 1 A. Under conditions where the extent of the current depression allows the estimation of $E_{1/2}$, the latter does not vary with ionic strength and concentration of polyamines at constant pH.

Some of the curves recorded at intermediate pH and concentrations of NaCl and ethylenediamine, namely curves *b* in Fig. 1 A, *c* in Fig. 1 B, and *b-d* in Fig. 1 C, showing the limiting portion being distorted by a current suppression, illustrate well how the limiting portion and the peak could be erroneously considered as a demonstration of two electrolytic processes (6). Note also the substantial difference between the potentials of background discharge of DC curves with eliminated and developed current suppressions (cf. subsequent discussion).

The extent of the current depression increases with increasing concentration of poly A. At low concentrations (below approximately 0.2 mM at pH 6.0 in 0.3 M NaCl and phosphate buffer), the depression is small enough so that it does not mask the limiting portion of the wave and i_{lim} is then directly proportional to the concentration of poly A. At higher concentrations (>0.5 mM) only a sharp peak can be seen, the height of which starts to decrease at still higher concentrations (>1.0 mM).

The extent of the current depression seems to be also affected by molecular weight, e.g., the onset of the depression is shifted by approximately 50 mV to more positive potentials by reducing the molecular weight from 3.3×10^6 to 3.2×10^5 while the $E_{1/2}$ remains constant (cf. Fig. 2 in reference 2). This phenomenon may be related to the degree of electrode coverage, which, analogously to polyuridylic acid (poly U) (unpublished results), may vary with molecular weight.

A characteristic pattern of changes including appearance and build-up of the

[3] May be referred to as maximum (7) or maximum-like curve (10).

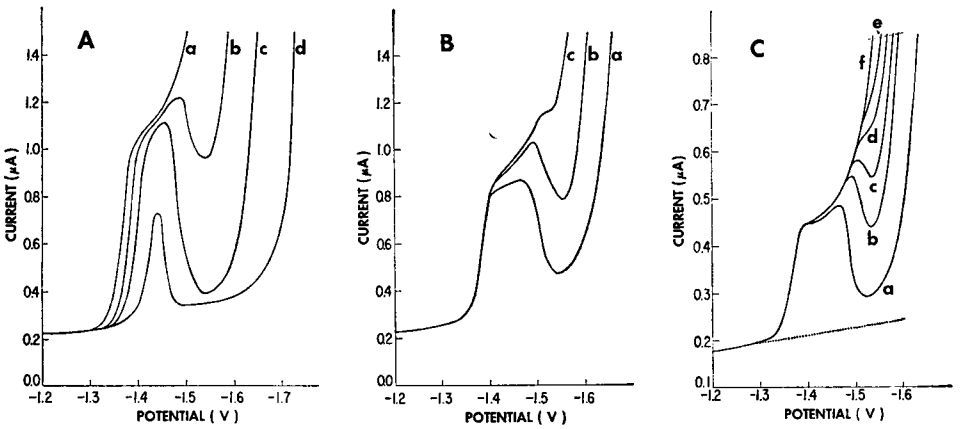

FIGURE 1 Effect of pH (A), NaCl (B), and ethylenediamine (C) on the shape of DC polarographic curves of poly A at 25°C. (A) 0.2 mM poly A ($15 \cdot 10^4$ daltons) in phosphate buffer (I = 0.05 M) and 0.4 M NaCl at pH 5.7 (*a*), 6.0 (*b*), 6.2 (*c*), and 6.6 (*d*). (B) 0.2 mM poly A ($52 \cdot 10^4$ daltons) at pH 5.9 in phosphate buffer (I = 0.1 M) and 0.1 (*a*), 0.4 (*b*), and 0.75 (*c*) M NaCl. The current depression was completely eliminated in 1 M NaCl (not shown), but the wave height was slightly lower than that in curve *c*. (C) 0.1 mM poly A ($1.1 \cdot 10^6$ daltons) at pH 5.9 in phosphate buffer (I = 0.1 M) and 0.2 M NaCl. Ethylenediamine (mM): 0.0 (*a*), 0.4 (*b*), 0.6 (*c*), 0.8 (*d*), 1.0 (*e*), and 1.5 (*f*). The area of current suppression of, e.g., curve *b* is defined by the curves *b* and *f* and the arbitrary line (dashed) which is drawn parallel to the residual-current line (dotted) at the distance of 0.6 μA (cf. Table I). Potentials are against saturated calomel electrode.

