Polarographic Studies on Copper(II) and Nickel(II) Ion Interactions with DNA

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(Received December 29, 1986)

Abstract

Differential pulse-polarography was used to study the interactions of Cu(II) and Ni(II) ions with calf-thymus DNA. Both binding sites of nucleic acid, *i.e.* phosphates and bases, are involved in metal ion coordination, with Ni(II) preferentially bound to phosphates and Cu(II) to bases. Nickel shows more potent renaturation ability than copper. The latter metal ion denatures DNA when it is present in excess. The fact that DNA undergoes the labilization process during the DPP reduction revealed that both metal ions bind to base donors more easily in partly unwound regions of nucleic acid.

Introduction

During the past decade there has been growing recognition that metal compounds are an important group of carcinogens. Nickel, besides chromium and cadmium, seems to be recognized as the most effective carcinogen [1–3] and nickel subsulphide, Ni_3S_2, is the most potent metal carcinogen that has been tested in experimental animals [1]. Many inorganic nickel compounds have been tested and their effect on a cell or tissue established [4]. It was shown that Ni^{2+} among others enters the nucleus and damages DNA molecules. Many nickel compounds induce single strand breaks and DNA–protein cross-links [5–7] and although the relationships between the DNA lesions and transformation is not known, it seems to be possible that DNA–protein cross-linking may facilitate misincorporation of nucleotides during DNA replication or repair, thus contributing to mutation [6].

The general features of metal ion effects on the structure of DNA are already well established. There are two major binding sites for metal ions in DNA molecule, phosphates and base donors. These two possible metal–DNA interactions may lead to very different structural effects in the DNA molecule [8]. The metal interaction with phosphates stabilizes the double helical structure while the metal binding to the bases destabilizes the ordered DNA hydrogen system and induces a nucleic acid denaturation process (decrease of melting point) [9]. Preference for the phosphate over the base binding decreases in the order $Mg(II) > Co(II) > Ni(II) > Mn(II) > Zn(II) > Cu(II)$ [10]. Early work showed that the Ni^{2+} ion has a stabilizing effect on DNA, thus interacting with phosphate donors [10, 11]. Recent studies on Ni(II) polynucleotide systems, poly(dG-dC) and poly [d(G-C)] [12, 13], have shown that carcinogenic nickel compounds are able to induce the B to Z conformational transition of polynucleotides at substoichiometric concentrations with respect to phosphate. The details of Ni(II)–DNA interactions are, however, still unknown, especially as far as a specific strong interaction of metal ion with nucleic acid is concerned, with possible carcinogenity of Ni(II).

Electrochemical techniques have been shown to be very sensitive for the study of DNA and metal–DNA systems [14–17], including differential pulse polarography (DPP) [14, 18, 19].

In this work we have employed the DPP technique to understand moore deeply the Ni(II)–DNA system and some results are presented below. Since cupric ions are thought to act predominantly at the bases [8–10], for comparative reasons we also performed polarographic studies of the Cu(II)–DNA system.

Experimental

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

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0020-1693/87/$3.50

DPP measurements were carried out on a pulse polarograph PP-04 (Telpod Krakow) with an X-Y recorder (Endim 620.02) using an MD-DME electrode with a drop time of 3 s. In all measurements the modulation amplitude was 50 mV and acetate buffer containing 0.05 M sodium acetate was used as electrolyte, pH = 5.6. The measurements were performed under argon and the wave potentials were related to a saturated Calomel electrode.

The concentration of native DNA was estimated spectrophotometrically.

Results and Discussion

During the polarographic process in acetate buffer, metal-free DNA undergoes a reduction at about −1.4 V. This process is usually assigned to a reduction of the adenine and cytosine residues of double-stranded DNA [20]. The relative height of a polarographic wave (limiting current) is suggested to be a measure of locally unwound parts of double-helical DNA, in which bases absorb at the mercury electrode and then undergo reduction [21, 22].

Totally denaturated DNA is reduced at considerably lower potentials (around −1.5 V). The fast local unwinding of the double helix is observed at higher electrode fields during the polarographic process [23]. The reduction mechanism of both bases mentioned above is relatively well known [24], and proceeds with four electrons for adenine and two electrons for cytosine, respectively. Reduction of these bases could damage the hydrogen bond system and induce partial opening of the double helical structure even after the DNA molecule is removed from the electrode. This effect was observed when the same solution of metal-free DNA was used for several DPP measurements.

The DNA solution left for about 100–120 min after the DPP measurements and then remeasured was characterized by a considerably higher polarographic wave (Fig. 1). This result indicates clearly that a reduction process produces chemical changes in DNA which lead to structural changes, e.g. strand breaks. These induce the unwinding process and make successive reduction more effective. 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 the measurements. This problem is described in more detail elsewhere [25].

