Polarographic and Spectroscopic Study on Pb^{+2} Ion Interaction with DNA

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The toxic effects of lead pollution seem to be already well established especially in industrial countries [1]. The complexity of lead poisoning [2, 3] has been compounded by the recent discovery of its carcinogenic action in animals [4].

In experiments with rats it was found that the injected lead accumulates inside the cell nuclei [5]. From the isotopic studies the authors were not able, however, to establish whether metal ions are directly involved in DNA binding and, if this is the case, what is the mode of lead binding to the nucleic acid.

The importance of lead binding inside the cell nuclei has also been shown in kinetic studies on isolated rat hepatocyte [6].

In this communication we present polarographic studies on the possible modes of interactions in the Pb^{+2}–DNA system.

Differential pulse polarography (DPP) was shown to be a very useful technique for the study of DNA [7–15] as well as metal ion behaviour [16–18]. The polarographic results were completed by the CD and absorption spectra including the measurements of the melting profiles.

Experimental

The measurements were carried out for a native calf-thymus DNA (WORTHINGTON) with protein content lower than 0.5%. All other chemicals used for the study were of analytical grade.

DPP measurements were carried out on a pulse polarograph PP-04 (TELPOD-KRAKOW) with a X–Y recorder (ENDIM 620.02) using a MD-DME electrode with a drop time of 3 sec. In all measurements the modulation amplitude was 50 mV and acetate buffer containing 0.15 or 0.05 M sodium acetate was used as an electrolyte. The measurements were performed under nitrogen atmosphere in conventional polarographic vessels. The wave potentials were related to a saturated calomel electrode.

The concentrations of native DNA were estimated spectrophotometrically.

CD spectra were recorded on a JASCO-J20 spectropolarimeter. The melting profiles were measured on a Beckman Acta CIII spectrophotometer at 260 nm. The studied solutions were buffered by 0.05 or 0.15 M acetate buffer (pH = 5.6).

Results and Discussion

During the polarographic process in acetate buffer lead-free DNA undergoes a reduction around −1.39 V (Table I). This process is usually assigned as a reduction of the adenine and cytosine residues of the double-stranded DNA molecule [19]. The relative height of a polarographic wave (h_{DNA}) is suggested to be a measure of the double-helical structure of nucleic acid [14, 15].

Pb^{+2}–DNA Solutions in 0.15 M Acetate Buffer

In 0.15 M sodium acetate h_{DNA} reaches a value close to the maximum (Fig. 1), which could indicate that most of the studied DNA is in a double-helical structure. The data presented in Table I show only a minor effect of Pb^{+2} ions (in the concentrations used) on the h_{DNA} values. The increase of Pb^{+2} to phosphate molar ratio (P) changes distinctly on the reduction potential of DNA from −1.398 V (P = 0.05) to −1.443 V (P = 10). The fact that the presence of Pb^{+2} ions causes a slight increase of h_{DNA} with time of solution storage and variation of the reduction potential of DNA (Table I) may indicate that lead ions compete with sodium ions in the interaction with DNA and that the main interaction site is the phosphate site. The latter interaction would stabilize the double-helical structure. Some additional stabilizing effect of the DNA structure in the presence of Pb^{+2} ions was also seen from the increase of the melting temperature of the nucleic acid from 83.6 (lead-free DNA) to 84.2 °C (P = 10). All the results presented clearly show the masking effect of sodium ions in the studied solutions and that is why the changes caused by Pb^{+2} ions are only minor though indicative.

The studies carried out on Pb^{+2} interactions with nucleosides and nucleotides have also shown that phosphate is a major binding site for the metal ion and the complexes formed are relatively stable [20, 21].

It should also be mentioned that the CD spectra did not show any distinct variation of $\Delta\epsilon$ values in the studied solutions.

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TABLE I. Polarographic Data[a] for Pb^{+2}–DNA Solutions, c_{DNA} = 25 μg/ml.