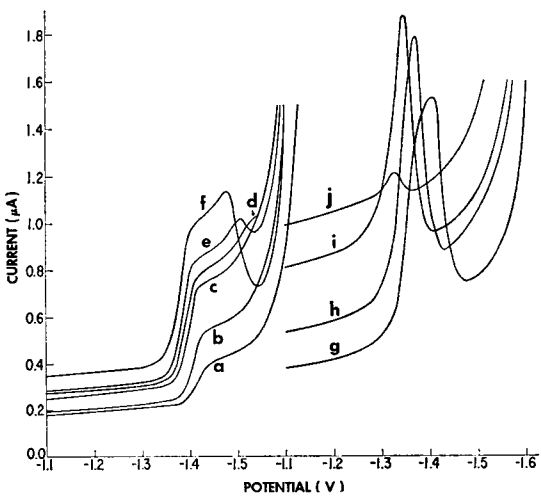

FIGURE 2 Effect of temperature on the shape of DC polarographic curves of 0.2 mM poly A ($49 \cdot 10^4$ daltons) at pH 5.9 in 0.4 M NaCl and phosphate buffer (I = 0.1 M). Temperature (°C): 2.8 (*a*), 6.7 (*b*), 14.5 (*c*), 17.5 (*d*), 22.0 (*e*), 29.5 (*f*), 48.1 (*g*), 59.0 (*h*), 68.8 (*i*), and 79.0 (*j*). The mercury column height was 64 cm. Potentials are against saturated calomel electrode which was held at the temperature indicated. The pH of the solution remained constant within about 0.1 pH over the entire range of temperatures employed.

current depression appears with increasing temperature (Fig. 2). The temperature coefficient of i_{lim} below 40°C, i.e. where the limiting portion is not masked by the current depression under given conditions, is +4.4%/degree which seems to be slightly higher than expected for diffusion controlled current (12).

Attempts have been made to determine the current-controlling factors of the electrochemical event of polynucleotides at the dropping mercury electrode. Based on the classical criterion of the dependence of limiting current i_{lim} upon mercury column height h a controversy has appeared as to whether the current yielded by native and denatured DNA at sufficiently low pH (where the current is independent of pH) is limited by diffusion (10, 11, 13) or is of an adsorptive nature (6). Similar uncertainty might arise in the case of poly A (reported as diffusion-controlled process [14]) since all reducible polynucleotides seem to follow the same pattern (7, 10). Limited diagnostic value of the $i_{lim} - h$ relationship in the case of DNA has been noticed at pH's in the vicinity of neutrality (11). We would like to point out that the above relationship has very limited significance in any case. The course of current changes at constant potential during the life of a mercury drop, the $i - t$ curve, is a sensitive indicator of instantaneous processes taking place at the electrode. Even under conditions where 0.2 mM poly A yields the normally shaped DC polarographic wave, e.g., low pH (pH 5.2, I = 0.65 M, T = 25°C) or low temperature (Fig. 2, curves *a–c*), the $i - t$ curves recorded on a single drop at potentials of the limiting portion are not parabolic (e.g., a parabola, $i = k \cdot t^{0.19}$, is expected for the diffusion-controlled current [15]) but assume shapes similar to those of curves *c* and *d* (without spermidine) in Fig. 3 B. The distortion of $i - t$ curves is particularly obvious at higher flow rates of mercury (with our capillary corresponding to h of about 70 cm or more). With decreasing h, the inhibition of the electrode process during the drop life becomes less pronounced (compare curves *c*, *c'*, and *c''* in Fig. 3 B) and finally all distortions may disappear, e.g., at h = 40 cm, pH 5.2, T = 25°C. Thus, the direct proportionality of i_{lim} to $h^{1/2}$ cannot be interpreted in terms of a solely diffusion-controlled process (in most cases, however, we obtained plots which were either curved or linear but not passing through the origin) unless other diagnostic criteria, e.g. $i - t$ curves, suggest the same interpretation.

