Formation of Complexes of Deoxyribonucleic Acid (DNA) with Copper(II) and Other Bivalent Metal Ions

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ABSTRACT: The complexation of deoxyribonucleic acid (DNA) with Cu(II) and other bivalent metal ions was studied at relatively low molar ratios of the metal ion to phosphoric acid residues (HL) of DNA ($T_{M^{2+}}/T_{HL}$) by potentiometry, ultraviolet, visible, infrared and circular dichroism spectrophotometry, and electron spin resonance spectroscopy. A Cu(II) ion forms a stable complex with DNA in the pH region above 7.3. In the pH region from 4 to 10, various bivalent metal ions can form complexes with DNA, the complexation ability being in the order of Cu(II) > Zn(II) > Co(II) > Ni(II) $\approx$ Mg(II). When $T_{Cu^{2+}}/T_{HL}$ is lower than 0.33, the structure of DNA (B-form) does not change by such complexation. Cu(II) ions are coordinated mainly with the phosphoric acid residues of the same DNA chain. However, this coordination bond is relatively weak.

KEY WORDS DNA / Cu(II)-DNA Complex / Coordination /
Conformation / Polymer-Metal Complex /

The interaction of DNA with metal ions, especially bivalent metal ions, has aroused interest in relation to the functions of nucleic acids, such as DNA replication, transcription of genetic codes, and enzyme reactions with DNA.¹ Thus, a number of studies^{2–18} have been carried out on this interaction, the bivalent metal ions treated being Co(II), Ni(II), Zn(II), Mn(II), Cu(II), and Cd(II). Among these ions, the Cu(II) ion has the strongest complexation ability with DNA, and is mainly coordinated with the base of DNA.^{2–15} However, the experiments have so far concerned molar ratios of total Cu(II) ions ($T_{Cu^{2+}}$) to monomeric units of DNA (T_{HL}), $T_{Cu^{2+}}/T_{HL}$, larger than 0.5. Under such conditions, the metal ions were in excess compared to ligands, so that a uniform coordination was difficult to be realized.

DNA has two kinds of coordination sites, phosphoric acid residues and bases, to bivalent

metal ions. When in excess, the metal ions can coordinate with both coordination sites. In most previous reports, it was reasonably assumed that metal ions coordinate with these two sites. Moreover, under such conditions of $T_{Cu^{2+}}/T_{HL}$ larger than 0.5, it is possible that the higher ordered structure of DNA is destroyed. Thus, it is important to discuss each of the two coordination sites separately at relatively low $T_{Cu^{2+}}/T_{HL}$.

In a series of papers,^{19–21} we studied the complex formation and coordination structure of several transition metal ions with polymeric ligands such as poly(vinyl alcohol), poly(α -amino acid)s, and silk proteins. In these reports, we proposed that metal-polymer complexes having a uniform coordination structure may be formed at values of $T_{M^{2+}}/T_{Ligand}$ ratio below 0.5.

The purpose of this paper is to characterize the complex formation of DNA with bivalent

metal ions, especially Cu(II) ion, at relatively low values of $T_{M^{2+}}/T_{HL}$ by potentiometry, ultraviolet, visible, and infrared spectroscopy, circular dichroism, and electron spin resonance.

EXPERIMENTAL

Materials

The DNA sample used was a sodium salt of calf thymus type I DNA purchased from Sigma. The proteins usually contained in commercial DNA were removed by extraction with freshly distilled phenol and reprecipitation from an ethanol solution, both at 4°C. The reagent used, including copper perchlorate and other inorganic chemicals, were of analytical grade and used without further purification. Partially phosphorylated poly(vinyl alcohol) (phos.-PVA) was prepared in the same way as in our previous paper.²² The degree of phosphorylation of phos.-PVA was found to be 30% by pH titration.

Formation of Cu(II)-DNA and Cu(II)-Phos.-PVA Complexes

All solutions were prepared with deionized distilled water. The concentration of DNA was determined from a UV absorption at 260 nm ($\epsilon = 6600$).²³ The pH of the solution was controlled by adding aqueous HCl or KOH. A Cu(II)-DNA complex solution ($\mu = 0.1$; $KClO_4$) was prepared by adding aqueous $Cu(ClO_4)_2$ (2×10^{-3} (mol l⁻¹)) to 10 ml of aqueous DNA [2.19×10^{-3} (mol of phosphoric acid residues (abbreviated as HL) l⁻¹)]. The value of $T_{Cu^{2+}}/T_{HL}$ was varied from 0.083 to 0.33. 5 ml of aqueous $NaClO_4$ (0.8 (mol l⁻¹)) and 0.01 *N* aqueous NaOH were added to make up a solution of 30 ml, and then the pH of the solution was adjusted to a desired value. This mixture was left standing overnight at 25°C in order to complete the complexation reaction. Complexes of DNA with other bivalent metal ions were formed in a similar way, and Cu(II)-phos.-PVA complex

solutions were obtained in the same way as mentioned above.

