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Synthesis and DNA Cleaving Activity of Copper-complex of Macrocyclic Compound Containing 1, 10-Phenanthroline

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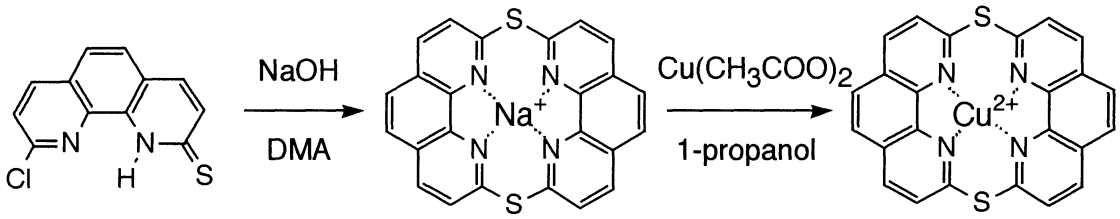
Copper-complexes of 1, 14: 7, 8- diethenotetrapyrido- [2, 1, 6-de: 2', 1', 6'-gh: 2", 1", 6"-kl: 2"', 1"', 6"'-na] [1, 5, 8, 12] tetraaza- [3, 10] dithiocyclotetradecine (SMC) consisting of two 1, 10-phenanthroline molecules bridged by sulfur, was synthesized. Interaction of Cu-SMC with DNA was examined by spectroscopic methods. Cu-SMC binds with DNA and cleaves DNA in the presence of a reducing agent under an aerobic condition.

Recently, there has been considerable interest in the specific DNA binding and cleavage activity mediated by metal complexes of 1, 10-phenanthrolines.[1-5] Sigman and coworkers reported that the copper complex of 1,10-phenanthroline, $Cu(phen)_2$, noncovalently binds to DNA and induces cleavage of DNA in the presence of a reducing agent under an aerobic condition.[2-5] We have been interested in the metal complexes of macrocyclic compound containing 1, 10-phenanthroline moiety, which are expected to have different properties and different DNA binding and cleaving activity from the metal-phenanthroline complexes, because the macrocyclic complex is imposed to have a plane structure.

Previously, we have reported the synthesis of several macrocyclic compounds containing two 1, 10-phenanthroline molecules and investigated properties of their metal complexes.[6,7] These metal complexes, however, have very poor solubility in water. On the other hand, metal complexes of macrocyclic compound (SMC) consisting of two 1,10-phenanthroline molecules bridged by sulfur has been found to be soluble in water. Here we report the synthesis of the copper complexes (Cu-SMC)of SMC, and their specific DNA binding and cleaving activity.

Cu^{II}-SMC(3) was prepared as follows: 1, 2-dihydro-9-chloro-1, 10-phenanthroline-2-thion (1) was synthesized by thiolation of 2, 9-dichloro-1, 10-phenanthroline with thiourea.[6] A mixture of (1) (0.6 g) and sodium hydroxide (0.1 g) in N, N-dimethylacetamide (30 ml) was refluxed at 166 °C for 2.5 h for cyclization. After cooling, Na^{I}-complex of SMC (2) was obtained in 71% yield as a yellow solid. The structure of Na^{I}-SMC (2) was characterized by ^1H NMR, mass spectrum and elementary analysis.[8] Metal substitution reaction of (2) (0.1 g) with cupric acetate (0.5 g) in 1-propanol (20 ml) at 97 °C for 2.5 h gave cupric complex of SMC(3) in 40% yield as a brown solid. The structure of Cu^{II}-SMC (3) was determined by the mass spectrum

and the elementary analysis.[9] The mass spectrum in the FAB mode, m/e=483 indicates that the compound is composed of SMC and copper. The elemental analysis also agrees with the structure. The IR spectrum of Cu-SMC (3) is similar to that of Na-SMC (2).



Scheme 1. Synthesis of Cu-SMC.

