Asian Journal of Chemistry Vol. 1, No. 2 (1987), 93–104

Pulse Polarographic Studies on Metal ion Interaction with DNA

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The application of the differential pulse-polarography and sweep voltammetry to the studies of the metal ion interaction with DNA have shown that the local labilization of the double stranded structure of nucleic acid caused by *e.g.* charged surfaces may be of critical importance for such interaction. The opening of the double helix stimulates the metal ion binding to nucleic bases. This interaction is of minor importance when the intact DNA structure is available for metal ions. The polarographic techniques are very useful in the study of the local *defects* of the structure of the DNA molecule and of their influence on the metal ion binding. Such situation may happen on the biological membrane which is electrically charged and whose electric charge may be imitated by the mercury electrode surface.

INTRODUCTION

Metal ions can play an essential role in biological processes, the association of divalent of metal ions with e.g. ribosomes, is a well established fact. Many of them are required in virtually all biological processes in which nucleic acids are engaged. During the past decade has been recognized that metal compounds may be also fatal for life e.g. being an important and dangerous carcinogens. Nickel, for example, besides chromium and cadmium, seems to be recognized as the most effective carcinogen and nickel subsulfide, Ni_3S_2, is the most potent metal carcinogen that has been tested in experimental animals[1–3].

Studies of the metal ion interaction with DNA are usually made on two levels: (i) addition of metal ions to natural DNA preparations and studies of the metal–DNA complexes obtained, and (ii) search for metals already present in DNA molecules extracted from various biological materials. The general features of metal ion effects on the structure of nucleic acids are already relatively well established. There are two major binding sites for metal ions in DNA molecule: phosphates and base donors. These two possible metal-DNA modes of interaction may lean to distinctly different structural effects in the nucleic acid molecule[4,5]. The metal interaction with phosphates stabilizes the double helical structure while the metal binding to the bases destabilizes the ordered DNA helical structure and induces a nucleic acid denaturation process (decrease of melting point)[6]. The spectroscopic studies reveal the overall picture of

such interaction which may be not very relevant in the indication of the metal DNA interaction in the living cell. In the latter case the nucleic acid structure may be locally disturbed by the environment e.g. charged membrane or highly charged structural proteins. These local changes in the DNA structure may have critical influence on the metal ion nucleic acid interactions[9,10]. The numerous studied on the electrochemical behaviour of nucleic acids revealed that the charged electrode may serve as a simple and quite interesting model of the charged interfaces[7-20].

The polarographic techniques may not only serve as a source of the electrically charged interfaces but also as the set of the methods which are able to register the local variations in the DNA molecule or locally important interactions with metal ions as it is shown in this work. The polarography was already proved to be quite useful in the study of the metal ion nucleic acid systems[9, 10, 12, 17-20] and the information was unique for the methods applied. In this work we have employed some polarographic techniques for the studies of Ni(II) and Cu(II) ion interactions with calf-thymus DNA mainly to try to understand the influence of the local labilization of the DNA double helical structure on such interactions.

EXPERIMENTAL

The measurements were carried out for native calf-thymus DNA (Serva, mean molecular weight 1.2×10^6 daltons), with protein content lower than 0.5%. The DPP measurements at pH 7[32] indicated[32] less than 1% of denatured DNA in the used samples. All other chemicals were of analytical grade. Differential pulse-polarographic (DDP) measurements were carried out on a pulse polarograph PP–04 (Telpod Krakow) with X–Y recorder (Endim 620. 02). The three electrode system comprising an MD–DME working electrode with a drop time of 3s, a platinum wire auxiliary electrode and saturated calomel electrode as reference electrode was used. In all measurements the modulation amplitude was 50 mV. Sweep voltammetric (SV) measurements were carried out on a PA–4 polarographic analyzer (Prague). The three-electrode system comprising a hanging mercury drop electrode (HMDE, 1.65 mm^2) as working electrode, a platinum wire as auxiliary electrode and saturated calomel electrode as reference electrode was used. Scan rate 0.2 Vs^{-1}, adsorption potential (U_i) from −0.1 to −1.3V, switching potential (U_s) −1.85V and accumulation time 120s without stirring was applied in the SV measurements.

Test solutions were deoxygenated with a slow stream of analytical grade nitrogen and, during the measurements, nitrogen was passed over a solution surface. DNA was thermally denatured by heating in the solutions at 100°C for 30 min with subsequent rapid cooling in an ice bath.