Aqueous solutions of DNA- and phos.-PVA-Cu(II) complexes were dialyzed against pure water and cast onto polyethylene films. The films obtained were dried for about three days at room temperature and further dried under vacuum for a few days.

Measurements

pH values of the complex solutions were measured within an accuracy of 0.05 by an Orion Research Model 701A digital potentiometer after the solutions had stood for at least 24 h.

Spectrophotometric measurements were carried out at 25°C by a JASCO UVIDEK-505 type spectrophotometer with a 5 cm quartz cell. Circular dichroism (CD) was measured by a JASCO J-40A CD spectrophotometer at room temperature using a 1 mm quartz cell. Infrared (IR) spectra of the complex films were obtained using a JASCO IR-A type spectrophotometer.

Viscosities of the complex solutions were measured by a Ubbelohde viscometer at 25 (± 0.05) °C.

Electron spin resonance (ESR) spectra of Cu(II)-DNA and Cu(II)-phos.-PVA complexes were measured at room temperature by a Varian E-102 type X-band spectrometer with 100 kHz field modulation. The microwave frequency was 9.521 GHz, the microwave power, 5 mW for complex films and 12.5 mW for complex solutions, and the modulation amplitude, 8 G. The samples were placed in a quartz tube of 2 mm in inner diameter.

RESULTS AND DISCUSSION

Complex Formation of DNA with Bivalent Metal Ions

The pH titration curves for aqueous solutions of DNA and DNA-bivalent metal ion complexes are shown in Figure 1, along with that for aqueous $Cu(NO_3)_2$. In this figure, α

Figure 1. Potentiometric titrations of aqueous DNA, $\text{Cu}(\text{NO}_3)_2$ and DNA-bivalent metal ion complexes: $\alpha = [\text{KOH}]$ (or $[\text{HCl}]/[\text{M}^{2+}]$; $T_{\text{M}^{2+}}/T_{\text{HL}} = 0.2$; $\mu = 0.1$ (KClO_4); $[\text{DNA}] = 1.81 \times 10^{-3}$ (unit mol l^{-1}) at room temperature. (a) DNA; (b) Ni(II)-DNA ; (c) Cu(II)-DNA ; (d) Co(II)-DNA ; (e) $\text{Cu}(\text{NO}_3)_2$.

denotes the molar ratio of added KOH or HCl to metal ion, HL the phosphoric acid residue of DNA, and $T_{\text{M}^{2+}}$ and T_{HL} the total molar concentrations of metal ions and HL, respectively. The pH of an aqueous DNA ($\mu = 0.1$; KClO_4) was lowered by the addition of aqueous $\text{Cu}(\text{ClO}_4)_2$, whose pH was the same as that of aqueous DNA. The Cu(II)-DNA complex solution showed the lowest pH titration curve over a wide range of pH among those for mixed solutions of bivalent metal ions and DNA. This indicates that protons are released from HL as a result of complexation of DNA with Cu(II) ions. The degree of proton release estimated from the titration curves obtained showed that the complexation ability of a bivalent metal ion with DNA under our experimental conditions is in the order of $\text{Cu(II)} > \text{Zn(II)} > \text{Co(II)} > \text{Ni(II)} \approx \text{Mg(II)}$. Sigel *et al.*²⁴ studied the complex formation between these bivalent metal ions and adenosine 5'-triphosphate (ATP) under the conditions $\mu = 0.1$ and $T_{\text{M}^{2+}}/T_{\text{HL}} < 1.0$, and found the same results as ours with HL. Since the

complexes formed between bivalent metal ions and ligands with amino groups are generally stabler in the order of $\text{Cu(II)} > \text{Ni(II)} > \text{Co(II)} > \text{Zn(II)}$,²⁵ we see that these metal ions coordinate HL rather than bases of the DNA chain. The coordination number, coordination structure, and equilibrium constants cannot be determined only by these titration curves, since there are two kinds of coordination sites on the DNA chain (HL and bases) and the interaction between a metal ion and DNA is relatively weak. This will be discussed in detail in a later part of this paper.