Reduction of Cu^{II}-SMC with ascorbic acid gave Cu^{I}-SMC, as recognized by UV spectra. Extinction coefficient of Cu^{II}-SMC at λ_{max} was $\varepsilon_{213\ nm}$=6093 and $\varepsilon_{289\ nm}$=2962, and of Cu^{I}-SMC, $\varepsilon_{349\ nm}$=3020 and $\varepsilon_{432\ nm}$=2481.

The binding activity of the Cu-complexes to calf thymus DNA was examined by means of UV absorption and circular dicroism spectrophotometric titration. Figure 1 shows the CD spectral titration of Cu^{I}-SMC with DNA. Neither Cu^{I}-SMC, nor DNA alone showed CD band in the region of 300-500 nm, while the

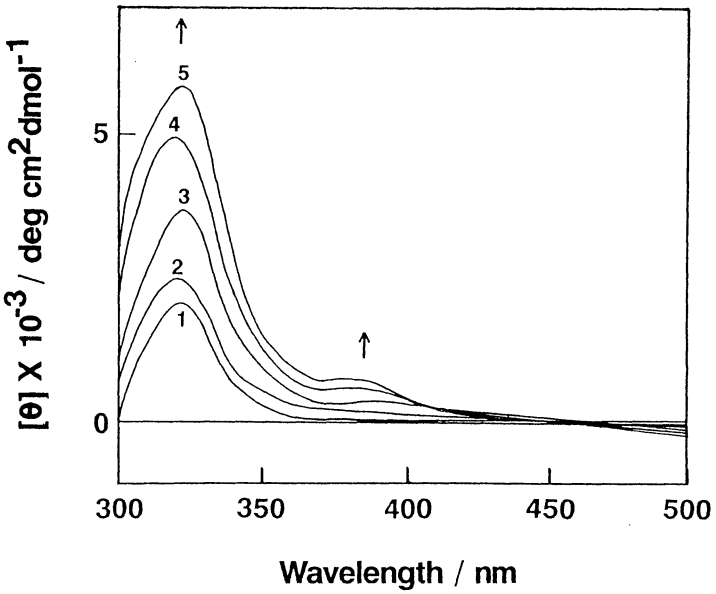


Fig. 1. Circular dicromism spectra of Cu^{I}-SMC (0.2 mM(=mol dm^{-3})) in 0.1 M Tris-acetate buffer pH 7.2 in the presence of calf thymus DNA: 1,1 mM; 2, 2 mM; 3, 3 mM; 4, 4 mM; 5, 5 mM. Calf thymus DNA concentration was determined spectrophotometrically using the molar absorption coefficient of $\varepsilon_{260\ nm}$= 6.55X10^3 $M^{-1}cm^{-1}$.

addition of DNA to the solution of Cu^{I}-SMC induced the positive CD spectrum in the same region. The result indicates that optical inactive Cu^{I}-SMC binds to double-stranded DNA. The binding constant, K was calculated from the increase of CD band at 320 nm by the method of Scatchard plot.[10-12] The estimated K of Cu^{I}-SMC with DNA is $4.0X10^{4}$, which is consistent with the K value of $4.2X10^{4}$, determined by the absorption spectral titration at 330 nm. The K value of Cu^{II}-SMC with DNA, estimated from the absorption spectral titration, was $1.5X10^{4}$. The mono-cationic Cu^{I}-SMC binds to DNA stronger than the di-cationic Cu^{II}-SMC, but slightly weaker than the corresponding cuprous phenanthroline complex.[13]