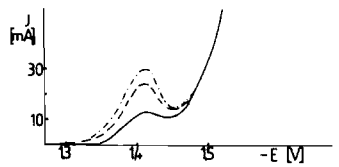

Fig. 1. Dependence of a limiting current I (mA) of a DNA reduction wave (Serva) on the number of successive measurements: (——) first, (– – –) second, (–·–·–) third measurement; the exposure time was below 5 h. The measurements were performed 2–3 h after each other. [DNA] = 50 μg/ml, 0.05 M sodium acetate buffer, pH 5.6.

Ni(II)–DNA Solutions

The addition of Ni(II) ions to solutions containing DNA has a marked effect on the polarographic wave height of DNA (h^{DNA}). It reduces the limiting current of the DPP wave, suggesting the stabilizing effect of Ni(II) ions on DNA double helical structure. This effect derives from the fact that metal ions interact mostly with DNA phosphates [14]. This leads to more effective hydrogen bonds between the bases, as well as to a decrease in the repulsive interactions between the negatively charged electrode and the phosphate chain, which is a major cause of the destabilization of the double helical structure of DNA on an electrode surface. The effect of metal ions on a polarographic picture of DNA does not depend on the exposure time or the metal-to-phosphate molar ratio used (Fig. 2). This is observed, however, only during the first DPP measurement for a given solution. A distinct dependence on the

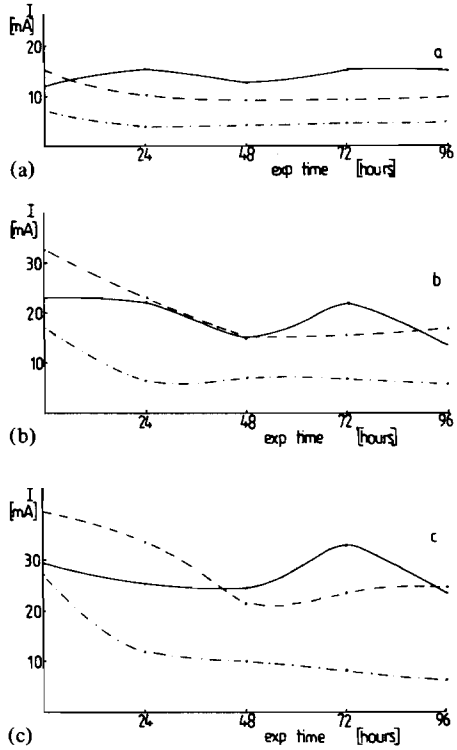

Fig. 2. Dependence of I (mA) of DNA on Ni(II) concentration; P = 0 (——), P = 1 (– – –), P = 5 (– –·–), and exposure time (in h) for the first, second and third successive measurements (a, b, c). The conditions are as in Fig. 1.

molar ratio or DNA-to-metal exposure time was observed when second or third consecutive DPP measurements were carried out with the same solution (Fig. 2b, c). In the latter case the excess of nickel ions is more effective in rewinding the open parts of the DNA caused by electrode potential and partial reduction of nucleic acid residues. This process is time dependent and reaches a kind of equilibrium after about 24 h (Fig. 2c).

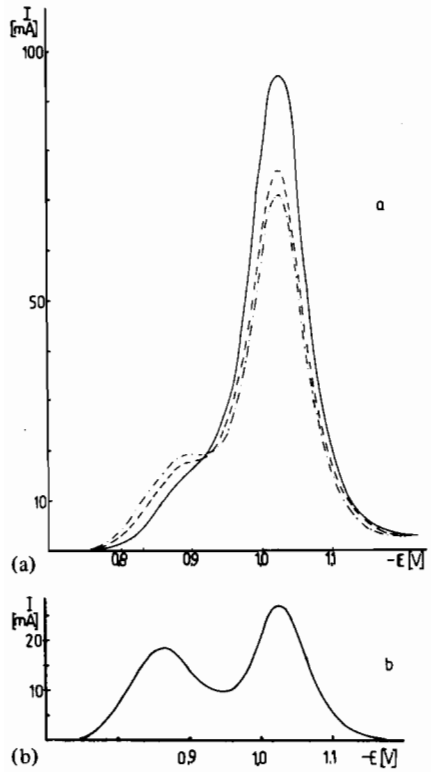

Fig. 3. The DPP reduction wave of Ni(II) obtained for the same sample measured three times in about 2 h intervals; (———) first, (– – –) second, (–·–·–) third measurements, respectively (a), and for the Ni(II)-denaturated DNA solution (b).