Figure 2 shows the electronic spectra (500–850 nm) for Cu(II)-DNA complex solutions ($T_{\text{Cu}^{2+}}/T_{\text{HL}} = 0.2$) at different pH. A new broad band appeared at about 600–800 nm when Cu(II) ions were added to aqueous DNA. When the pH of the solution was lower than 6.3, the wavelength at the peak of this broad band (λ_{max}) was 740 nm and independent of pH. With an increase in pH, however, λ_{max} shifted to a shorter wavelength and the absorbance at λ_{max} (D_{max}) increased simultaneously. At pH

Figure 2. Electronic spectra of Cu(II)-DNA complexes in aqueous solutions of different pH: $T_{\text{Cu}^{2+}}/T_{\text{HL}}=0.2$; $\mu=0.1$ (KClO_4); $[\text{DNA}]=1.50 \times 10^{-3}$ (unit mol l^{-1}) at room temperature; cell length = 5 cm. (a) pH = 7.28; (b) pH = 6.82; (c) pH = 6.53; (d) pH = 6.43; (e) pH = 6.30.

Figure 3. pH dependence of D_{690} and λ_{max} for Cu(II)-DNA complexes in aqueous solution: $T_{\text{Cu}^{2+}}/T_{\text{HL}}=0.2$; $\mu=0.1$ (KClO_4); $[\text{DNA}]=1.50 \times 10^{-3}$ (unit mol l^{-1}) at room temperature; cell length = 5 cm. (a) D_{690} ; (b) λ_{max} .

7.3, λ_{max} was observed at about 690 nm. A further increase in pH did not affect λ_{max} and D_{max} .

The pH dependence of λ_{max} and the absorbance at 690 nm (D_{690}) is shown in Figure 3. λ_{max} and D_{690} changed in the pH region from 6.3 to 7.3, and were constant for other pH.

These results indicate that no complex was formed at pH below 6.3 and a stable complex of Cu(II)-DNA was formed at pH above 7.3.

The molecular extinction coefficient (ϵ) for the Cu(II)-DNA complex calculated from the D_{690} value at pH = 8.0 was about 60 ($\text{l mol}^{-1} \text{cm}^{-1}$). This ϵ value and λ_{max} allow the

Figure 4. Relationship between absorbance (D_{260} and D_{690}) and $T_{Cu^{2+}}/T_{HL}$ in Cu(II)–DNA complexes in aqueous solution: pH = 8.0; $\mu = 0.1$ (KClO₄); [DNA] = 1.76×10^{-3} (unit mol l⁻¹) at room temperature; cell length = 5 cm for D_{690} and 1 cm for D_{260} . (a) D_{260} ; (b) D_{690} .

absorption band to be assigned to a $d-d$ absorption band of the coordinated Cu(II) ion. In general, λ_{max} for the $d-d$ absorption band of a Cu(II) ion coordinated with nitrogen atoms is located at a wavelength shorter than 650 nm. λ_{max} of a Cu(II) ion coordinated with oxygen atoms of the HL of partially phosphorylated poly(vinyl alcohol) (phos.-PVA) was found between 700 and 800 nm.²² The value of ϵ in the Cu(II)–DNA system was comparable to that of the Cu(II)–phos.-PVA complex system. It was reported that in the Cu(II)–ATP system,²⁴ λ_{max} of the $d-d$ absorption band was about 670 nm. Therefore, it may be concluded that Cu(II) ions are coordinated with the HL of DNA. The result that λ_{max} was observed at a relatively long wavelength suggests that the Cu(II) ion is coordinated tetragonally with four oxygen atoms of the HL of DNA.

Figure 4 shows the absorbance at 260 nm (D_{260}) and D_{690} as a function of $T_{Cu^{2+}}/T_{HL}$ in the Cu(II)–DNA system at pH = 8.0. The value of D_{690} is proportional to $T_{Cu^{2+}}/T_{HL}$, indicating that a quantitative complex formation occurs up to $T_{Cu^{2+}}/T_{HL} = 0.33$. In contrast, the absorbances at the $d-d$ absorption bands of Cu(II) and Ni(II) ions coordinated with fibroin

or other flexible polymeric ligands are usually proportional to $T_{M^{2+}}/T_{Ligand}$ in the region below 0.125, and then level off.^{26,27} This fact was attributed to the steric hindrance of a flexible polymer chain and/or to the decrease in concentration of active coordination sites due to neighboring inactive residues. Since DNA maintains a double helical structure (B-form under our experimental conditions), the effect of the steric hindrance on the complex formation may not be important. Therefore, more Cu(II) ions may have a greater tendency to associate with DNA than the more flexible polymeric ligands. On the other hand, D_{260} was independent of $T_{Cu^{2+}}/T_{HL}$, indicating that the concentration of DNA could be determined from D_{260} even in the presence of Cu(II) ions.