Polyamines are known to stabilize the secondary structure of natural and synthetic polynucleotides, e.g. against thermal denaturation, through "strong," stoichiometric interactions (in contrast is the "weak" backbone-phosphate charge neutralization by monovalent cations where about a 10^2-fold excess of the latter is required), e.g., references 16–18. The double-stranded structure of acid poly A, however, is destabilized by diamines which specifically interact with negatively charged phosphates (19) and thus interfere with the helix-stabilizing interstrand hydrogen bond between phosphate and amino groups and with the electrostatic interaction between the negative charge of the phosphate group oxygen and positive charge of the protonated nitrogen N(1) of the purine nucleus (19, 20). Absorbance-temperature profiles,

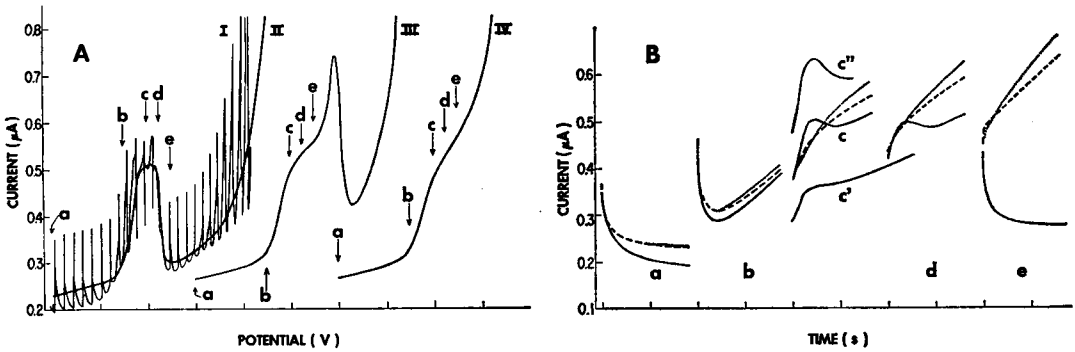

FIGURE 3 Effect of spermidine on the shape of DC polarographic curves (A) and $i - \bar{t}$ curves (B) of 0.1 mM poly A ($3.3 \cdot 10^6$ daltons) at pH 6.2 and 25°C in phosphate buffer (I = 0.1 M) and 0.2 M NaCl. (A) Spermidine (μM): 0.0 (I and II), 15 (III), and 40 (IV). Curves II–IV were recorded in the current-sampled mode at a sample duration of 15 ms and a controlled drop time of 1 s. Curve I, being shown for comparison, was recorded in the natural mode at a natural drop time. The potential scale is 100 mV/division; each curve starts at −1.3 V (against mercury pool electrode). The mercury column height h was 64 cm in all cases. The arrows indicate potentials at which the $i - t$ curves shown in B were recorded −1.300 V (*a*), −1.450 V (*b*), −1.500 V (*c*), −1.525 V (*d*), and −1.550 V (*e*). Concentration of spermidine (μM): 0.0 (——), 15 (---), and 40 (·····). All curves were recorded at h of 64 cm except c' (45 cm) and c'' (80 cm). The time scale is 2 s/division.

sedimentation coefficients, and gel filtration all failed to detect, however, any interaction of polyamines with the neutral form of poly A (16, 21, 22). Similarly, under our conditions (cf. Table I) where poly A exists in the neutral form (3), we did not observe any significant spectral changes induced by polyamines. Increased resistance of poly A to ribonuclease in the presence of polyamines (23) and our polarographic data, as subsequently discussed, indicate, however, that the interaction of poly A with polyamines is specific in contrast to alkali metal ions whose concentration has to be, by four to six orders, higher than that of polyamines to cause the same effect.