In all measurements the acetate buffer containing 0.05 M of sodium acetate was used as a supporting electrolyte (pH 5.6). The concentration

of DNA (native or denatured), was 25 or 100 mgl^{-1} in DPP and SV measurements, respectively and was estimated spectrophotometrically. Measurements were performed at 25°C.

RESULTS AND DISCUSSION

In deoxynucleic acids the electroactive groups are bases. Adenine and guanine can be oxidized on graphic electrodes[21,22], while adenine and cytosine can be reduced at mercury electrodes[8,16,23].

There are two major models trying to explain the behaviour of DNA at charged interfaces. In the approach of Nurnberg *et al.*[8,11,15,18] and Palecek *et al.*[7,12,13,16,23] native DNA adsorbed at the electrode undergoes changes in the double helical structure, up to total strand separation. According to this model only single stranded parts of DNA undergo the polarographic reduction. The model of Berg *et al.*[24,25], on the other hand, presumes no denaturation of double helical structure at the electrode surface. The reduction of protonated DNA is possible for all conformational states according to this approach.

Assuming the former model, the relative height of a polarographic wave (limiting current), is a measure of locally unwound parts of double helical DNA, in which bases adsorb at the mercury electrode and next undergo reduction[13,16]. The amount of the local defects caused by the single polarographic reduction is rather low and can not be easily measured. The subsequent repetition of the e.g. DPP measurements leads, however, to the considerable increase of the DPP peak characterizing the reduction of unwound bases available for the electrode surface[10] (Fig. 1c). The effect of even small amount of defects caused by the polarographic reduction processes (several consecutive measurements) can also be seen in the DPP polarograms when the DNA solutions measured several times were left for 5 or 6 hours and remeasured[9] (Fig. 2). In order to obtain more precise idea about the cause of the labilization of the DNA structure we have performed several different experiments. Some results of these measurements are presented in Fig. 1. It should be mentioned that incubation of DNA in acid medium (pH 5.6) at room temperature within 96 hours has only slight effect on the DPP peak height of DNA[9] (Fig. 2a). The effect of mercuric ions on the double helical structure of nucleic acid was found to be negligible unless the solutions were remeasured (Fig. 1a). The peak height of DNA reduction was, however, increasing considerably when the metal-free DNA containing solutions were measured repeatedly i.e. DNA was undergoing the subesequent reductions (Fig. 1c)[9,10], or the electrode potential was varied certain number of times between 0 and −1.0 V without the nucleic acid reduction and then DNA was reduced (Fig. 1b). The final picture of the DNA reduction pattern in the two latter experiments was found, to be distintly different as it is seen in Fig. 1b and c. The consequent potential sweep between 0 and −1.0 V led to

