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Binding of Cu(II) to DNA in the Presence of Li(I), Na(I) and Mn(II)

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Complexation of Cu(II) with DNA has been studied by EPR and UV techniques in the presence of Li(I), Na(I) and Mn(II) both in unoriented samples (solutions) and in partially oriented ones (fibres). This study shows that Cu(II) binding to the bases of DNA is influenced by the concentration and by the nature of the cations present. All the ions investigated cause a reduction of the amount of Cu(II) bound to the bases which is proportional to their concentration. The effect, at any given concentration of the ions, follows the order Mn(II) ≫ Li(I) > Na(I). Since the stabilisation of DNA double helix by these ions follows the same order, it is suggested that Cu(II) binding to the bases depends on the possibility that the double helix can be deformed, at least locally.

Introduction

Several authors have observed that nucleic acids extracted from cells tightly bind trace amounts of transition metal ions which are not readily removed even by repeated purifications or by prolonged dialysis against complexing agents [1–4]. The binding of transition metal ions to nucleic acids and, in particular, of Cu(II) to DNA has been, therefore, the object of numerous investigations [5–12] aiming at the identification of specific binding sites for this ion.

A problem which has not yet been developed in detail concerns the role played by mono- and divalent cations in directing Cu(II) binding to the different potential sites on the DNA. The aim of this paper is to give an answer to some aspects of this question through a study of the influence which Li(I), Na(I) and Mn(II) at several concentrations exert on the binding of Cu(II) to the bases in DNA. Both unoriented samples, such as solutions, and partially oriented ones, such as fibres of DNA, have been investigated. Among the techniques which could be used, we have chosen UV differential spectroscopy and EPR spectroscopy for the study of solutions and fibres respectively.

Experimental

DNA was sedimented in a SW41 rotor of a Beckman Spinco Mod. L65 ultracentrifuge. UV spectra were recorded on a Pye Unicam Mod. 1700 UV/Visible spectrophotometer. A home made X-band spectrometer was used to measure EPR spectra. The spectrometer was equipped with a Varian rectangular cavity operating in the TE_{102} mode.

Calf thymus DNA was from Sigma, salts were analytical grade from Merck.

Purification of DNA

DNA was purified from residual proteins which are usually found in commercial products by two extractions with freshly distilled phenol and precipitated from an alcoholic solution. All these operations were performed at 4 °C.

Preparation of DNA Fibres

Cu(II) and Mn(II) containing DNA fibres were prepared by a modification of the procedures described in the literature [13, 14]. A 0.1% DNA solution in 0.0055 *M* LiCl or NaCl was ultracentrifuged at 40,000 rpm for 16 h at 4 °C. A drop of the DNA gel which was recovered at the botton of the ultracentrifuge tubes was suspended on the rounded off ends of two tiny glass rods facing each other. A drop of a 0.05 *M* water solution of $CuCl_2$ or $MnCl_2$ was then carefully deposited on the DNA gel. The alignment of the fibrils in the fibre was favored by slightly stretching the DNA gel during the slow evaporation of the water at room temperature. These operations were performed under a microscope. The degree of order of the fibres was checked with a polarizing microscope.

EPR Measurements

EPR spectra were recorded at room temperature. Samples were prepared by aligning, under the microscope, approximately 10 fibres on a diamagnetic support which was then fixed on a quartz rod and introduced in the resonant cavity. DPPH was used as field marker.

UV Measurements

UV measurements were taken at room temperature on 2.32×10^{-4} *M*(P) DNA solutions in 0.002 *M*, 0.01 *M* and 0.1 *M* NaCl or LiCl after the addition of appropriate amounts of $CuCl_2$ to give different [Cu(II)]/[P] ratios within the range 0.10–7.50. Reference solutions were identical to the samples except that they did not contain $CuCl_2$. OD readings were taken at 280 nm, where the difference spectra display a maximum. Samples containing both Mn(II) and Cu(II) were prepared in the same way and [Mn(II)]/[P] ratios varied within the range 0.10–7.50.

Results and Discussion

Two of the EPR spectra recorded on Cu(II)–DNA fibres are reported in Fig. 1. Spectra a and b were obtained respectively from Na(I) and Li(I) containing fibres. The complexity of EPR fibre spectra of Cu(II)–DNA is comparable to that observed by other authors on Cu(II)–DNA solutions [15, 16]. This is a consequence of the superimposition of at least three distinct absorptions with different and unresolved g corresponding to 1) Cu(II) bound to the bases, 2) to the phosphates and 3) the free ion.