The factor(s) preventing the limiting current yielded by poly A from reaching its maximum value appears to be fully eliminated by polyamines (Figs. 1 C and 3). In the homologous series of diamines $H_2N(CH_2)_nNH_2$ the efficiency (expressed as the [P]/[NH_2] ratio obtained for the minimum polyamines concentration eliminating completely the current suppression, Table I) increases with their total chain length (sum of methylene and amino groups residues), number of methylene groups, and basicity (Table I). This simple relationship does not apply to more complex polyamines, e.g. the efficiences of 1,8-diaminooctane, spermidine, and 1,2-bis(aminoethylamino)ethane being 0.3, 2, and 12, respectively, differ from each other within a 40-fold range although the total chain length of the corresponding polyamines is the same. Also, the basicity of the more complex polyamines does not seem to be directly related to their efficiency. At the 0.5 mM level of poly A compared with the 0.1 mM level, the efficiency values are higher (Table I) and the a vs. [P]/[NH_2] plots exhibit much sharper decrease of a, the area of depression, with increasing concen-

TABLE I

EFFICIENCY OF POLYAMINES IN ELIMINATING THE CURRENT SUPPRESSION ON DC POLAROGRAPHIC CURVES OF POLY A

Polyamine	$([P]/[NH_2])_{min}$		pK_{a1}	pK_{a2}
	0.1 mM poly A	0.5 mM poly A		
$NH_2(CH_2)_2NH_2$	0.04		10.1	7.0
$NH_2(CH_2)_4NH_2$	0.04		10.8	9.6
$NH_2(CH_2)_5NH_2$	0.06		11.0	10.0
$NH_2(CH_2)_6NH_2$	0.08		11.1	10.0
$NH_2(CH_2)_8NH_2$	0.3	0.8	11.0	10.1
$[NH_2(CH_2)_3]_2NH$	2		11.0	10.1
$NH_2(CH_2)_3NH(CH_2)_4NH_2$	2	6	9.7	7.7
$[NH_2(CH_2)_2NH]_2(CH_2)_2$	12		9.9	9.2

Solution of poly A ($1.1 \cdot 10^6$ daltons) in phosphate buffer (I = 0.1 M) and 0.2 M NaCl, pH 5.94 was titrated at 25°C in a polarographic cell by the same solution containing polyamine in a suitable concentration (0.1 – 10 mM). The values of $([P]/[NH_2])_{min}$, which were determined from the a vs $[P]/[NH_2]$ plots, were those for the lowest polyamine concentration at which $a = 0$, i.e., the current suppression was completely eliminated. The area of current suppression a was measured with a planimeter in arbitrary units and defined (see Fig. 1 C) as restricted by the curve exhibiting current suppression, the curve with eliminated suppression and an auxiliary line which was drawn parallel to the residual current line at the distance of 0.8 μA and 4.0 μA for curves yielded by 0.1 mM and 0.5 mM poly A, respectively. The suppression was considered as eliminated when the curve had the usual shape of a DC polarographic wave showing no distortion below the auxiliary line and whose shape did not change significantly after a further small increase of the polyamine concentration. The values of pK_a (20°C) were taken from the literature (24, 25).

tration of polyamine. This seems to indicate that the efficiency of polyamines increases with concentration of poly A; there is no precipitation at these concentration levels of poly A by polyamines.

It is probably a combination of several factors which determines the efficiency of polyamines. (*a*) Chain length: longer polyamines being more flexible can adjust better to the hydrodynamically nonrigid, single-stranded molecule of neutral poly A. Although the methylene residues do not interact directly with negative charges of the phosphate groups, they can sterically shield them from the negatively charged electrode provided the total mass of methylene groups is sufficiently large. (*b*) Number of amino groups per chain unit and geometry of their distribution, e.g. of two molecules of the same chain length, 1,2-bis(aminoethylamino)ethane having four amino groups with a $(CH_2)_2$ residue between each two adjacent amino groups is much more effective than spermidine which has three amino groups spaced by $(CH_2)_3$ and $(CH_2)_4$ residues.