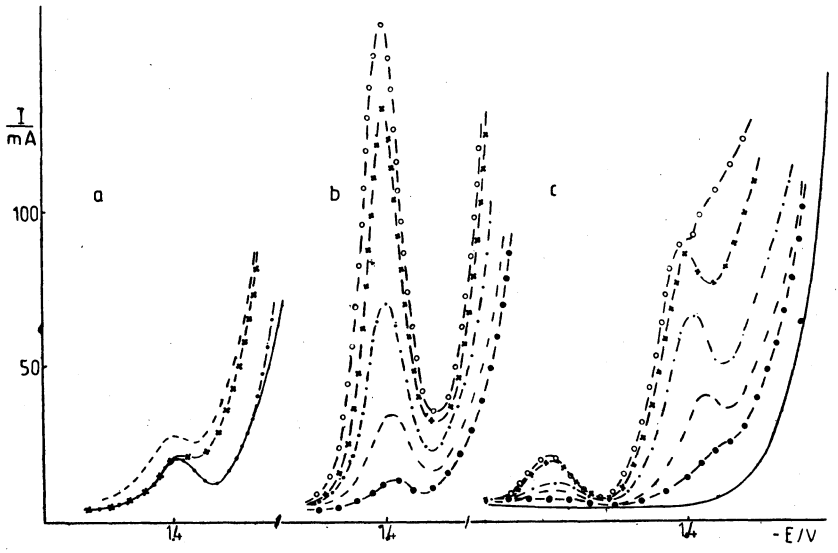

Fig. 1. (a) DPP reduction peak of freshly prepared DNA solution (—) a freshly prepared DNA–Hg(II) (P=0.1) solution (-·-), a DNA–Hg(II) solution measured 6 h after the preparation (first measurement (-×-), a DNA–Hg(II) solution measured 4 consecutive times and remeasured after being kept 14 h in room temperature (- - -); (b) Dependence of the DPP reduction peak height on a number of the potential sweeps (0 to −1.0 V) without reduction of DNA molecule. The DPP measurements were performed after 1 (-⊕-), 20 (- - -), 40 (-·-), 60 (-×-) and 80 (-o-) sweeps; (c) Dependence of the DPP reduction peak height on a number of the consecutive reduction measurements (0 to −1.6 V). The DPP measurements were performed after 1 (-⊕-), 20 (- - -), 40 (-·-), 60 (-×-) and 80 (-o-) DNA reduction measurements. Solid line represents buffer background. In all these experiments 25 mg dm^{-3} of calf thymus DNA in 0.05 M sodium acetate buffer (pH 5.6) was used with pulse amplitude 50 mV and drop time 3 s.

considerable increase of the DNA reduction peak at around −1.4 V indicating the increase of the number of the labilized fragments of DNA. The subsequent reduction processes caused severe damages of the nucleic acid structure and besides the peak at −1.4 V the two other ones were observed at −1.21 V and −1.45 V (Fig. 1c), which correspond to the reduction of the small fragments of nucleic acid and denatured DNA, respectively[9, 10]. Since in some cases the measured DNA samples were exposed on the metal mercury derived from the electrode drops for several hours (upto 6 hours) we have performed the measurements of the DNA being exposed on the metal mercury for 24 hours (thin layer of a DNA solution above pool of metal mercury with and without stirring). This measurement indicated only minor (less than 10%) variation of the metal mercury on a DNA peak height. Thus, the charged electrode as

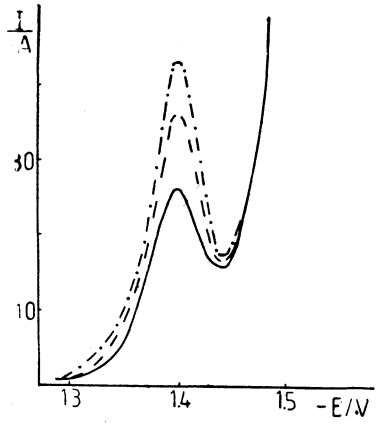

Fig. 2 Dependence of the height of the metal-free DNA reduction peak on the number of successive measurements: (—) first, (– –) second and third (–·–) measurement: the waiting time between the consecutive measurements was 5 hours at room temperature. [DNA]=50 mg dm^{-3}, 0.05 M sodium acetate buffer, pH 5.6 pulse amplitude 50 mV, drop time 3 s.

well as the reduction process may have the critical influence on the DNA structure and its picture in the electrochemical measurements. It is evident from the Figures 1c and 2 that the DNA molecules interacting with mercury electrode desorb very slowly. It takes several hours (waiting time between the measurements) to observe desorption of considerable amount of damaged DNA molecule from the mercury surface[33]. To prove that desorption of damaged DNA from mercury electrode really exists and influences the picture of DPP peak we have performed within one hour the 17 DPP measurements of native DNA in 0.2 M ammonium formate buffer (pH 6.5) and after the measurements we have removed all solution from the cell. DNA-free buffer was added to the remaining mercury pool and the polarographic and spectroscopic measurements were made to check whether DNA adsorbed on mercury drops desorbs and what is its state. After 30 min. of washing with the buffer the concentration of nucleic acid desorbed from mercury was estimated to be 2.5 mg/l (spectrophotometrically) and the DPP peak at —1.419 V was observed. The height of this peak increased with time (twice after 60 min). The measurement made after 20 h of washing of mercury with the buffer have shown very strong broad DPP peak at about —1.05 V corresponding to the reduction of oligonucleotides or nucleotides. This was supported by the spectrophotometric measurements showing strong absorption at 230 nm which is

characteristic for the nucleotide absorption. This behaviour of the DNA damaged by the polarographic reduction and then desorbed from the mercury electrode pool corresponds well to the discussion of Fig 1b and 1c given above. The effect of the mercury ions on the structure of DNA can be synergistic one with the electric field as it is discussed below for the other metal ions. These defects caused by the electrochemical measurements remind the destabilization of the DNA double stranded structure by different modes of irradiation[20, 23, 26-28] at least as far as number of defects is concerned. Generally it can be concluded that the relative height of the polarographic wave depends strongly on the number of consecutive measurements carried out with a given solution, as well as on the resting time between these measurements[9, 10]. It should be mentioned here that the relative increase of peak height of the thermally denatured DNA caused by the conscutive measurements is considerably lower (about one third) than that found for the native DNA molecule. The defects caused by the polarographic process create the situation in which e.g. metal ion can interact with DNA molecule differently at labilized parts and at intact double helical parts.

