Differential Pulse-polarographic Approach to Zinc(II)– and Cadmium(II)–DNA Systems

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Abstract

The differential pulse-polarographic studies have revealed that the double helical structure of nucleic acid undergoes severe destabilization process during the DPP reduction on a mercury electrode. The opening of the double helix depends on the number of strand breaks caused by the reduction of adenine and cytosine residues in the DNA molecule. Zinc showed to be very effective in preventing the opening of the double helix and its renaturation ability is much higher than that of cadmium. The latter metal seems to be not very specific in its interaction with the intact DNA molecule but the labilization of the nucleic acid structure e.g. by the DNA interaction with charged membranes in the living cell or by some physical means (ionizing irradiation), allows for Cd(II) ions to penetrate effectively the base binding sites.

Introduction

It has been already shown that the contact of nucleic acid with the charged mercury electrode may cause the labilization or unwinding of the double helical structure of DNA. The opening of the double helix could derive from the strong repulsions between the negatively charged phosphate groups and electrode surface [1] on which the hydrophobic bases are anchored. The opening process of the DNA molecule is thought to be relatively slow but it can be accelerated by some effects e.g. by small gamma-radiation doses [2]. The similar effect could also be caused by the reduction process performed on the mercury electrode during e.g. differential pulse polarography (DPP) (see also [3]). During the polarographic process two of the DNA residues, adenine and cytosine, undergo the reduction with four and two electrons, respectively [4]. The effective reduction of the DNA molecule may proceed, however, only when its double helical structure is partly destabilized. The unwinding of some regions of the DNA molecule allows the reducible groups of the bases to be oriented towards the electrode and then reduction proceeds [1, 5–7].

The interaction of the charged mercury electrode with the adsorbed DNA molecule, on the other hand, was found to be a very good model to simulate the nucleic acid interaction with cell membranes. Thus, the electrochemical methods besides of their powerful analytical ability can help to understand the studied system e.g. metal–DNA, in the cell environment *i.e.* on membranes in the living cell and on protein envelopes of the DNA. In this respect, the study on the metal nucleic acid system may help to explain some details of the interaction between metal ion and DNA and serve as a dynamic model of this interaction and its consequences during the interaction of nucleic acid with the naturally charged environment.

Experimental

The measurements were performed for a native calf-thymus DNA (Serva or Worthington), with protein content below 0.5%. All other chemicals were of analytical grade. DPP measurements were carried out on a PP-04 Pulse Polarograph (Telpod Krakow) with a X–Y recorder (Endim 620.02) using a MDDME electrode with a drop time of 3 s. In all measurements the modulation amplitude was 50 mV. An acetate buffer containing 0.05 M of sodium acetate was used as an electrolyte with pH = 5.6. The measurements were carried out under argon and the wave potentials were related to a saturated calomel electrode. The concentration of the native DNA in all measurements, 25 μg/ml, was estimated spectrophotometrically. The absorption spectra were recorded on a Unicam SP 800 B spectrophotometer. The circular dichroism (CD) spectra were recorded on a JASCO-J20 spectropolarimeter.

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Results and Discussion

Metal-free DNA

Calf-thymus DNA (native) undergoes the reduction process in DPP at about −1.4 V (acetate buffer, pH = 5.6). The relative height of the DPP peak (a limiting current), is routinely used as a measure of amount of adenine and cytosine residues present in the labile fragments of nucleic acid. An intact double helix of a native DNA shows only residual DPP signal, deriving from the partially open fragments of a double helical structure. The destabilization process proceeds in the electric double layer formed at the mercury electrode [5, 8, 9]. The totally open (e.g. by thermal denaturation) nucleic acid offers the maximum signal available. The electrochemical methods contributed also to the elucidation of the destabilization of a double stranded DNA structure by another physical effect e.g. ionizing irradiation [2, 10–12]. Since during the reduction process the adenine and cytosine residues undergo the dramatic chemical modification it seems to be logic to expect that a substantial labilization of the double helical conformation may also happen when polarographic process would be performed. The variation of the DPP peak of DNA during the subsequent measurements is shown in Fig. 1. It is easy to see that the reduction wave at −1.4 V increases systematically up to the maximum value reached in the 40–50th subsequent measurement (Fig. 2). The results obtained for two different calf-thymus DNAs as received from Serva (Figs. 1 and 2) and Worthington (Fig. 3) though vary in the values exhibit the same behaviour of both