Interesting observations were also made for the nickel DPP wave (Fig. 3). The reduction of metal ion in the presence of DNA is characterized by two overlapped waves (Fig. 3) which correspond to at least two binding sites of nickel ion in the DNA molecule. The height of the major peak is only slightly dependent on the exposure time but it decreases considerably during the second or third consecutive measurement of the same solution (20 to 30%) (Fig. 3). The minor wave, which is seen as a shoulder during the first measurement, becomes quite a distinct peak during the third one (Fig. 3). The increase of the latter peak height is of the same order as the decrease of the major peak height.

Comparison of the DNA behaviour discussed above with that of metal ion shows that the minor wave of the metal ion which limits current increases when more effective unwinding of DNA is observed (see above), corresponds to the metal ion interacting with open parts of a double helix, *i.e.* with bases. Thus, the other peak assigned to the metal ion corresponds to nickel being in fast exchange between phosphates and unbound aquo-ion.

Thus, the DNA unwinding caused by the electric potental (e.g. that of cell membrane) or induced by double strand breaks (caused by chemical or physical factors), promote effectively the metal–base interaction and all consequences derived from that fact, e.g. renaturation of nucleic acid induced by metal ion bound to purine or pyrimidine bases. Strong evidence for the interpretation presented above was obtained when thermally denatured DNA was measured in the presence of nickel ions. The DPP polarogram shows the lower potential peak as very distinct with a relative height almost equal to the other one representing the native or renatured nucleic acid (Fig. 3).

Copper(II)–DNA Solutions

Cupric ions have much stronger ability to bind with DNA than nickel ions. A variety of methods has been applied to understand the Cu(II) binding modes in the DNA molecule [26, 27]. Although there is general agreement that this metal ion interacts preferentially with nucleic acid bases, difficulty still exists in the unequivocal elucidation of the exact nature of this interaction.

The effect of cupric ion on the DPP reduction wave for DNA is immediately seen after addition of metal salt to DNA solution. The peak height of the DNA reduction decreases considerably and its magnitude is not distinctly affected by the exposure time of the molar ratio (Fig. 4). The molar ratio as well as the exposure time are, however, important when second or third successive measurements are performed with the same solution (Fig. 4b, c). The metal ion does not affect the relative height of the DPP peak at short exposure times, indicating low renaturation ability of this metal ion (see also Ref. 25) under these conditions (Fig. 4b, 4c). The solutions remeasured after a DNA exposure on Cu(II) ions from 24 to 72 h are characterized by a considerably lower reduction wave for DNA and the different effects of two molar ratios applied in this study. At an exposure time of 72 h or more the relative height of the DNA peak is again molar ratio independent. There are two possible reasons for the limiting current decrease of native DNA: the rewinding process of the partly open double helix (see above), *i.e.* renaturation of nucleic acid structure; and a denaturation process which is usually observed for higher metal ion concentrations [26, 27]. Both of these antagonistic effects of metal ion on nucleic acid structure are usually suggested to happen in this system