Ni(II)-DNA System

The addition of nickel(II) ions to solutions containing DNA exercised a marked effect on the DPP wave height of nucleic acid. The limiting current of the DPP wave decreases, suggesting the stabilizing effect of this metal on the double helical structure of DNA. The stabilization of the DNA double stranded structure derives from the metal ion interaction with nucleic acid phosphates[4, 6, 17]. Such interaction leads to more effective hydrogen bonds between the bases, as well as to decrease of the repulsive interactions between the negatively charged electrode and the phosphate chain, which is the major cause of the destabilization of the double helical structure on the electrode surface. This effect of nickel ions on the DNA molecule does not depend on the exposure time or the metal to DNA phosphate molar ratio (P). These observation could be made only during the first DPP measurement for a given solution. A distinct dependence on the molar ratio or DNA to metal exposure time was observed, however, when the second or third consecutive DPP measurements were carried out with the same solution[9]. The consecutive polarographic reductions were introducing the local breakes in the double helix which during the resting time between the two measurements were inducing local unwinding. These local breaks, enlarged with time, contain the easily available bases which can serve as the effective donors for metal ions. Thus, nickel ions may bind DNA preferably via the phosphate donors along the intact double helix and to bases especially in the parts labilized by the reduction process. This behaviour is clearly seen as far as DPP wave of nickel(II) reduction is considered. The DPP peak of Ni(II) of

freshly prepared solutions consists of major component whose reduction potential is close to that of DNA—free solutions and slight shoulder at higher potential (—0. 9V), (Fig. 3). The latter component increases with number of the consecutive measurements of the same solution. It becomes the major reduction wave of metal ion when nickel(II) is added to the

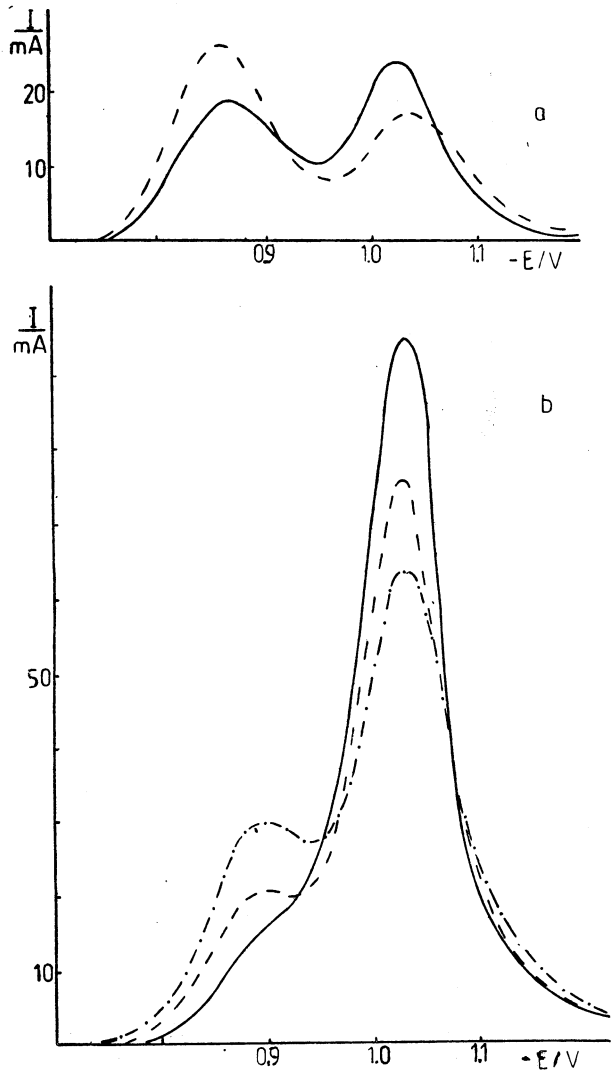

Fig. 3 The DPP reduction wave of Ni(II) ions obtained for the Ni(II)–DNA solutions (P=1) remeasured three times in about 4 hours intervals: (—) first, (– – –) second, (–·–) third measurement, respectively (b) and for Ni(II)–denatured DNA solution (a). The solutions between the subsequent measurement were kept at room temperature. For the other conditions see Fig. 2.

denatured DNA solution (Fig. 3). The two distinct reductions wave of nickel ions in the presence of DNA indicate two different binding sites of this metal with nucleic acid: the phosphates and the bases[9]. The latter ones become easily available when the base pairing system is damaged by some external effect. This behaviour of the Ni-DNA system could support the model suggesting the labilization of DNA double helix at the electrode surface as proposed by Palecek et al. at least for the conditions used in this work.