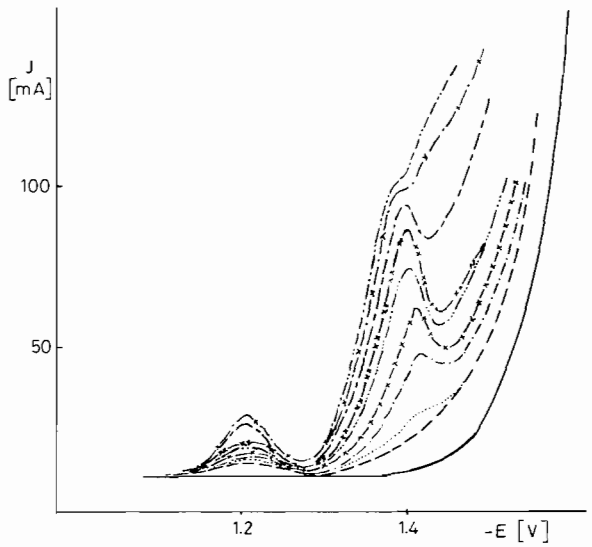

Fig. 1. DPP polarograms of DNA (Serva). Dependence of the limiting current I (mA), on the number of consecutive measurements: (——) 1st, (·····) 10th, (—·—) 20th, (—×—) 30th, (—···—) 40th, (— — —) 50th, (—·:·—) 60th, (×——·) 80th, (—··—) 85th measurement.

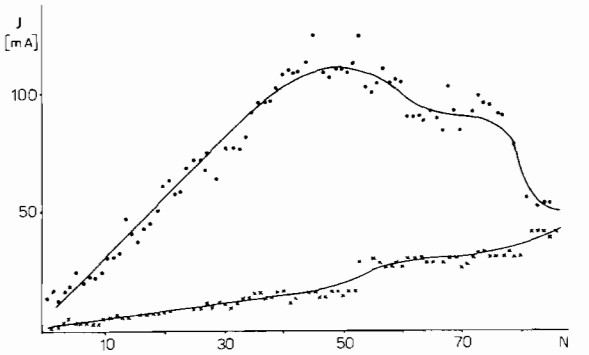

Fig. 2. Dependence of I (mA) of DNA (Serva), on the number of consecutive measurements (N), for peak I ($E_{1/2}$ = −1.21 V), (×××), and peak II ($E_{1/2}$ = −1.406) (·····), respectively. [DNA] = 25 μg/ml, 0.05 M sodium acetate, pH = 5.6.

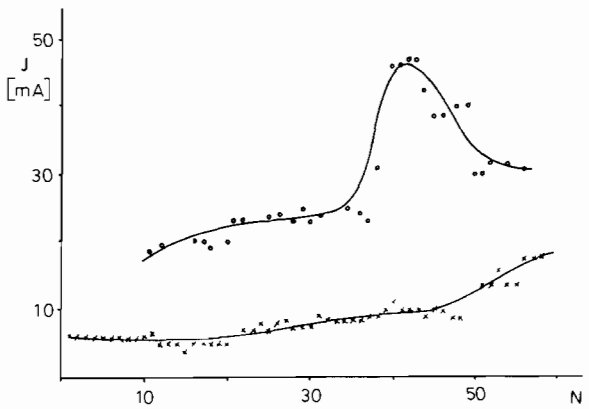

Fig. 3. Dependence of I (mA) of a reduction wave of Worthington DNA on the number of consecutive measurements (N) for peak I ($E_{1/2}$ = −1.21 V) (×××), and peak II ($E_{1/2}$ = −1.406 V) (·····), respectively. Concentrations as in Fig. 2.

nucleic acids during the polarographic process. The main DPP peak at −1.403 V is accompanied by two other polarographic waves at −1.21 V, observed already after several initial measurements of the same DNA solution, and at *ca.* −1.5 V seen as a shoulder in an electrolyte background (Fig. 1) which is seen when the main DNA peak decreases substantially (after *ca.* 80 subsequent measurements). The latter wave can be easily assigned as a reduction wave of the denaturated DNA or its fragments [13]. The other reduction wave could be assigned as the wave corresponding to the short fragments of the DNA produced on the mercury electrode during the polarographic process. The latter assignment was proved by the DPP measurement of the sonicated DNA which exhibits main reduction peak just about −1.2 V. The denaturation and fragmentation of the native DNA on the mercury electrode was also seen in the absorption spectra in the 200–300 nm region which is quite sensitive to a