The $i - t$ curves recorded at representative potentials along the current-voltage curve (Fig. 3) demonstrate (*a*) the suppression of the current during the drop life, (*b*) more pronounced suppression at more negative potentials, and (*c*) elimination

of the current-suppressing factor(s) by spermidine. Curves *c* and *d* resemble $i - t$ curves of electrode processes inhibited by surface active substances (15, 26). The $i - t$ curves, however, which were obtained even at extreme concentrations of the surfactant (26), e.g. at the complete surface coverage, do not bear any resemblance to curve *e* recorded at the potential of the maximal current suppression (Fig. 3 A, curves I and II). In fact, curve *e* is almost identical in shape with curve *a* (Fig. 3 B) which represents, in principle, the variation of the charging current with drop age without any substantial contribution from the faradaic current (12). This seems to indicate that the suppression of the current is the result of depletion of poly A from the electrode surface and double layer. At the given potential, the magnitude of current suppression would be given by the degree of the depolarizer depletion. Changes in the shape of $i - t$ curves caused by polyamines (Fig. 3 B) demonstrate the restoration of a hypothetical uninhibited electrode process in situations where the inhibition was partial (curves *c* and *d*) or total (curve *e*). In the latter case, the charging current is clearly replaced by the faradaic current. The current, however, yielded at early stages of the drop life in the presence of a polyamine is lower than in its absence. If we assume that in the absence of polyamines the initial, ascending part of the $i - t$ curves *c* and *d* follows more or less closely the hypothetical course of the current uninhibited by any factors, i.e. diffusion-controlled current at sufficiently low pH,[4] then the current suppression can be attributed to the following. Poly A diffusing to the electrode increases its apparent molecular weight M (decreases its diffusion coefficient D) due to the association with polyamines. Consequently, the diffusion current i_d obeying the proportionality (2)

$$i_d \sim M^{-1/4} \sim D^{1/2}, \tag{1}$$

decreases. Polyamines also may act as weak surface active agents in which case they would cause the current to decrease at the early stages of the drop life (26). AC polarograms (Fig. 4), however, do not indicate any significant adsorption of polyamines at potentials of interest.

Thus, the course of the observed $i - t$ curves recorded in the presence of polyamines is the result of two antagonistic effects (Fig. 3 B): (*a*) the restoration of the hypothetical current (seen as an increase in current near the end of the drop life) and (*b*) the suppression of the current (seen as a decrease in current near the beginning of the drop life). Both of these processes are proportional to the concentration of polyamine.

AC polarography is well suited for the study of the capacity of the electrode-electrolyte boundary and the effects of adsorption on it (27, 28).[5] As indicated by

[4] A kinetic current is expected at higher pH's near neutrality where the reaction is limited by the rate of protonation of the adenine moiety (8, 9).

[5] The differential capacity of the electrode double layer C_E is simply related to the experimentally