The opening of the helical structure of adsorbed native DNA leading to the reduction of the DNA bases is especially effective at the HMDE. In this case the time of contact of nucleic acid with the charged electrode surface (waiting time) is relatively long and the effect of the electrical field on the DNA structure can be easily observed[11]. Thus the application of the sweep voltammetry can supply another evidence that the local labilization of the double stranded structure may distinctly influence the interaction modes between metal ions and nucleic acid. Figure 4 shows the dependence of the SV reduction peak height of DNA (native and denatured), on the molar ratio of added nickel(II) ions. It is just evident that low ratios of metal ion (P=0.5 and 1) do not influence the peak height distinctly. Excess of metal (P=5), however causes the considerable increase of the DNA reduction wave to the values observed for the denatured nucleic acid. The interesting observations were also made for the variations of the reduction potential of DNA and the peak height of the reduction of nickel(II) with the value of P. None of these magnitudes changes distinctly for P=1 and 0.5 while both of them vary for P=5. This behaviour of SV results can indicate that at low ratios of Ni(II) the major interaction site of metal is that at phosphate chain. These interactions stabilize the double stranded structure of DNA molecule. The binding to phosphates does not change considerably the metal ion environment which both in acetate buffer with and without DNA consists of octahedrally bound oxygens. The stabilizing effect of low nickel(II) ratios was found earlier by the spectroscopic techniques[4,6]. The excess of metal ions may, however, compete in binding to base donors. As it was shown above and earlier[9], intact double helical structure of DNA is unaccessible easily for metal ions and even after several hours no direct effect of Ni(II) ions on DNA bases was observed. The DNA molecule adsorbed on a mercury electrode for several minutes (waiting time in our experiment was 120 s), at different initial potential was, however, labilized and metal ion interaction was stimulated. Already at low initial potentials the peak height of the DNA reduction in the presence of Ni(II) ions, (P=5), reaches the values close to those obtained for the denatured nucleic acid (Fig. 4). It strongly indicates that cooperative effect of the electric field and metal ions labilizes the double helical structure quite effectively. None of these factors can influence on the nucleic acid structure alone to the extend

observed in this experiment. The interpretation given above is supported also by the results obtained for the Ni(II)–denatured DNA containing solutions. The height of the SV peak of the reduction of denatured DNA

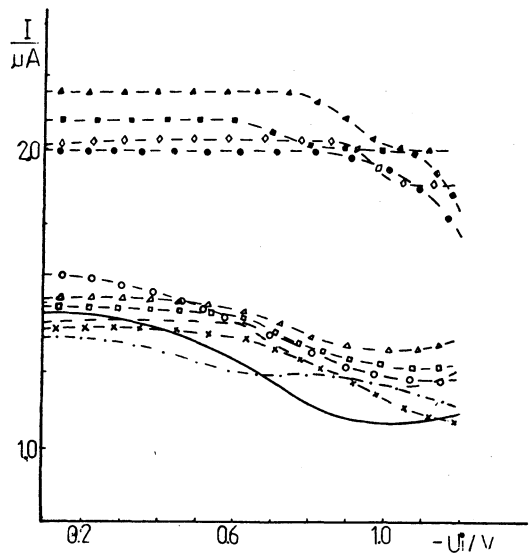

Fig. 4 Dependence of an SV reduction peak height of DNA for: native metal-free DNA (—), native DNA with Ni(II) for P=0.5: exposure time: 0 (– – –), 24 (–··–), 48 hours (–×–): P=1: exposure time: 0 (–o–) 24 (–□–), 48 hours (–△–): P=5: exposure time: 0 (–⊕–), 24 (–■–) and 48 hours (–▲–) and denatured metal-free DNA (–◇–) on an initial potential U_i. The solutions between the subsequent measurements were kept at room temperature the measurements were performed with HMDE (surface area 1.65 mm²) in 0.05 M sodium acetate buffer (pH 5.6), waiting time 120 s, scan rate 200 mV s^{-1} [DNA]= 100 mg dm^{-3}.

in the presence of the Ni(II) ion excess remains unchanged on the level of the metal free denatured DNA, while for P=1 and 0.5 its value decreases immediately or after some time after the mixing of metal with thermally denatured DNA (Fig. 5). This may indicate some renaturing ability of the Ni(II) ions, as well.