TABLE I. Absorption Spectra Data of DNA (Serva) after Given Number of Successive Measurements

Number of measurement	230 nm ϵ	260 nm ϵ	280 nm ϵ	$\frac{230}{260}$	$\frac{260}{260}$	$\frac{280}{260}$
0	0.218	0.540	0.292	0.403	1	0.540
20	0.330	0.620	0.350	0.530	1	0.564
40	0.415	0.715	0.400	0.580	1	0.559
60	0.620	0.895	0.520	0.692	1	0.581
80	0.980	1.36	0.790	0.720	1	0.581

nucleic acid conformation state. The increased number of the subsequent DPP measurements done in the respective solution was followed by substantial increase of absorption at 230 and 260 nm and the 230 to 260 nm absorption ratio (Table I). These two results indicate clearly the breaking down of double helices as well as the labilization and denaturation of the DNA structure [14].

The results presented above indicate that even the short contact of nucleic acid with charged electrode (several seconds) can be very effective in the unwinding of DNA double-stranded structure. This process is very effectively promoted by the partial reduction of the DNA molecule. The reduction of adenine and cytosine residues causes the breaking of the hydrogen bond system between adjacent bases and induces the opening of double helical structure. The appearance of the short fragments of DNA also indicate that the polarographic process causes numerous strand breaks in nucleic acid molecule. The fact that the even small number of the strand breaks induced the considerable labilization of the DNA 'native' structure was seen when already after first DPP measurement the solution was left for one or two hours and remeasured (see ref. 3). The DPP peak of DNA obtained in the second measurement for the solution resting for a few hours is considerably higher than that obtained in remeasured sample immediately after the first DPP reduction (the method applied in this work).

Zn(II)–DNA

Zn(II) ions are preferentially bound to the phosphate sugar backbone although some involvement in the base coordination was also suggested [15, 16, 20]. Zinc(II) was found to decrease the melting temperature at high metal to DNA–P molar ratios, *i.e.* it was facilitating the thermal denaturation process, and to induce the reformation of the DNA double helix upon cooling, *i.e.* the renaturation process. The latter process was explained by the formation of metal bridges between complementary bases of opposite strands in the unwound state. The preferential interaction of Zn(II) ions with bases has been suggested to occur with GC sites with chelate formation via the N-7 of guanine and the phosphate residue [15]. Zinc, on the other hand, can perform an important biological function in the processes involving DNA [17, 18, 20].

The effect of Zn(II) ions on the DPP picture of DNA depends on the Zn(II):DNA–P molar ratio, on the exposition time and on the number of a consecutive measurement made with the same solution *i.e.* on the state of unwinding of DNA (see above). The low metal ratios e.g. P = 0.5 (*i.e.* $\frac{1}{2}$ Zn(II) to 1 DNA phosphate), usually promote the unwinding process starting on the electrode, especially at shorter exposition times e.g. up to about 50 h for the first and the second measurement of the same solution. The relative DPP peak height increased in the presence of metal ion even twice (Fig. 4a, b). At longer exposition times the decrease of this peak was observed for all molar ratios. The very effective variations were observed for the solutions in which the third subsequent DPP measurement was carried out. The zinc ions decrease the peak height drastically (down to 20–30% of the initial value). The latter result indicates the renaturation ability of zinc ion. The metal ions bind the bases in the unwound parts of DNA molecule promoting the rewinding process while resting in solution. The involvement of metal ions in the interstrand bridging induces the reformation of the double helix. On the other hand, however, such Zn(II)–DNA complex may be stable enough to be a species which undergoes successive reduction. The intercrossing metal ion could reduce the amount of DNA bases available to reduction and decrease the DPP reduction peak of DNA. This situation could explain the considerable variations of the reduction potential of DNA observed for higher metal ratios for long exposition times (Fig. 5). For P = 3 the DNA DPP peak shifts to −1.372 V and for P = 8 there are two reduction waves seen at −1.370 and at −1.458 V. Thus, the DPP results seem to indicate that low amounts of Zn(II) ions interact with phosphates decreasing their negative charges and promoting better adsorption of DNA on a mercury electrode. Similar increase of DPP DNA peak height was observed when sodium ions were added to the DNA solution [19]. It clearly supports the phosphates as the binding sites for zinc at low values of P. The excess of Zn(II) leads to the effective renaturation