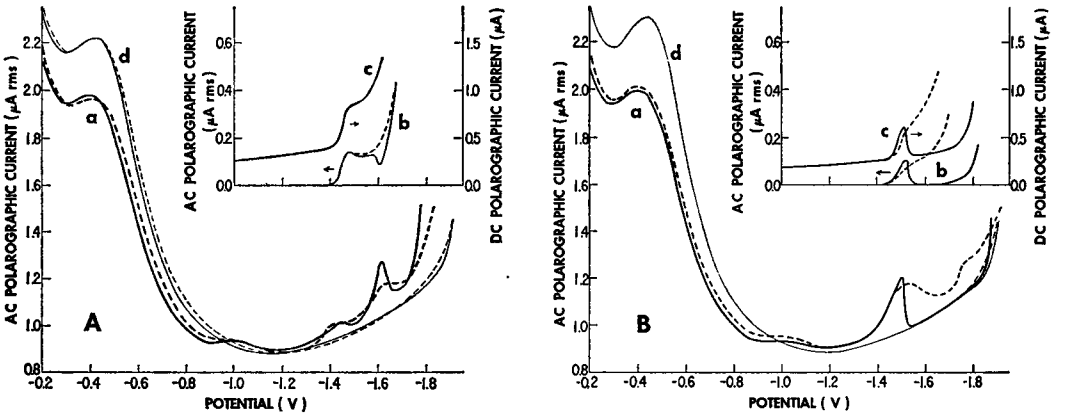

FIGURE 4 Effect of spermidine on the adsorption-desorption pattern of poly A at pH 5.7 (A) and 6.5 (B). Solution of poly A ($3.3 \cdot 10^6$ daltons), 0.2 mM, in McIlvaine buffer (I = 0.25 M) and 0.2 M NaCl, alone (——) and 40 μM in spermidine (- - -); background electrolyte alone (——) and with spermidine (- - -). Curves *a* and *d* represent the 90° out-of-phase current component. The inphase component (curve *b*) and DC polarogram (curve *c*) are shown for comparison. The potential scale of curves *b* and *c* is aligned with that of curves *a* and *d*. All curves were recorded in the current-sampled mode at 25°C and h = 64 cm. Potentials are against mercury pool electrode. rms, root mean square.

AC polarography, poly A (7, 29) similarily to its monomeric unit (9) is strongly adsorbed at the dropping mercury electrode over a wide potential region centered around the potential of zero charge of the electrode. Interpretation of total AC polarograms in terms of adsorption at the negative potentials where poly A is reduced is complicated by the concomitant presence of faradaic and capacitive currents. Of some help is phase sensitive AC polarography where, for example, the capacitive current may be eliminated by recording the component in phase with the input alternative voltage. The 90° out-of-phase component contains full contribution from the capacitive current while the contribution from the faradaic current is reduced; here also (Figs. 4 and 5) depression of the current below the base line of background electrolyte indicates adsorption around the zero charge potential.

measured quantity, AC, $i_\sim$,

$$i_\sim = \omega \cup C_E, \tag{2}$$

where the angular frequency, $\omega = 2\pi\nu$. The Eq. 2 is valid provided that the superimposed voltage has a frequency ν and amplitude U in the order of 100 Hz and 10 mV, respectively, and the background electrolyte is sufficiently conductive ($I > 0.1$ M). C_E is defined in terms of C, specific capacity per unit area and q, area of the electrode surface at time t and flow rate m,

$$C_E = q \cdot C = 0.85\ (m \cdot t)^{2/3} \cdot C. \tag{3}$$

The double layer capacity is a sensitive indicator of changes taking place in the double layer, e.g., adsorption of a substance results in a decrease of C_E and consequently of $i_\sim$.

With increasing negative potential the course of the current reaches higher values than the background electrolyte demonstrating a desorption of poly A. At pH 6.5 (Fig. 4 B) there is one desorption peak at −1.50 V while at pH 5.7 (Fig. 4 A) two peaks appear at −1.45 V and −1.62 V. At higher poly A concentrations (Fig. 5), the AC polarographic curves are slightly more complicated but basically the same pattern is evident.

Comparison of the potential range and extent of reduction of poly A, i.e. the position and shape of DC polarographic curve (Fig. 4, curve *c* and Fig. 5) and the inphase (faradaic) AC component (Fig. 4, curve *b*), with the potential range and extent of desorption, i.e. the position and shape of desorption peak(s) (Figs. 4 and 5), indicates that poly A undergoes desorption at potentials which coincide with its reduction. The extent of consequent inhibition of the reduction process depends on the completeness of desorption. At pH 6.5, the faradaic current is fully inhibited at potentials more negative than the desorption peak where the oxidized form (and any other adsorbant) is completely desorbed (Figs. 4 B and 5 B). At pH 5.7, the more positive desorption peak at about −1.45 V, which parallels the reduction process, is relatively small (Figs. 4 A and 5 A) and probably only a part of the oxidized form is desorbed so that the inhibition of the reduction process is only partial, i.e., the current oscillations at the limiting portion of the DC polarographic wave are distorted but the total current is not markedly suppressed (Fig. 5 A). The more negative desorption peak at about −1.6 V indicates further desorption of the oxidized form

