Interaction of DNA and Cobalt(II) Chloride in the Presence of Heteroaromatic Compounds

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ABSTRACT

The effect of cobalt (II) chloride ($CoCl_2$) on DNA helix conformation was investigated by circular dichroism (CD) measurements in the presence of 4-aminopyridine (4AP), 4-aminoquinoline (4AQ) and 9-aminoacridine (9AA). CD spectra for DNA in the presence of 4AQ-$CoCl_2$ and 9AA-$CoCl_2$ mixtures were different from those of DNA with each of $CoCl_2$, 4AQ and 9AA. The spectrum of DNA and 4AP-$CoCl_2$ mixture was the same as that of DNA-$CoCl_2$. The different effects on CD may be due to a reaction between DNA and Co(II)-4AQ or Co(II)-9AA complexes.

1. INTRODUCTION

The genetic activity of cobalt compounds has been studied on salts of cobalt(II). Cobalt(II) chloride ($CoCl_2$) was inactive in a *Bacillus subtilis* rec assay (Kanematsu *et al.* 1980; Nishioka 1975) and in a *Salmonella typhimurium* mutation test (Hollstein *et al.* 1979), but active in an *Escherichia coli* DNA repair test (Warren *et al.* 1981). It was also reported that Co(II) cation exhibited the activity of DNA binding (Eichhorn and Shin 1968) and reduced the fidelity of DNA synthesis (Sirover and Loeb 1976) in an in vitro system. Only a few information concerning the mode of action of $CoCl_2$ on DNA was given in such reports.

In a previous paper, we found for the first time that $CoCl_2$ was mutagenic in the Salmonella test system when combined with heteroaromatic compounds such as 4-aminoquinoline (4AQ), 9-aminoacridine (9AA) and harman (Ogawa *et al.* 1986). It has also been reported that the combined mutagenic activity is presumably the result of an equimolar complex forming between Co(II) cation and heteroaromatic bases (Ogawa *et al.* 1986, 1987, 1988). Of these, the combined mutagenicity for mixtures of $CoCl_2$ and heteroaromatic compounds showed an inverse correlation with the strength of their coordinate bonds judging from the spectral data. These results indicate that the heteroaromatic bases may work as "carriers" of Co(II) ion in the membrane transport, and whether Co(II)-heteroaromatic base complexes themselves or Co(II) cation released from the complexes within the cell may exert

a mutagenic effect. Therefore, we decided to investigate the effect of Co(II)-heteroaromatic base complexes to DNA in vitro. For this purpose, we characterized the spectral properties for mixtures of DNA and $CoCl_2$ in the presence of heteroaromatic compounds by means of circular dichroism (CD) measurements.

2. MATERIALS AND METHODS

Calf thymus type I DNA (Sigma Chemical Company, St. Louis, MO, U. S. A.) was used in this study. The DNA was treated twice with phenol and then precipitated with ethanol. Concentrations of DNA are expressed as the molar basis of DNA-phosphate (DNA-P).

Chemicals used were reagents of the highest grade purchased from Wako Pure Chemicals Industries Ltd. (Osaka) or Nacalai Tesque Inc. (Kyoto). The chemicals were made at 1 mM $NaClO_4$ (pH 5.5−5.6) in unbuffered systems before use.

CD spectra were recorded in a spectropolarimeter (J-500A, Japan Spectroscopic Co., Ltd., Tokyo) in 1 mM $NaClO_4$ at room temperature.

3. RESULTS AND DISCUSSION

The CD measurements show strong evidence for binding effects and conformational changes in the double helical structure of DNA. As seen in Fig. 1, the CD spectrum of calf thymus DNA (7.6×10^{-5} M DNA-P) has a positive maximum at 278 nm and a negative maximum at 245 nm in 1 mM $NaClO_4$ solvent system (pH 5.5 − 5.6). $CoCl_2$ and heteroaromatic compounds gave no CD band (data not shown). The spectrum for an equimolar mixture of 7.6×10^{-5} M each of DNA and $CoCl_2$ decreased the intensity of the positive CD band around 281 nm (Fig. 1A). This may be due to the interaction of the Co(II) ion with the sites of the bases and/or the phosphate residue in DNA. On the other hand, in the spectra for mixtures of the concentrated ratio of 1 4AQ or 0.1 9AA per DNA-P (7.6×10^{-5} M) in 1 mM $NaClO_4$, the intensity of the CD bands changed and increased around 275 nm for the DNA-4AQ mixture and around 268 nm for the DNA-9AA mixture (Fig. 1A). These results indicate that 4AQ and 9AA intercalate into DNA, supporting that their optical activities are increased on CD measurements; 9AA has already been known one of DNA intercalating agents.