Figure 5 shows the effects of added Mg(II) ions on the Cu(II)–DNA complex formation. The value of D_{690} which reflects the coordination bonding of Cu(II) ions with the HL of DNA decreased gradually with an increase in Mg(II) ion concentration. This suggests that the coordination bonding between Cu(II) ions and the HL of DNA are broken by the addition of Mg(II) ions. In other words, Mg(II)

Figure 5. Effects of added Mg(II) ions on the complex formation of Cu(II) with DNA; $T_{Cu^{2+}}/T_{HL}=0.2$; pH = 8.0; [DNA] = 4.55×10^{-3} (unit mol l $^{-1}$) at room temperature; cell length = 5 cm.

and Cu(II) ions can be competitively coordinated with the HL of DNA at the same time. However, since the strength of coordination bonding between Mg(II) ions and the HL of DNA was much weaker than that between Cu(II) ion and the HL of DNA, much more Mg(II) ions than Cu(II) ions were necessary to exchange metal ions of the complex with DNA. When the concentrations of Mg(II) and Cu(II) ions were comparable, the latter ions were preferentially coordinated with the HL of DNA. Thus, the interaction between Cu(II) ions and the HL of DNA is relatively weak and an electrostatic interaction between the HL of DNA and Cu(II) ions also operates in complex formation of DNA with Cu(II) ions. This is in contrast to the fact that a metal-exchange reaction occurred with difficulty in a system consisting of Cu(II) and Mg(II) ions and polymeric ligands containing amino groups. Therefore, it is apparent that Cu(II) ions interact mainly with the HL of DNA.

For the formation of coordination bonds between the bases of DNA and bivalent metal ions, it seems necessary that hydrogen bonds between the bases and/or base stackings be destroyed and the double helical structure of DNA be deformed. If this is the case, bivalent

metal ions may be advantageous to coordinate preferentially with the HL of native DNA. When the double helical structure of DNA is destroyed, the bases become free, and coordinate preferentially bivalent metal ions, since the equilibrium constant for the coordination between a bivalent metal ion and the base of DNA is larger than that between a bivalent metal ion and the HL of DNA.

Coordination Structure of Cu(II)–DNA Complex

The circular dichroism (CD) spectra of DNA and Cu(II)–DNA complexes are shown in Figure 6. Under our experimental conditions, DNA takes on the B-form structure, as can be seen from a negative peak around 245 nm, a positive peak around 280 nm, and the difference between the molar extinction coefficients for left- and right-handed circular polarized light ($\Delta\epsilon = \epsilon_L - \epsilon_R$; units in l mol $^{-1}$ cm $^{-1}$) at these peaks.²⁸ When Cu(II) ions were added to aqueous DNA, CD spectra essentially remained unchanged. Moreover, the reduced viscosity of aqueous DNA changed only slightly by the addition of Cu(II) ions, in marked contrast to a drastic decrease in reduced viscosity occurring a sys-

tem consisting of flexible polymeric ligands and Cu(II) ions. Thus, the double helical structure, B-form, of DNA undergoes no

substantial changes by complexation with Cu(II) ions under the experimental conditions that $T_{\text{Cu}^{2+}}/T_{\text{HL}} < 0.33$ and $\mu = 0.1$. If a Cu(II) ion is coordinated with four oxygen atoms of more than two HL of different DNA chains, the reduced viscosity may increase because of cross-linking. Therefore, most Cu(II) ions are considered to be coordinated with the HL on the same DNA chain. According to Förster *et al.*,²⁹ the structure of DNA is destroyed by complexation with Cu(II) ions. However, their experiments were done at relatively low ionic strengths, *e.g.*, lower than $\mu = 0.01$, and higher $T_{\text{M}^{2+}}/T_{\text{HL}}$ than in ours. In our work, because the ionic strength was higher, the strength of the coordination bonds was weaker, so that the structure of DNA was not destroyed.

