Polarographic Reduction of Adenine and Cytosine Residues in Denatured DNA

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Polarographic reduction of adenine and cytosine moieties in denatured DNA was followed by means of differential (derivative) pulse polarography. The measurements of the reduction currents yielded by samples of denatured DNA differing in guanine+cytosine (G+C) content showed that both cytosine and adenine residues were reduced in DNA in the pH range of 6.0–8.7. The contribution of their reduction to the total reduction current of denatured DNA differs in the range pH 6.0–8.7, however; the contribution of the current of adenine residues to the current of DNA decreases with increasing pH as compared with the contribution of cytosine residues. It was also shown that in the region of neutral pH reduction current of denatured DNA is independent of the G+C content in DNA.

The polarographic reducibility of DNA is conditioned by the presence of adenine and cytosine in the polynucleotide chain [1]. In the region of neutral pH, where the majority of polarographic measurements with DNA were carried out, only cytosine of all DNA constituents is reducible as a monomer [2]. Monomeric adenine is reducible only at pH < 6 (ref. 3). Therefore it was supposed originally [4] that at pH > 6 only cytosine residues are reduced polarographically in DNA. Later, however, it was shown [5] that polyadenylic acid yielded reduction currents also in the region of neutral pH. It has been, however, further supposed by other authors [6] that at neutral pH only cytosine residues are reduced in DNA. In the present paper we have attempted to show whether adenine bound in denatured DNA is also reduced in the region of neutral pH. Measurements of reduction currents yielded by DNA samples differing in the content of guanine + cytosine (G+C) have shown that the adenine residues in denatured DNA are reduced and that the contribution of their reduction currents to that of DNA is changed in dependence on pH.

Materials and Methods

DNA samples of *Bacillus alvei* (33% G+C), *Micrococcus luteus* (72% G+C) and *calf thymus* (42% G+C) were isolated and characterized as in previous papers [7, 8]. *E. coli* DNA (50% G+C) was made available to us by courtesy of ing. E. Lukášová. Double-helical DNAs were sonicated for 9 min at a concentration of 540 μg/ml in 0.015 M NaCl with 1.5 mM sodium citrate, pH 7 (SSC/10) at 40 °C in a nitrogen atmosphere using a ULA 1000 ultrasound generator (Laboratorní přístroje Praha), at roughly 750 W and a frequency of 1 MHz; a longer period of sonication did not lead to any further decrease of DNA viscosity. Denaturation of DNA was carried out by heating DNA at a concentration of 540 μg/ml in SSC/10 at 100 °C for 10 min, and subsequent quick cooling in an ice bath. DNA concentration was estimated spectrophotometrically. Chemicals used for the background solutions for polarography were all of analytical grade.

The differential (derivative) pulse-polarographic measurements were carried out with a Model 174 Polarographic Analyzer, supplied by Princeton Applied Research Corporation. A three-electrode system was used including a dropping mercury electrode (DME), a Pt-counter-electrode of appreciable area, and saturated calomel electrode (SCE). Pulse-polarographic measurements were performed at the following settings: scan rate 1 mV/s, scan range 0.75 V, initial potential −1.15 V, modulation amplitude 50 mV, drop time 5 sec. Alternating current (a. c.) polarographic measurements were carried out with a GWP 563 Polarograph [9]; details of the measurements have already been published [10, 11]. All polarographic measurements were made in an argon atmosphere at 25 °C; the DME had, at a mercury column height of 35 cm, a mercury flow rate of 1.02 mg/s (measured in distilled water with an open current circuit and at 25 °C) and drop time of 5.0 sec. All potentials are reported vs. SCE.

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Spectrophotometric measurements were performed on a Zeiss VSU 2-P apparatus; pH values were measured with a PYE Universal instrument. Viscosity was measured with a four-gradient Ubbelohde type viscometer [12].

Results and Discussion

In our experiments thermally denatured DNAs with different G + C content were used. Reduction polarographic currents of DNA were measured by means of differential pulse polarography. Considering that samples of denatured DNA differing in G + C content but having exactly the same molecular weights can be obtained only with difficulty, we tried to eliminate the influence of molecular weights on reduction currents (resulting in different rates of polynucleotide transport to the electrode surface).