Next, we performed the CD measurements for an equimolar mixture of DNA and $CoCl_2$ in the presence of heteroaromatic compounds. To a solution of $CoCl_2$ was first added 4AP (4-aminopyridine), 4AQ or 9AA and then DNA was added 30 min later. In Fig. 1B, the CD spectra for the ternary mixture of DNA, $CoCl_2$ and 9AA were shown. The spectra were different from those of each DNA-$CoCl_2$ and DNA-9AA mixtures. Similar phenomenon was also observed when 9AA was replaced by 4AQ (Fig. 1C). These results suggest that those mixtures showed the different interaction to DNA judging from the spectral data for DNA with each of $CoCl_2$, 4AQ and 9AA. It may be due to synergism of intercalating and binding to DNA. In the spectra of DNA-4AP and DNA-$CoCl_2$-4AP mixtures, the CD bands exhibited the same as that for DNA alone or DNA-$CoCl_2$, indicating that the 4AP can not be affected for DNA (Fig. 1A and 1D).

We have shown previously that the combined mutagenicity of $CoCl_2$ and heteroaromatic compounds in the Salmonella test system is due to the formation of moderate to weak complexes such as Co(II)-4AQ and Co(II)-9AA and that the combined mutagenicity may be due to a reaction of DNA and Co(II)-heteroaromatic base complexes judging from the spectral data (Ogawa et al. 1986). While Co(II)-4AP formed a very stable complex, resulting in it to be non-mutagenic. The CD spectral data for the ternary mixtures of DNA, $CoCl_2$ and 4AQ or 9AA showed apparent differences from those of DNA with each of $CoCl_2$, 4AQ or 9AA. These observations indicate that the CD spectral change in the mixtures of DNA and $CoCl_2$ in the presence of heteroaromatic compounds correlate with the combined mutagenicity

for mixutre of $CoCl_2$ and 4AQ or 9AA in the Salmonella test system. The CD technique may therefore be available for the screening of metal compounds and complexes for their suspected mutagenicity or carcinogenicity. Further studies will be necessary to search the mode of action of metals on DNA.

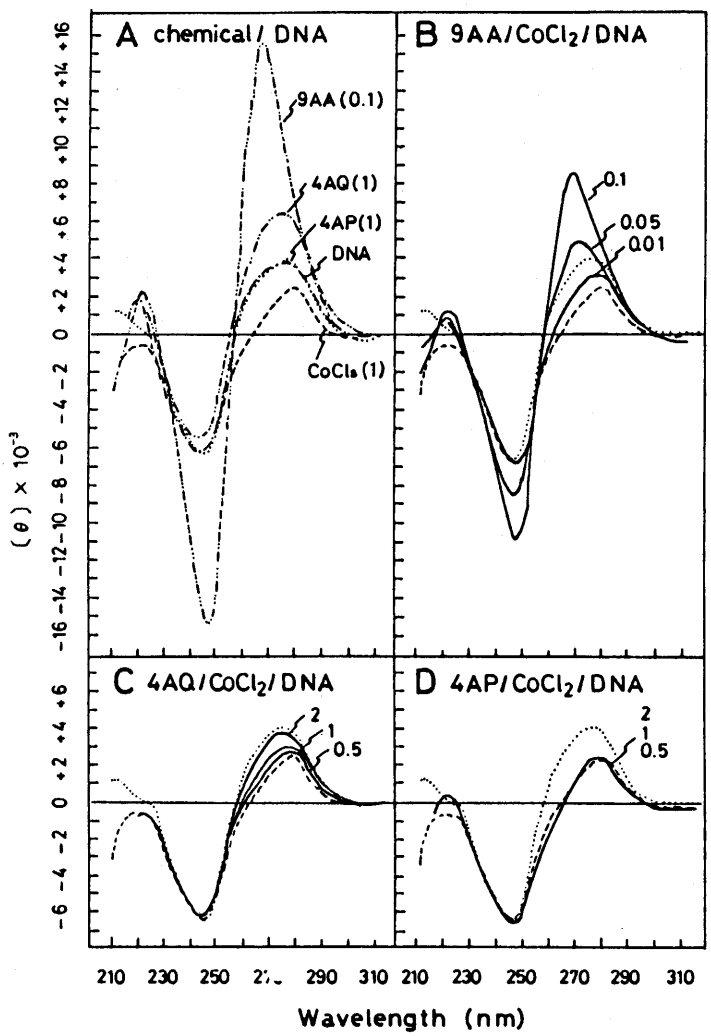

Fig. 1. CD spectra of DNA with each of $CoCl_2$, 4AP, 4AQ and 9AA at 1 mM $NaClO_4$ (A). The spectra for an equimolar mixture of DNA and $CoCl_2$ in the presence of 9AA (B), 4AQ (C) or 4AP (D). Numbers indicate ratio of chemicals to DNA-P (7.6×10^{-5}M).

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