P	exposition time in hours	$E_{1/2}$ Pb^{+2} [V]	reduction potential of DNA [V]	h_{DNA} [A.U.]
0.15 M acetate buffer				
0			−1.396	6.7
0.5	1	−0.435	−1.385	6.3
	25	−0.447	−1.378	6.6
1	0	−0.439	−1.388	6.2
	70	−0.456	−1.405	6.9
10	0	−0.475	−1.443	6.8
	60	−0.488	−1.442	7.0
0.05 M acetate buffer				
0			−1.385	see Fig. 2
0.05	0	−0.423	−1.387	
	72	−0.420	−1.384	
1	0	−0.431	−1.387	
	96	−0.428	−1.391	
10	0	−0.456	−1.416	
	64	−0.453	−1.420	
15	0	−0.469	−1.434	
	72	−0.470	−1.436	

[a]The polarographic wave of Pb^{+2} did not allow the precise measurement of the parameters of a reduction wave of DNA for P > 15.

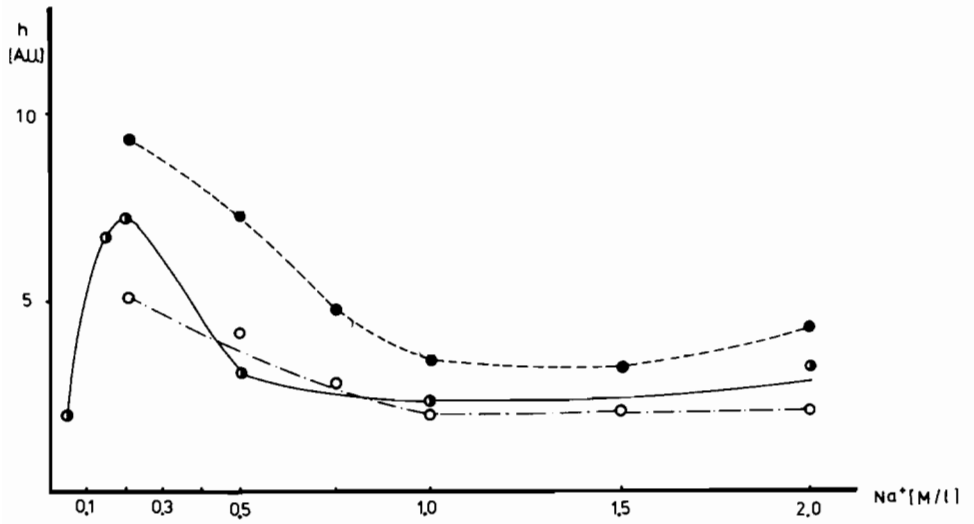

Fig. 1. Dependence of h_{DNA} on Na^+ ion concentration in acetate buffer (pH 5.6), ○ c_{DNA} = 50 μg/ml, ◑ c_{DNA} = 25 μg/ml, ● c_{DNA} = 100 μg/ml.

Pb^{+2}–DNA Solutions in 0.05 M Acetate Buffer

The h_{DNA} value in the solutions containing 0.05 M Na^+ ions is about one third of that found for DNA in 0.15 M buffer (Fig. 1). Addition of Pb^{+2} ions to that solution causes a distinct increase in h_{DNA} which depends on the Pb^{+2} ion concentration as well as on the time of the DNA exposition on metal ions (at 25 °C under N_2 atmosphere) (Fig. 2). These results indicate that the direct involvement of lead ions in the stabilization of a double-helical structure of DNA is considerable (increase of h_{DNA}).

The kinetics of this interaction is rather slow and the maximum h_{DNA} values were obtained *e.g.* for P = 10 after 72 hours. The stabilization of a double-stranded structure of the DNA molecule indicates that the phosphate is the main interaction site of the Pb^{+2} ion in the studied solutions [22].