Reduction currents of denatured DNA can have the character of adsorption currents [13] independent of the transport rate, and of depolarizer concentration in the bulk of solution [14]. The measurements have to be performed, however, with the DME surface fully covered by adsorbed molecules of oxidized form of denatured DNA [13] in order that the reduction currents should have the character of adsorption currents. It has been already shown [13] that the value of the lowest concentration of denatured DNA at which full coverage of the DME was achieved decreased with increasing drop time as well as with the increasing diffusion coefficient of DNA. We were able to carry out our pulse-polarographic measurements at the highest drop time 5 sec. Furthermore, we could not use higher DNA concentrations than approximately 400 μg/ml, because at higher concentrations the measurements were unfavourably influenced by aggregation and precipitation of DNA. However, at DNA concentrations lower than 400 μg/ml, and drop time 5 sec, it was not possible to achieve full coverage of the DME surface for all DNA samples. Therefore we decreased the molecular weight of all DNA samples by sonication [15], and thus increased the diffusion coefficients (Fig. 1). The polarographic measurements were then carried out for all samples of denatured sonicated DNA at two concentrations (270 and 320 μg/ml). Considering that the reduction current was the same at both concentrations, it is apparent (Fig. 1) that we measured in the region

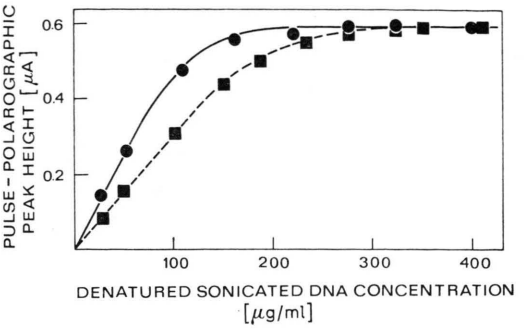

Fig. 1. Dependence of the pulse-polarographic peak height on the concentration of denatured calf thymus DNA in 0.3 M ammonium formate with Britton-Robinson buffer, pH 6.0. (●—●), sonicated DNA; (■—■), nonsonicated DNA.

where the current magnitude did not depend on the rate of depolarizer transport.

The measurement of the dependence of polarographic current of denatured DNA on their G + C content was carried out in a medium of 1.0 M ammonium formate (Fig. 2). This medium makes it

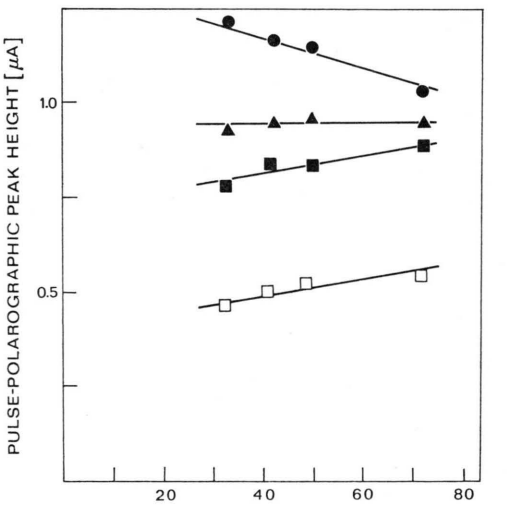

Fig. 2. Dependence of the pulse-polarographic peak height of denatured sonicated DNA on the G + C content of DNA at different pH. DNA at a concentration of 270 μg/ml in 1.0 M ammonium formate with Britton-Robinson buffer. (●—●), pH 6.0; (△—△), pH 7.4; (■—■), pH 8.0; (□—□), pH 8.7.

possible to follow the polarographic reduction of denatured DNA even in the alkaline pH region, up to approximately pH 9 (ref. 8, 16). It was possible to measure only to pH 6 in the acid pH region, however. At pH < 6.0 the pulse-polarographic curve corresponding to DNA reduction merged with the curve of background electrolyte discharge. As follows from Fig. 2, at pH 7.4 the polarographic

current of denatured DNA was practically independent of the G + C content in DNA. At pH 6.0 the current increased with the decreasing G + C content, whereas at pH > 7.4 it decreased. The shape of the pulse-polarographic curve and the summit potential of the pulse-polarographic peak were independent of the G + C content at given pH. With increasing pH the summit potential shifted to more negative values, similarly to the half-step potential of the direct current polarographic step [8].