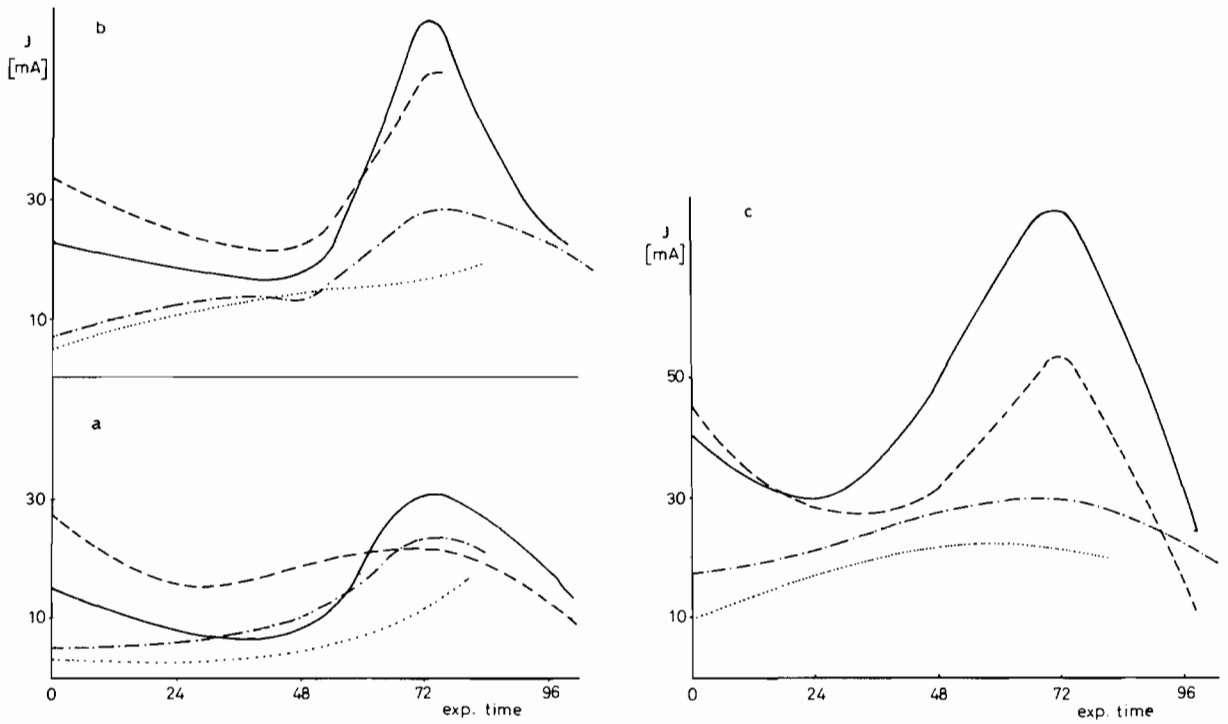

Fig. 4. Dependence of I (mA) of a reduction wave of Worthington DNA on a Zn(II) concentration, P = 0 (——), P = 0.5 (— — —), P = 3 (— · —), P = 8 (·····) and the exposition time (in hours) for the first, second and third measurement (a, b, c) performed 2–3 h after each other. Concentrations as in Fig. 2.

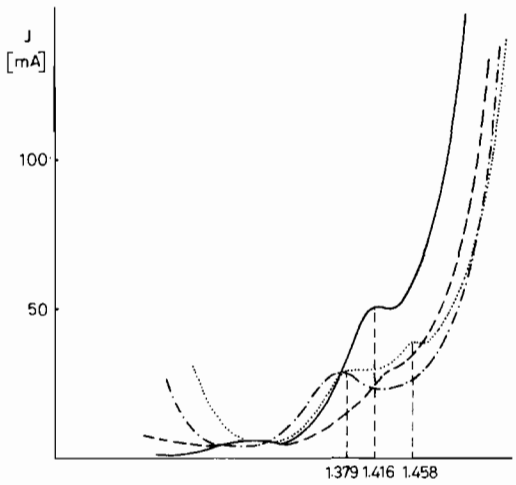

Fig. 5. DPP polarograms of Zn(II)–DNA solutions obtained for P = 0 (——), P = 0.5 (— — —), P = 3 (— · —) and P = 8 (·····) during the third successive measurement for exposition time 96 h. Other data as in Fig. 3.

process of DNA unwound by electrolysis. Metal ion binding to bases is effectively induced by the opening of DNA double helical struture which proceeds during the reduction process on mercury electrode. The interaction mode may strongly depend on different factors e.g. exposition time, metal to DNA–P molar ratio and on the state of the DNA structure *i.e.* amount of the labilized regions which could be induced e.g. by charged membranes or structural DNA proteins.