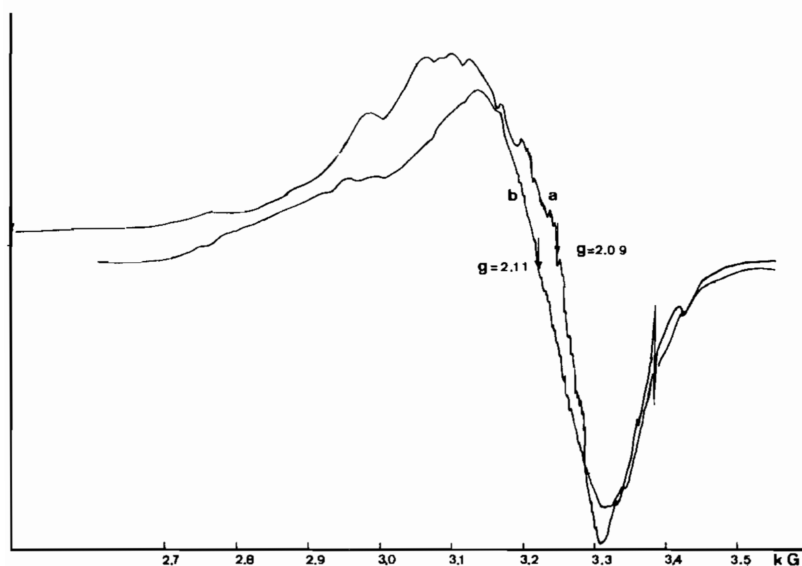

Figure 1. X-band EPR spectra of Cu(II)–DNA fibres containing a) Na(I) and b) Li(I).

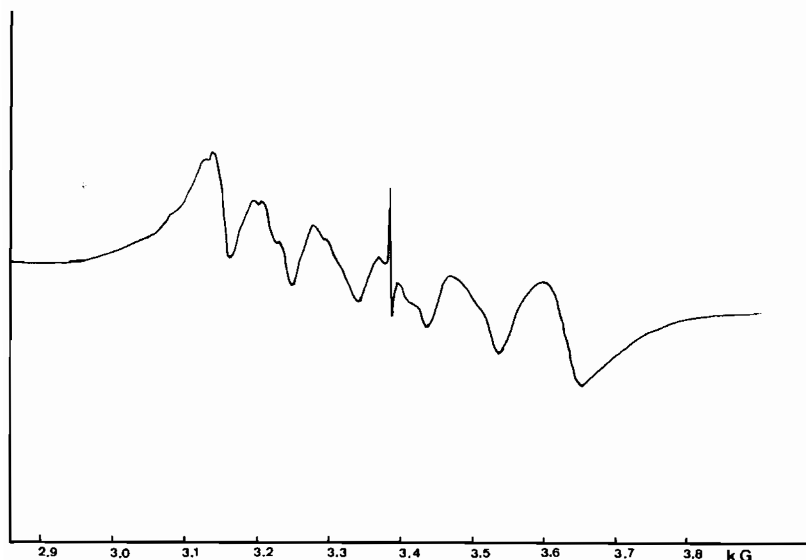

Figure 2. X-band EPR spectrum of Mn(II)–DNA fibres containing Li(I).

The spectrum is not simplified at Q band as we could establish in preliminary experiments. We shall not attempt, therefore, an interpretation of the spectra in terms of spin Hamiltonian nor a computer simulation, given the number of parameters which should be varied systematically.

We shall, however, point out the salient points which can be related to some structural characteristics of the complexes. In the $g_\perp$ region a pronounced superhyperfine structure can be observed while the hyperfine structure is almost absent. This is indicative of the delocalisation of Cu(II) unpaired electron onto the ligands and, therefore, of the covalent character of the metal–ligand bond. This feature is common to both samples containing Li(I) and Na(I). The two spectra differ on the contrary in that in b) (Li(I) containing Cu(II)–DNA) there is a smaller contribution by the component characterised by the lowest g value, which has been identified as Cu(II) bound to the bases [15, 16]. This is evidenced by the lower value of apparent g measured for Na(I) containing Cu(II)–DNA fibres (g_{app} = 2.09) compared to those containing Li(I) (g_{app} = 2.11). Moreover, in the last mentioned spectrum the superhyperfine structure is less pronounced. It follows from this that, in the presence of Li(I), Cu(II) binding to DNA bases is inhibited compared to Na(I).