Figure 7 shows infrared absorption spectra of Cu(II)-DNA complex film. Differing from DNA, absorption peaks appear at 1170 (cm^{-1}), 1075 (cm^{-1}), 988 (cm^{-1}), and 900 (cm^{-1}). By comparison with the IR spectra of Cu(II)-phos.-PVA complex film, these peaks may be assigned to $\nu_{\text{P}=\text{O}}$ (1170 cm^{-1}), $\nu_{\text{P}-\text{O}}$ (1075 and 988 cm^{-1}), and chelate ring deflection (900 cm^{-1}), respectively. Therefore, it may be concluded that Cu(II) ions are coordinated with the HL of DNA.

X-Band electron spin resonance spectra

Figure 6. Circular dichroism spectra of DNA and Cu(II)-DNA complexes in aqueous solution: pH=8.0; $\mu=0.1$ (KClO_4); $[\text{DNA}]=1.76 \times 10^{-3}$ (unit mol l^{-1}) at room temperature. (a) $T_{\text{Cu}^{2+}}/T_{\text{HL}}=0.33$; (b) $T_{\text{Cu}^{2+}}/T_{\text{HL}}=0.2$; (c) $T_{\text{Cu}^{2+}}/T_{\text{HL}}=0.14$; (d) DNA.

Figure 7. Infrared spectra of DNA and Cu(II)-DNA complex films: $T_{\text{Cu}^{2+}}/T_{\text{HL}}=0.2$. (a) Cu(II)-DNA; (b) DNA.

Figure 8. X-Band ESR spectra of Cu(II)-DNA and Cu(II)-phos.-PVA complex films: $T_{\text{Cu}^{2+}}/T_{\text{HL}}=0.1$ at room temperature. (a) Cu(II)-DNA; (b) Cu(II)-phos.-PVA.

(ESR) of Cu(II)-DNA and Cu(II)-phos.-PVA complex films are shown in Figure 8. The apparent g values ($g_{\perp}$) of Cu(II)-DNA and Cu(II)-phos.-PVA complexes were 2.08 and 2.09, respectively. The apparent g value of the Cu(II)-DNA complex solution, $T_{\text{Cu}^{2+}}/T_{\text{HL}}=0.10$, $\mu=0.1$ (NaClO_4), $\text{pH}=2.8$, where no complex was formed, was comparable to the value at $\text{pH}=8.0$ (other experimental conditions were the same), in which the formation of Cu(II)-DNA complex may be complete. From this result, it may be concluded that the coordination bond of Cu(II)-DNA is very weak. When Cu(II) ions are coordinated with imidazolyl groups, the $g_{\perp}$ value is smaller than 2.06.³⁰ Similarly, if Cu(II) ions are coordinated with the bases of DNA, a $g_{\perp}$ value smaller than 2.06 may be obtained. Thus, Cu(II) ions are considered to be coordinated with the HL of DNA. The hyperfine structure in the $g_{\perp}$ region was not apparent and a $g_{\parallel}$ value higher than the $g_{\perp}$ value was obtained. These results suggest that the tetragonal coordination structure of the Cu(II)-DNA complex is formed under the present experimental conditions. The ESR

spectrum of a Cu(II)-phos.-PVA complex was very similar to that of the Cu(II)-DNA complex. These findings indicate that Cu(II) ions are coordinated relatively weakly with the HL of DNA.

Many different results have been reported in regard to the complex formation of bivalent metal ions with DNA.²⁻¹⁸ Differences in the species of bivalent metal ions, the source and structure of DNA, and the experimental conditions such as pH , $T_{\text{M}^{2+}}/T_{\text{HL}}$ and ionic strength may account for these difference.

CONCLUSION

In this study, the coexistence of two coordination structures and the conformational change of DNA were eliminated by choosing the experimental conditions as $T_{\text{M}^{2+}}/T_{\text{HL}} < 0.33$ and $\mu=0.1$. The following results were obtained: (1) Bivalent metal ions are coordinated preferentially with four oxygen atoms of more than two HL of the same DNA chain instead of the bases of DNA, probably in a tetragonal arrangement. (2) The coordina-

tion force is relatively weak. (3) The structure of DNA (B-form) does not change on complexation. (4) Complexation of bivalent metal ions with DNA is enhanced in the order of Cu(II) > Zn(II) > Co(II) > Ni(II) $\simeq$ Mg(II).

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