We have then investigated DNA cleaving activity of Cu-SMC in the presence of mercaptopropionic acid (MP) and oxygen using pBR322 DNA. The reaction mixtures (6 μl) containing Cu-SMC (0.2-0.05 mM), MP(5 mM) and DNA pBR322 (0.3 μg) were mixed in 0.1M Tris-borate pH 7.2 buffer, and incubated for 1h at 37 °C. The reaction was quenched by adding EDTA (1 mM) to the mixture. Cleaving pattern of pBR322 DNA by the Cu-SMC, analyzed by agarose gel electrophoresis after staining with ethidium bromide as shown in Fig. 2. The closed circular DNA (form I) is cleaved to the nicked (form II) and the linear (form III) DNA by the Cu-SMC in the concentration of 0.02-0.2 mM. The ratio of form II and form III DNA increased with increasing the concentration of Cu-SMC (lane 5-7). In lane 4, DNA was degraded to small pieces in the presence of 0.2 mM of Cu-SMC (lane 4). No significant cleavage was observed in the absence of mercaptopropionic acid, or when cupric acetate was used instead of Cu-SMC. In comparison with $Cu(phen)_2$, DNA binding and cleaving activity of Cu-SMC was found to be slightly lower under the same condition.

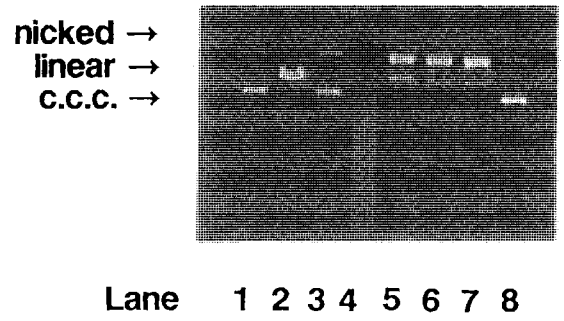


Fig. 2. Strand scission of pBR322 DNA by Cu-SMC. The detail is described in the text. Lane assignments: 1, DNA control, c.c.c. pBR322; 2, DNA control, linear pBR322; 3, Cu-SMC 0.2 mM; 4 to 7, Cu-SMC 0.2, 0.15, 0.1 and 0.05 mM, respectively + MP 5 mM; 8, MP 5 mM. Agarose gel (0.9 %) was prepared in a buffer (pH 7.2) composed of Tris-borate (0.1 M), EDTA (0.002 M). The electrophoresis was carried out at 100 V for 1 h with bromphenol blue as a marker. The gel patterns were developed by soaking the gels in ethidium bromide (1 mg/1 ml). After dying, the gel was irradiated UV (short wave), then the pictures were taken.

The result likely reflects the unique geometry of Cu-SMC. $Cu^{I}(phe)_2$ forms tetrahedral structure, in which two phenanthroline molecules coordinate to Cu^{I} ion orthogonal each other.[13] On the other hand, Cu^{I}-SMC can not form tetrahedral structure because two 1,10-phenanthroline molecules are imposed to a plane structure by sulfur bridging. Thus, Cu^{I}-SMC binds weaker to DNA from the minor groove than $Cu^{I}(phen)_2$, if one phenanthroline moiety of Cu^{I}-SMC intercalates to DNA, since $Cu^{I}(phen)_2$ tends to bind DNA by intercalation without steric restriction as suggested by James M. Veal and coworkers.[13]

Further work is now under progress to shed more light on the structure of the metal complex of SMC and its reaction.

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8) For (II), mp 372 °C (d.p.); MS(FAB) m/e=443 (Na-SMC); IR(KBr)(cm^{-1}):1584, 1440, 1119; ^{1}HNMR(CF_3COOD-$(CD_3)_2SO$, TMS): δ 8.49(s,1H), 9.27(s,1H), 9.32(s,1H) ppm; Anal. Found: C, 59.62; H, 3.10; N, 12.04%. Calcd for $C_{24}H_{12}N_4S_2NaCl$: C, 59.99; H, 2.82; N, 11.70%.
9) For (III), mp 385 °C (d.p.); MS(FAB) m/e=483 (Cu-SMC); IR(KBr)(cm^{-1}):1569, 1473, 1130, 1028; Anal. Found: C, 56.17; H, 3.14; N, 9.96%. Calcd for $C_{24}H_{12}N_4S_2Cu(C_2H_3O_2)_2$: C, 55.86; H, 2.99; N, 9.81%
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