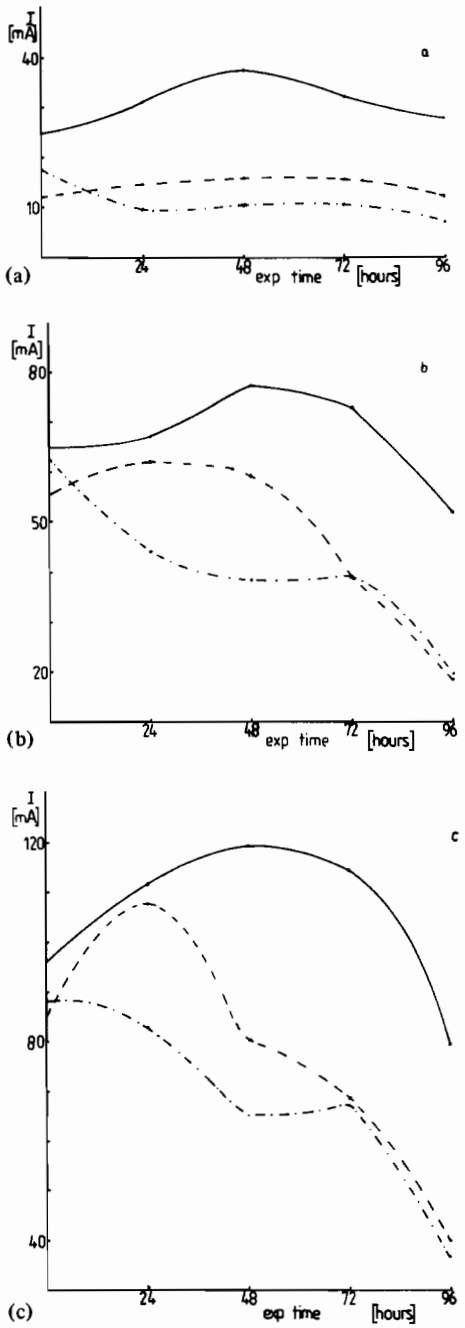

Fig. 4. The limiting current dependence of DNA on Cu(II) ion concentrations, (– – –) $P = 1$, (–·–·–) $P = 5$, and exposure time for first, second, and third successive measurements (a, b and c, respectively). [DNA] = 25 μg/ml, 0.05 M sodium acetate buffer, pH 5.6. Solid line corresponds to metal-free DNA.

[27]. It is difficult, however, to distinguish between these processes using the DNA reduction wave only.

Additional information may be obtained from the metal ion reduction wave. The DPP peak of the metal ion for solutions with a high excess of Cu(II) is very similar to that in the DNA-free solutions, indicating that most of the metal ion is present in an unbound state and its DPP peak covers the minor peak of Cu(II) bound to DNA. The distinct variations in relative DPP peak height as well as in the reduction potential of metal ion in the presence of nucleic acid was observed for the 1:1 phosphate to Cu(II) molar ratio (Figs. 5 and 6). The DPP peak height of Cu(II) decreases considerably in the presence of DNA during the first DPP measurement and still more drastically during the respective successive remeasurements (Fig. 5). This DPP wave height is only slightly dependent on the exposure time.

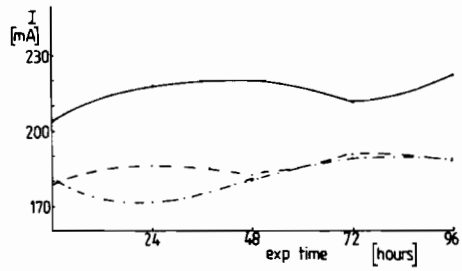

Fig. 5. Dependence of DPP peak height of Cu(II) on exposure times and number of successive measurements; (——) first, (– – –) second, and (–·–·–) third measurement ($P = 1$). The relative height for Cu(II) in acetate buffer equals 268 mA.

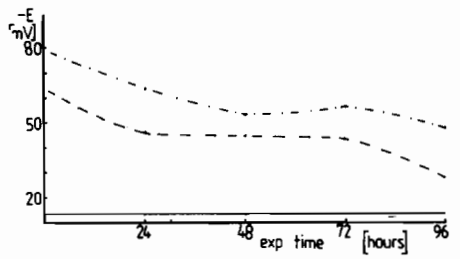

Fig. 6. Dependence of the Cu(II) ion reduction potential for $P = 1$, on exposure time and number of successive measurements of the same solution; (——) first, (– – –) second, and (–·–·) third measurement. Reduction potential of Cu(II) in the buffer equals –15 mV.

The very distinct decrease of this value may indicate the involvement of Cu(II) ion in the binding of bases even in low molar ratio solutions shortly after contact with nucleic acid. The additional opening caused by the charged electrode and resting time after the first or any other successive DPP measurement induces this interaction, as was the case in the Ni(II)–DNA system (see above). The strong involvement of cupric ions in the binding of bases is also seen by the considerable variation of metal ion reduction potential. The reduction potential of Cu(II) obtained from successive measurements of the same solution is considerably more negative than that

obtained for this metal ion in buffered solution (Fig. 6). This very effective involvement of Cu(II) ions in the interaction of bases seems to indicate clearly that the major species existing in the Cu(II)–DNA solutions, especially after the unwinding of nucleic acid by the DPP process, is the denatured Cu(II)–DNA complex. The DPP peak parameters of Cu(II), *i.e.* reduction potential and peak height as well as the DNA peak parameters described above for solutions treated only once, show some renaturating ability for this metal ion as well.

Conclusions

The discussion presented above seems to indicate that the DPP technique is not only very useful for the metal–DNA system as an analytical method, but it also can help to imitate the influence of a charged cell membrane, which may modify dramatically the DNA molecule interaction with ions or molecules (see e.g. Ref. 28). Both metal ions which were studied in this work have rather complicated ways of interaction with DNA, particularly when the nucleic acid has been partly damaged or modified by cell membrane structure. Cu(II) and Ni(II) ions interact easily with base donor systems, especially within unwound parts of nucleic acid. This may lead to some renaturation or denaturation processes as described for example in Ref. 27.

Acknowledgement

This work was financially supported by the Ministry of Science, Higher Education and Technique, Project RP 13.1.5.

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