For all samples of denatured DNA the a. c. polarograms were also recorded. In a medium of 1.0 M ammonium formate, pH 8.7, we could observe a decrease of a. c. caused by an adsorption of the polynucleotide even at potentials lying between those of the a. c. polarographic peaks 1 and 2 (ref. 11) (Fig. 3). This confirmed our assumption

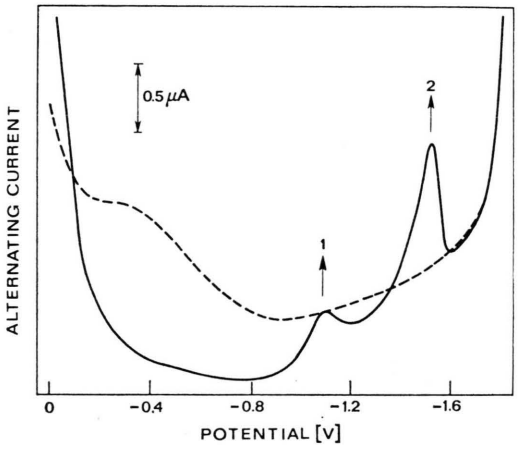

Fig. 3. A.c. polarogram of denatured sonicated *E. coli* DNA in 1.0 M ammonium formate with Britton-Robinson buffer, pH 8.7. DNA concentration 270 μg/ml. (———), DNA; (- - - - -), background electrolyte.

that total desorption of the oxidized form of denatured DNA from the DME surface took place only at potentials of the a. c. polarographic peak 2 (Fig. 3). The same behaviour was observed for single-stranded polycytidylic acid [5]. As follows from our study of the electrode process to which denatured DNA is subject on DME, the magnitude of reduction current depends on the difference between the potentials of reduction and desorption of DNA [13]. The measurements reported here showed that the summit potential of peak 2 (indicating DNA desorption) did not depend on the G + C content at given pH so that it is probable that the adsorbability of individual samples of denatured DNA did not have any influence on the magnitude of the reduction current of DNA.

The respective contributions of polarographic reduction currents of adenine and cytosine residues of DNA to the total reduction current yielded by denatured DNA at given pH can be expressed (under conditions of our measurements) in the equation

$$i = k_C x_{G+C} + k_A (100 - x_{G+C}), \qquad (1)$$

where k_C is the reduction current (in μA) of cytosine residues bound in a hypothetical sample of denatured DNA with G + C = 1% at given pH, k_A is the reduction current (in μA) of adenine residues bound in a hypothetical sample of denatured DNA with G + C = 99% at given pH, i is the total reduction current of denatured DNA at given pH, and x_{G+C} is the percentage of G + C in a given DNA sample. By rearranging equation (1) we obtain

$$i = (k_C - k_A) x_{G+C} + 100\, k_A . \qquad (2)$$

From the relation (2) it follows that for $x_{G+C} = 0\%$ $i = 100\, k_A$ and for $x_{G+C} = 100\%$ $i = 100\, k_C$. The values of k_C and k_A obtained by the extrapolation of the curves in Fig. 2 to the limit values of x_{G+C} are listed in Tab. I. The lines in Fig. 2 intersect the horizontal coordinate neither at 0% nor 100% G + C in any of the media used. Therefore it can be concluded that in the range of pH 6.0 – 8.7 (where the total reduction current of denatured DNA already decreases with increasing pH [8] (Fig. 2)) both adenine and cytosine residues are reduced in DNA. However, the values k_A/k_C (Tab. I) show that the contribution of adenine residues to the total reduction current of denatured DNA decreases with increasing pH in the range of pH 6.0 – 8.7 as compared with the contribution of cytosine residues.

Tab. I. Dependence of the values k_A and k_C obtained from Fig. 2 and of the ratio k_A/k_C on pH.

pH	k_A [μA]	k_C [μA]	k_A/k_C
6.0	1.34	0.97	1.38
7.4	0.95	0.95	1.00
8.0	0.73	0.92	0.79
8.7	0.40	0.60	0.67

The increase of the current with the decreasing G + C content in DNA at acid pH (when total protonation of adenine and cytosine moieties on mercury electrode can be expected [3, 17]) is probably

connected with the fact that monomeric adenine is reduced by four electrons [3], whereas monomeric cytosine only by three electrons [17]. At pH 6.0 the ratio k_A/k_C is approximately equal to 4/3 (Tab. I). *i.e.* to the number which would be obtained if monomeric adenine and cytosine were reduced under conditions when both bases are fully protonated at the electrode [3, 17]. Thus it is probable that at pH 6 already not only cytosine residues, but also adenine residues yield the maximum value of reduction current. The values of k_A and k_C (Tab. I) also demonstrate that the reduction current of denatured DNA is independent of the G + C content in DNA in the vicinity of neutral pH. The value of the ratio k_A/k_C estimated at pH 6 (Tab. I) indicates that the polarographic reduction of bases bound in a polynucleotide will not differ significantly from the polarographic reduction of monomeric bases. The direct determination of electron consumption at the reduction of denatured DNA by means of electrolysis on a mercury pool electrode has not yet given satisfactory results, since during the electrolysis a precipitate appeared that contained both reduced and non-reduced denatured DNA [8].

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