The presented above interpretation is supported by spectroscopic data. As it was mentioned above the successive DPP measurements were unwinding and denaturating DNA and these effects were reflected in the UV spectra of DNA (Table I). The addition of Zn(II) to the DNA solutions resulted in drastic decrease of the variations of the absorption spectra at 230 and 260 nm caused by the denaturation process.

Cd(II)–DNA System

The earlier studies on the cadmium interaction with nucleic acid have suggested that this metal ion interacts at both possible sites *i.e.* at the phosphates and the bases [16, 20, 21]. The electrochemical approach did not indicate, however, any specific interaction of Cd(II) with nucleic acids [21]. The lack of any major effect of cadmium ions on DNA DPP picture was also discovered in this work. The addition of metal to the DNA containing solution did not affect distinctly the reduction potential or peak height of DNA at any molar ratio used or exposition time, especially during the first measurement of a given solution (Table II). Though some stabilizing effect of Cd(II) ions on DNA double helical structure is observed the renaturation ability of cadmium is

TABLE II. Polarographic Data for Cd(II):DNA) (Worthington) Solutions[a]

P	*N*	Exposition time							
		0		24		48		72	
		I (mA)	+*E* (V)	*I* (mA)	+*E* (V)	*I* (mA)	+*E* (V)	*I* (mA)	+*E* (V)
0	1	14	−1.409	18	−1.405	21	−1.406	16	−1.404
	2	35	−1.402	51	−1.403	34	−1.405	31	−1.407
	3	62	−1.403	73	−1.405	57	−1.403	45	−1.403
0.5	1	0	–	24	−1.406	17	−1.411	14	−1.407
	2	26	−1.407	50	−1.408	32	−1.405	41	−1.405
	3	42	−1.403	55	−1.407	59	−1.404	69	−1.408
3	1	19	−1.409	18	−1.407	14	−1.411	18	−1.416
	2	31	−1.406	47	−1.403	29	−1.404	38	−1.408
	3	53	−1.405	63	−1.403	63	−1.404	73	−1.410
8	1	17	−1.415	10	−1.416	14	−1.413	14	−1.424
	2	43	−1.410	30	−1.411	23	−1.427	33	−1.420
	3	68	−1.410	40	−1.413	56	−1.416	56	−1.418

[a]c_{DNA} = 25 μg/ml; 0.05 M acetate buffer; pH = 5.6; *E* = reduction potential; *I* = limiting current of reduction peak; *N* = number of successive measurement; exposition time in hours.

considerably lower. Similarly to Zn(II), however, Cd(II) binding to DNA bases is more effective when double stranded structure of nucleic acid is destabilized by the DPP reduction process.

The CD spectra of the Cd(II)–DNA solutions for metal to phosphate molar ratios below 3 ($P < 3$), are almost the same as for the metal-free DNA. For $P > 3$ the positive Cotton effect decreases considerably and shifts to 280 nm. This result may indicate the change of nucleic acid conformation from B to C. The very high excess of cadmium ions ($P > 15$), leads to simultaneous decrease of both Cotton effects.

Conclusions

The discussion presented in this work together with the earlier results obtained for Cu(II)– and Ni(II)–DNA systems [3], seem to reveal some new features of the metal–DNA interaction, e.g. the more effective interaction with bases when nucleic acid undergoes the labilization (partial unwinding) caused by any physical effect, e.g. interaction with charged membranes. All four metal ions seem to have the renaturation ability, the strongest in the case of zinc and the weakest in the case of copper. Copper was found to exerase the strongest denaturation effect when present in excess. It is likely that the effective binding of some metal ions to bases found by the methods of melting curves [16], was due to the fact that temperature was labilizing the double helix and so the metal ions could enter the base sites inducing additional weakening of the nucleic acid structure. This situation could explain the lack of specificity for Cd(II) and its interaction with RNA loops whose structure may resemble the labilized structure of DNA [21].

It is also important to conclude that the application of e.g. DPP technique to follow the melting temperatures of nucleic acids [22] may lead to severe errors in the values of the measured melting points. The destabilization of a DNA structure by the subsequent reduction processes occurring on a charged electrode during a polarographic measurement may diminish distinctly the real value of the melting temperature of nucleic acid.

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