Spectra recorded on Mn(II)–DNA and Mn(II), Cu(II)–DNA fibres are reported in Figs. 2 and 3 respectively. Mn(II)–DNA fibre spectrum is characterised by a six line hyperfine structure centered around g = 2 and by a pronounced asymmetric line broadening which increases with increasing magnetic field. The hyperfine coupling constant varies from 62 to 125 G. This kind of pattern is usually found for Mn(II) systems with ZFS anisotropy and long correlation times [17, 18] and is indicative of the limited rotational motion of Mn(II) binding site.

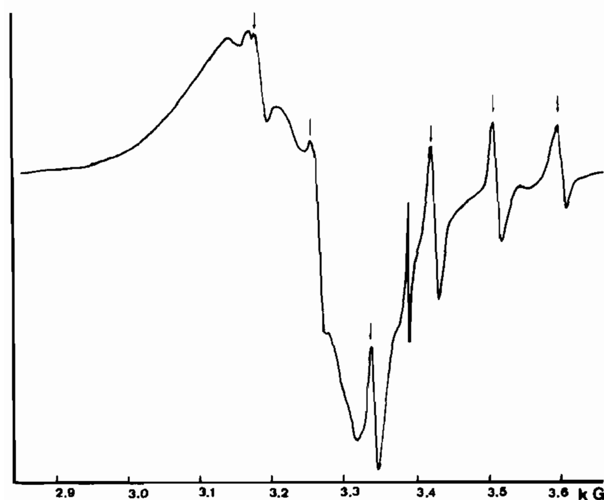

Figure 3. X-band EPR spectrum of Mn(II), Cu(II)–DNA fibres containing Li(I). The arrows indicate Mn(II) hyperfine components.

The EPR spectrum of Cu(II), Mn(II)–DNA fibres (Fig. 3) can be considered, to a first approximation, as arising from the superimposition of Cu(II) and Mn(II) absorptions. The exchange interaction between the ions is, therefore, weak and this rules out the formation of mixed metal dimers in which a strong coupling would produce a two state system characterised by total spins of two and three respectively with consequent disappearance of the typical absorption patterns of the isolated ions [19]. Nonetheless, the details of Mn(II) absorption differ from those seen in Fig. 2 in that the hyperfine structure is almost symmetric and there is a considerable line sharpening. This can be a consequence of faster tumbling of Mn(II) which is accompanied by a reduction of second order effects. We can, therefore, say that the presence of Cu(II) increases the mobility of Mn(II) in the complex.

The results of UV differential spectroscopy measurements on Cu(II)–DNA solutions in the presence of three concentrations of LiCl and NaCl are summarised in Fig. 4. The amount of Cu(II) bound to the bases is a function of [Cu(II)]/[P] and the increase of NaCl and LiCl concentrations is