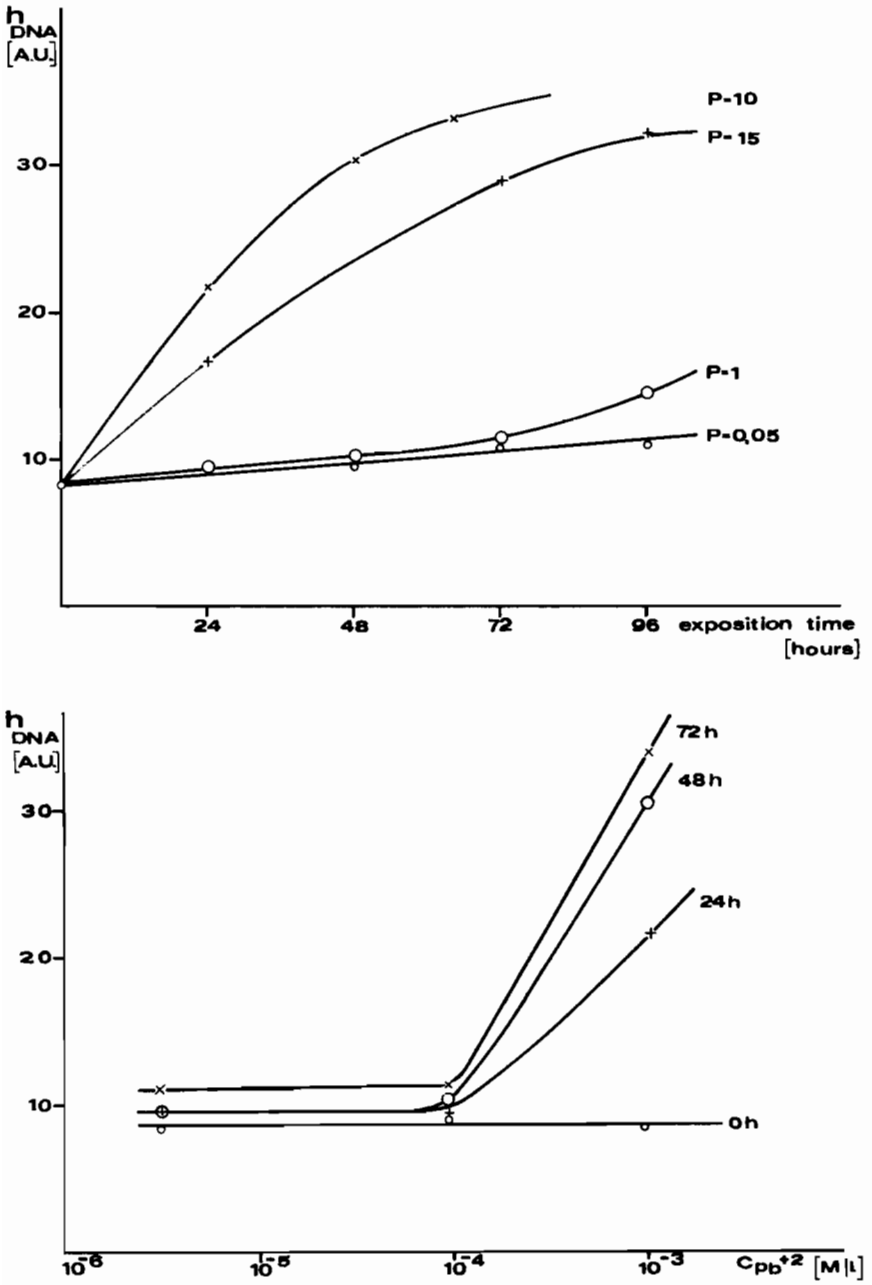

Fig. 2. Dependence of h_{DNA} on Pb^{+2} ion concentration and exposition time (solution storage) (c_{DNA} = 25 μg/ml, 0.05 M acetate buffer).

The stabilizing effect of Pb^{+2} ions on the double-helical structure of DNA which is suggested by the increase in h_{DNA} is supported by the CD spectra. The negative Cotton effect at 243 nm increases when lead is added up to P = 10 from $\Delta\epsilon = -3.0$ for Pb^{+2}-free solution to $\Delta\epsilon = -4.0$ for P = 10 (Table II). The variation of the positive band at 278 nm is more complicated. It increases slightly its $\Delta\epsilon$ from 2.5 (P = 0) to 3.0 (P = 1) and then decreases to 2.4 for P = 10. The latter result may indicate that Pb^{+2} ions in fact stabilize the helical structure of DNA and also influence the conformation of double-stranded DNA. For small P values the presence of Pb^{+2} ions favours the B form of DNA while for the higher P values (10–15) lead ions shift the conformational equilibrium towards the C form. This effect has been found for several metal ions though at much higher concentrations [23]. The CD spectra recorded for P = 15 and 100 show that for P > 10 both bands decrease when P increases (Table II). The latter result