It should be mentioned here that the similar experiments with Pb(II)–DNA have shown that lead ions behaved very differently and excess of metal

ions led to the drastic decrease of the SV peak due to aggregation of the DNA caused by an excess of Pb(II)[29].

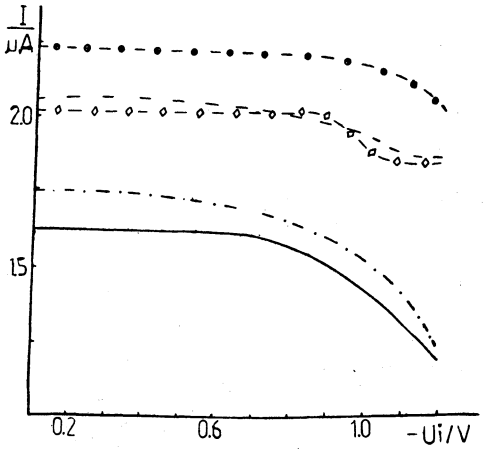

Fig. 5 Dependence of a SV reduction peak height for: denatured metal-free DNA (-◇-); denatured DNA Ni(II) for: P=0.5 exposure time 0 hours (—), P=1 exposure time 0 (- - -), and 24 hours (-·-), and P=5 exposure time 0 hours (-⊕-). For the other conditions see Fig. 4.

Cu(II)-DNA System

Cu(II) ions are much more effective in the interaction with nucleic bases than Ni(II) ions. A variety of methods has been applied to follow this interaction[30, 31] and it seems to be generally accepted that even small amount of cupric ions may destabilize the double stranded structure of nucleic acid. The effect of cupric ions on the DPP reduction wave is immediately seen after addition of metal salt to DNA solutions, even at equimolar amount (P=1). The strong involvement of cupric ions in the binding of bases was suggested especially when DNA structure was labilized by the polarographic reduction[9]. The consideration of the DNA and Cu(II) ion DPP parameters of the reduction peak suggested also some renaturing ability of this metal ion. The figure 6 shows that already for the equimolar solutions (P=1), the effect of the cupric ions on an SV peak was considerable. Its height increases up to the values characteristic for the denatured DNA when the exposure time (DNA on metal ions) increases up to 72 hours. These results again suggest that interaction of metal ion with bases was more effective when DNA structure was labilized by the electrically charged surface even when the metal ion can bind nucleic acid very strongly.

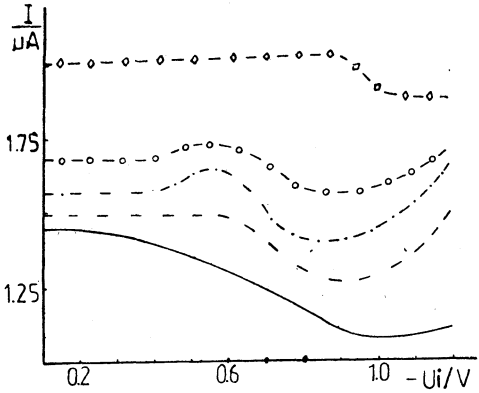

Fig. 6 Dependence of a SV peak reduction height of DNA for native metal-free DNA (—), deatured metal-free DNA (-◇-), and Cu(II)-denatured DNA for P=1 and exposure time 0 (- - -), 24 (-·-) and 48 hours (-o-). The other conditions as in Fig. 4.

The other important conclusion which can be derived from these studies[9,10], concerns the application of the melting profiles to the study of the metal ion interactions with nucleic acid: the results of such investigations just show the interaction of metal ion with thermally denatured DNA, i.e. with DNA whose bases are exposed to metal ions due to the denaturation process[31].

ACKNOWLEDGEMENTS

This work was financially supported by the Ministry of Education, (Project RP.II.13.1.5). Authors also wish to thank Prof. E. Paleček for helpful discussion of the results obtained in this work.

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[*Invited paper*, *Received*: 7 November, 1988] AJC-18