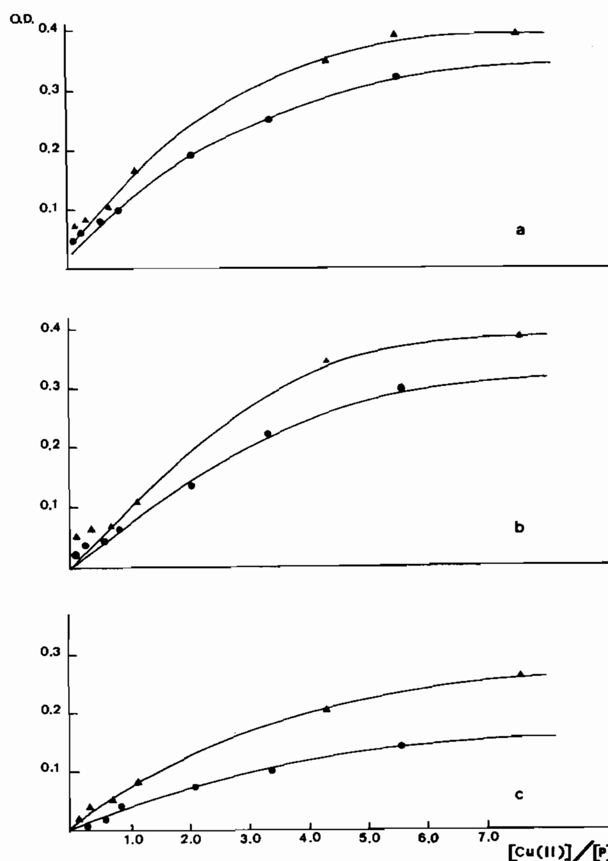

Figure 4. OD at 280 nm in difference spectra obtained from solutions of Cu(II)–DNA *vs.* [Cu(II)]/[P]. Solutions contained Na(I) (▲) or Li(I) (●) at molar concentrations 0.002 *M*, 0.01 *M*, and 0.1 *M*, in a, b and c respectively.

accompanied by a decrease of $OD_{280\ nm}$ which corresponds to a reduction of Cu(II) complexation on the bases of DNA. This feature is especially pronounced in 0.1 *M* salts. A similar effect, of comparable magnitude, is observed when $CuCl_2$ is added to DNA solutions containing $NaNO_3$ at increasing concentrations. The reduction of Cu(II) binding to the bases with increasing salt concentration in the DNA solution must, therefore, be essentially attributed to the cations. This experiment, moreover, suggests that $CuCl_2$ binding to the bases is not produced through a substitution reaction with liberation of chloride ions since in this case chlorides, compared to other anions, would cause a much greater decrease of Cu(II) binding to DNA bases through a common-ion effect.

At any concentration of monovalent ion and at any [Cu(II)]/[P] ratio the amount of Cu(II) bound to the bases is lower in the presence of Li(I) compared to Na(I). This is in agreement with the EPR results mentioned above. The measurements performed under similar conditions on DNA solutions containing both Mn(II) and Cu(II) gave the results reported in Fig. 5. Even very low [Mn(II)]/[P] ratios strongly reduce Cu(II) binding to the bases of DNA. This is not due to a partial occupation by Mn(II) of available sites for Cu(II) binding of the bases since difference spectra of Mn(II)–DNA solutions show no absorption at 280 nm. It is very likely, therefore, that Mn(II) interferes with Cu(II) binding on the bases through the stabilisation of the double helix by binding to the phosphates.

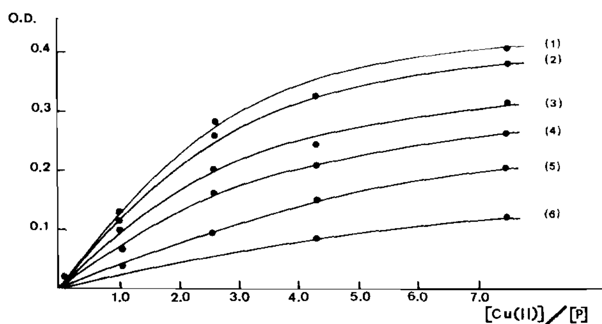

Fig. 5. OD at 280 nm in difference spectra of Cu(II), Mn(II)–DNA solutions containing 0.002 *M* LiCl *vs.* [Cu(II)]/[P]. Curves 1–6 correspond in the order to the following (Mn(II)]/[P] ratios: 0, 0.11, 0.28, 1.09, 4.31, 7.51.

Conclusions

Altogether the results of this investigation clearly show that Cu(II) binding to the bases of DNA is influenced by the nature of mono- and divalent cations present, both in solution and in fibres. They all cause a reduction of Cu(II) binding to the bases which is a function of their concentration. The effectiveness of the inhibition varies from ion to ion and for those examined in this paper follows the trend Na(I) $<$ Li(I) $\ll$ Mn(II). Since the double helix stabilisation by these ions follows the same order, we can conclude that the possibility of Cu(II) complexation to DNA bases exists only when the double helix can be deformed, at least locally.

The pronounced inhibition by Mn(II) of Cu(II) complexation on the bases is also realised through a mechanism of this type and involves the interaction of the divalent ion with the phosphates of the backbone of the polynucleotide chain.

The differences observed in the line shape of Mn(II) EPR absorption in Mn(II)–DNA and Cu(II), Mn(II)–DNA indicates that these two divalent ions mutually influence their binding to DNA and this is indicative of the fact that transition metal ion binding to DNA is very sensitive to the nature of accompanying ions.

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