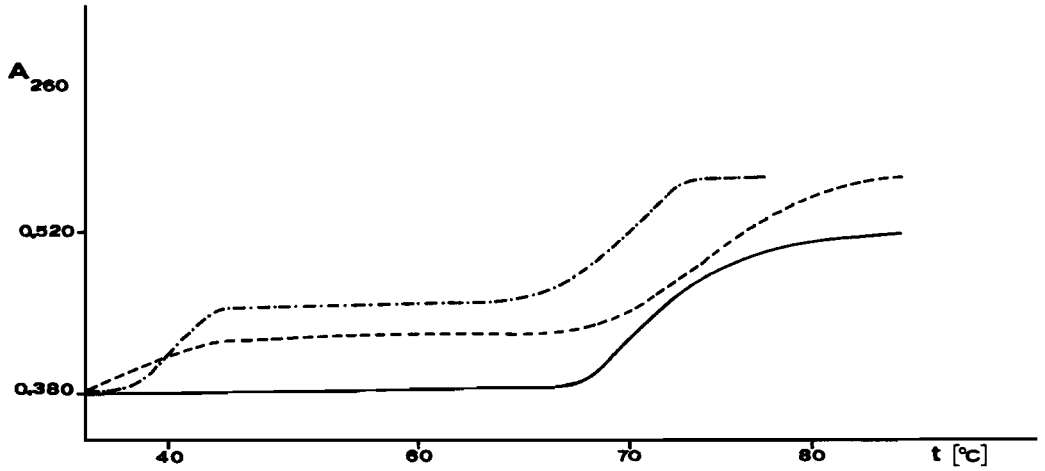

Fig. 3. Melting profiles of Pb^{+2}–DNA systems for P = 0 (——), 15 (– – –) and 50 (–·–), c_{DNA} 25 μg/ml, 0.05 M acetate buffer.

TABLE II. Δε Values for Pb^{+2}–DNA Solutions for Different P Ratios, c_{DNA} = 25 μg/ml, Exposition Time 48 h, in 0.05 M Acetate Buffer.

P	Δε 243 nm	Δε 278 nm
0	3.0	2.6
1	3.4	3.0
10	4.0	2.4
15	3.5 (?)[a]	2.3
100	2.0 (?)[a]	1.8

[a]Due to high absorption of Pb^{+2} ions these values could not be assigned with accuracy.

TABLE III. T_m Values for Pb^{+2}–DNA Solutions.

P	T_m, (°C)
0	72.4
1	75.7
10	74.6
15	74.3
	T_{m_1}[a] = 37.0
50	69.2
	T_{m_1}[a] = 41.0

[a]T_{m_1} = temperature of the first phase transition.

indicates the destabilizing effect of Pb^{+2} ions, *i.e.* their possible interaction with the base donors of DNA [22]. The possibility of the interaction of Pb^{+2} with base was indicated earlier in the model studies with ATP [21].

The polarographic and CD results are supported also by the melting profiles carried out for the Pb^{2+}–DNA solutions (Table III). The lead-free DNA in 0.05 M acetate buffer is characterized by T_m 72.4 °C. Addition of Pb^{+2} ion increases this temperature considerably *e.g.* for P = 1 to 75.7. The solutions with P = 10 have slightly lower T_m values but still distinctly higher than that for P = 0. These results clearly show the stabilizing effect of the lead ion of the DNA structure for the P values lower than 10. The melting profiles obtained for the high excess of Pb^{+2} ions show a considerable decrease of T_m values to *e.g.* 69.2 °C for P = 50 (observation of the melting profile at 340 nm excludes any precipitation of DNA). Additionally, for P ⩾ 15 the new phase transition appears around 40 °C (Table III, Fig. 3). This rather unusual result may indicate a more specific interaction of Pb^{+2} ion with DNA. The decrease of the T_m generally indicates the interaction of metal ion with bases of nucleic acid which destabilizes a double-helical structure [22]. The low temperature phase transition could be due to the melting of the d A–T rich regions of DNA *i.e.* Pb^{+2} ions interacting preferentially with the G–C regions makes a A–T region available for thermal denaturation at a relatively low temperature. More detailed studies on the two phase transitions presented in Fig. 3 are still in progress and the results will be published elsewhere.

Conclusions

Pb^{+2} ions, like some other divalent metal ions, interact with DNA in two different ways, *i.e.* via the phosphate chain leading to stabilization of a double-helical structure and via base donors (likely in the G–C rich regions), destabilizing a double-stranded structure. The latter interaction causes

the unusual two phase transition observed in the melting profiles. The latter result is still unclear though it may suggest some specificity in the Pb^{+2}–DNA interaction.

The comparison of the T_m values for the different P ratios with those of h_{DNA} obtained from the polarography studies shows that the latter parameter may characterize the double-helical structure though this relation does not seem to be simple (see also Fig. 1).

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