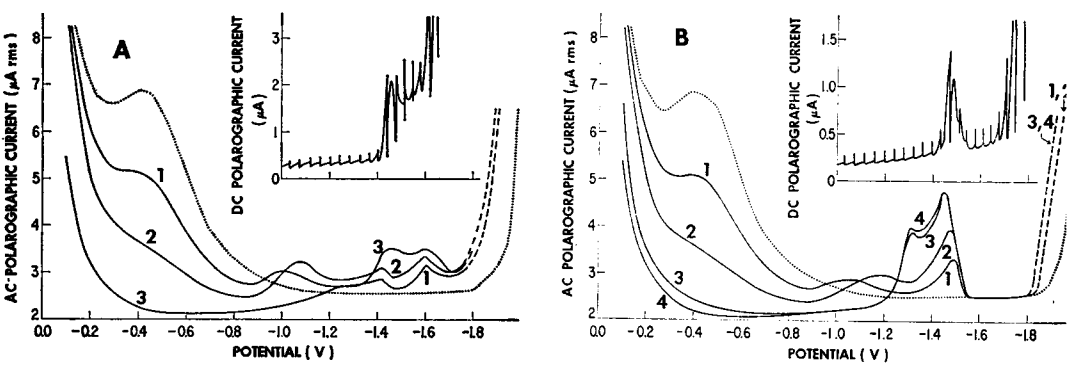

FIGURE 5 Effect of concentration of poly A on the adsorption-desorption pattern at pH 5.7 (A) and 6.5 (B). Concentration (mM) of poly A ($3.3 \cdot 10^6$ daltons) solution (——): (A) 0.22 (1), 0.40 (2), 1.0 (3), and 1.3 (trace identical with 3); (B) 0.22 (1), 0.40 (2), 0.67 (3), 1.0 (4), and 1.3 (trace identical with 4). Background electrolyte (·····): McIlvaine buffer (I = 0.25 M) in 0.2 M NaCl. AC polarograms (90° out-of-phase component) were recorded with the natural drop time at h = 64 cm and 25°C. The curves shown are the envelopes of the maxima of current oscillations. The oscillations were strongly distorted at high negative potentials (- - -). DC polarogram of 2.0 mM poly A, being shown for comparison, is aligned with the potential scale of AC polarographic curves; a relatively high scan rate of 10 mV/s was used in order to display the shape of current oscillations. Potentials are against mercury pool electrode.

(and possibly concomitant desorption of the reduced form which became adsorbed at slightly less negative potentials, see a trough between two desorption peaks). In any case, the latter adsorption-desorption processes hardly influence the reduction process since they occur at high negative potentials where the reduction of poly A is masked by the background discharge (Fig. 4 A, curves *b* and *c*, Fig. 5 A insert).

Spermidine, which itself does not seem to be adsorbed under given conditions, does not influence significantly the adsorption pattern of poly A, but it seems to affect adversely the process of desorption as indicated by the lower and more extended shape of the desorption peaks (Fig. 4). It is interesting to notice that at pH 6.5 spermidine changes the shape of the AC curve (at potentials more negative than −1.4 V) in such a way that the shape becomes very similar to that of the AC curve at pH 5.7 in the absence of and particularly in the presence of spermidine (Fig. 4). Analogously, similar changes occur in the DC pattern. This again suggests a causal relationship of adsorption-desorption and faradaic events at the electrode. The AC polarographic data also further support the idea that polyamines, by interacting with poly A, screen the negative charges of the phosphate groups, as already discussed, minimizing thus the repulsion from the negatively charged electrode. Consequently, they facilitate the diffusion transport of fresh depolarizer to the electrode so that an increased concentration of the depolarizer shifts the adsorption-desorption equilibrium towards adsorption.

SUMMARY

Poly A was studied at various conditions, e.g. pH, ionic strength, concentration, temperature, presence of polyamines, etc., using DC polarography, phase sensitive AC polarography, and measuring the $i - t$ curves on the growing mercury drop. The data obtained can be summarized and interpreted in the following way.

(*a*) Under most conditions the current yielded by poly A does not reach the values which it would if all of the depolarizer diffusing towards the electrode from the bulk of solution would reach the electrode and undergo reduction. As a result, the DC polarographic curves exhibit a more or less developed current depression at potentials of the limiting portion of the hypothetical undistorted wave (in extreme situations only a sharp peak can be seen).

(*b*) Poly A is strongly adsorbed at potentials near the zero charge of the electrode. At sufficiently negative potentials where poly A is reduced, the adsorption-desorption equilibrium shifts in favor of desorption. This is at least partially a consequence of (*i*) a decreasing concentration in the electrode double layer of the oxidized form due to its reduction and (*ii*) repulsion of poly A molecules with negative charges distributed along the sugar-phosphate backbone from the negatively charged electrode. Similarly, polyanionic polyphosphates, which are adsorbed on the electrode primarily through electrostatic forces between the positive charge of the electrode and the negative charge of the polyphosphate, desorb from the negatively charged

electrode and the adsorption-desorption process is affected by screening the phosphate group charges with mono- and divalent cations (30). While adenine and its nucleosides and nucleotides exhibit an adsorption-desorption pattern which is similar to that of poly A (9, 31, 32), their DC polarographic curves do not show current suppression. This suggests that desorption itself is not the only cause of the current suppression. (The desorbed poly A could be reduced provided it is not repulsed from the electrode.) The repulsion of the desorbed poly A molecules and those diffusing from the bulk of the solution can (in extreme situations) prevent the depolarizer from approaching close enough to the electrode for the electron transfer to occur. This explanation emphasizing the role of desorption and repulsion is supported by the effects of numerous factors, all causing the limiting current to decrease, e.g., increasing pH, temperature, negative electrode potential, and flow rate of mercury, and decreasing ionic strength and buffer capacity; vice versa, the current suppression can be at least partially eliminated by reversing these conditions. Some of these factors can be expected to mediate their effects directly through exposing the negative charges on phosphate groups, e.g., by decreasing the counterion concentration (increasing pH and decreasing ionic strength) and by transforming the single-stranded, stacked helical structure into random coil (increasing temperature).

The potential of background discharge is shifted to positive values in proportion to the concentration of poly A (7) and adenine derivatives (9); it seems that both the oxidized and reduced forms can cause the shift. Elimination of the current suppression on DC polarographic curves of poly A always results in a shift of the background discharge potential to positive values; the magnitude of the shift proportional to the extent of current suppression is always higher than that caused by a mere change of the background electrolyte composition, e.g., decrease of pH or increase of ionic strength. This observation is in agreement with the suggested unavailability of poly A to the electrode at potentials of the depression.

(*c*) Shielding of the negative charges on phosphate groups is most effectively achieved by polyamines. This reduces the extent of desorption and repulsion and facilitates the transport of depolarizer to the electrode. Although complex formation between neutral poly A and polyamines has not been detected by hydrodynamic and gel-chromatographic methods (16, 22), and polyamines do not affect the secondary structure of neutral poly A (21), our polarographic data do indicate specific interaction. This interaction, however, seems to involve only phosphate groups and not the purine moiety, e.g., spectral properties are not changed and the reduction sites of the adenine moiety are not blocked. Polyamines in contrast to inorganic salts are effective in low concentrations comparable with those of poly A. They do not seem to mediate their effect through the direct interaction with the electrode. Polyamines with longer chain length and higher density of amino groups per molecule are generally more effective. Their effectiveness also seems to depend on the geometry of the amino group distribution.

(*d*) The same factors, in principle, as outlined above are believed to cause the decrease of current yielded by poly C and DNA because of the close similarity of the polarographic behavior of these polymers (7, 10) to poly A.

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