Synthesis of a New Ruthenium Complex of a Macrocyclic Ligand Containing 1,10-Phenanthroline, and Its Photo-Mediated Covalent Binding to DNA

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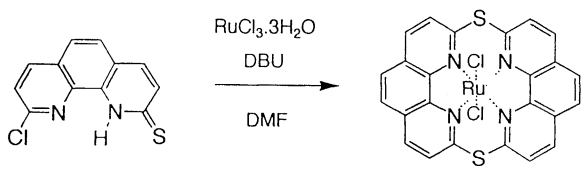
(Received September 2, 1996)

A ruthenium complex of a macrocyclic compound, consisting of two 1,10-phenanthroline molecules bridged by sulfur, was synthesized by template-directed macrocyclization on the ruthenium ion. The complex links to DNA by photo-mediated substitution of the coordinated chloro ion with the heterocyclic base of DNA and induces DNA cleavage.

There has been considerable interest in the specific DNA binding and cleaving activity of the metal complexes of phenanthrolione derivatives.[1,2] Sigman and coworkers reported that the copper complexes of 1,10-phenanthroline bind to DNA non-covalently and cleave DNA in the presence of a reducing agent under an aerobic condition.[1,3,4] Barton and coworkers have shown that ruthenium complexes of 1,10-phenanthroline derivatives recognize the different forms of DNA and induce cleavage of DNA upon irradiation of UV light.[2,5,6] The structure of each complex and the kind of metal contained likely determine the mode of interaction of the metal complex with DNA of a particular conformation.[1-8] Despite extensive studies, the structural features of the binding of DNA with the metal complexes have remained unclarified. To gain further information on the binding and cleavage of DNA with metal complexes, and to explore a new DNA binding and cleaving molecule, we undertook synthesis of new metal complexes of a macrocyclic ligand containing 1,10-phenanthroline.

Previously we have reported the preparation of copper complexes of a macrocyclic compound consisting of two 1,10-phenanthroline molecules bridged by sulfur, smc.[9] The copper complex, Cu(smc), the ligand of which has a planar structure, possesses different properties as well as different binding ability to DNA from those of the copper-phenanthroline complex which has a tetrahedral structure.[10] Here we report the synthesis of a ruthenium complex of the macrocyclic compound, Ru(smc)Cl_2, and its photo-mediated covalent binding to DNA. The DNA cleaving activity of Ru(smc)Cl_2 is also reported.

The complex, Ru(smc)Cl_2, was prepared by ruthenium ion template-directed macrocyclization. Thus, a mixture of 9-chloro-1,10-phenanthroline-2(1H)-thion (**1**) (100 mg), 1,8-diaza-bicyclo-[5.4.0]undec-7-ene (0.2 ml) and $RuCl_3 \cdot 3H_2O$ (100 mg) in 10 ml of dimethylacetamide was stirred for 1 h at 80 °C. After cooling, the resulting black precipitates were collected by filtration, and washed with water, methanol and ether to give Ru(smc)Cl_2 in 71 % yield. Mass spectrum in the FAB mode and elemental analytical data of the ruthenium complex agree with the structure of Ru(smc)Cl_2.[11] The IR spectrum of Ru(smc)Cl_2 is similar to that of a sodium or copper complex of smc.[10]



Scheme 1. Synthesis of Ru(smc)Cl_2

The UV spectrum of the complex in 0.1 M Tris buffer (pH 7.2) converted gradually from λmax at 263 nm (ε=3225) and 356 nm (ε=2065) to λmax at 265 nm (ε=2675) and 343 nm (ε=1016) on standing at room temperature, probably because of ligand substitution reaction of the coordinated chloro ion with water. The substitution reaction was accelerated greatly by irradiation of UV light of >310 nm.[12] The complex showed strong fluorescence at 550 nm (Ex=275 nm) and the fluorescence intensity decreased by addition of DNA.

The interaction of Ru(smc)Cl_2 with calf thymus DNA was investigated by UV absorption and fluorescence spectrophotometric titration. Figure 1 shows the spectrophotometric titration of Ru(smc)Cl_2 with DNA before and after irradiation of UV light of >310 nm. Irradiation of UV light enhanced the absorbance as shown in Figure 1b. The binding constant, K was estimated from the change of absorbance at 350 nm by the method of Scatchard plot.[5,13] The estimated K of the Ru-smc complex with DNA was 2.5 x10^4 and 5.2 x10^4, before and after UV irradiation, respectively. The K values obtained from the spectrophotomeric method showed fair agreement with those estimated from the fluorescence titration method, 1.7 x10^4 and 3.8 x10^4, before and after UV irradiation, respectively. The interaction of the ruthenium-smc complex with DNA is several times stronger than that of the ruthenium-phenanthroline complex, Ru(phen)$_3$.[5]

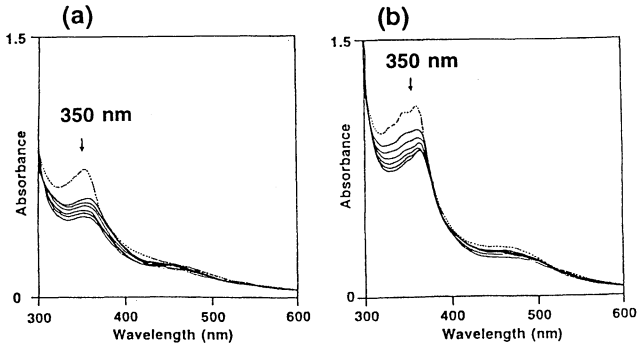


Figure 1. Spectrophotometric titration of Ru(smc)Cl_2 with DNA. Ru(smc)Cl_2 concentration is 0.2 mM. The DNA concentration ranges from 0 at the top dotted curve to 5 mM at the bottom curve. (a) The sample solution was kept for 2 h at dark for equilibration and (b) at the irradiation of UV light (>310 nm) for 72 h.

The DNA binding and cleaving activity of the Ru(smc)Cl_2 was assessed by conversion of a closed circular form of pBR322 DNA to a relaxed form or to a nicked and linear form

of DNA, which was analyzed by agarose gel electrophoresis after staining with ethidium bromide. The reaction mixture (10 μl) containing Ru(smc)Cl_2 (0.2 mM) and pBR322DNA (0.3 μg) in 0.1 M Tris-borate (pH 7.2) was incubated for 1- 6 h at 25 °C with irradiation of a 12-W mercury UV lamp using a short-wave cut filter below 310 nm. The light intensity was 200 $\mu W/cm^2$ as measured by an UV radiometer. The reaction without irradiation was also carried out under the same condition as a control. Essentially no cleavage of DNA was observed by irradiation of UV of >310 nm in the absence of the complex (lanes 10, 11). Lanes 2, 3, and 4 show that the electrophoretic mobility of the closed circular DNA was retarded gradually with increasing reaction time with Ru(smc)Cl_2. Irradiation of the sample solution greatly accelerated the mobility shift as shown in lanes 5, 6, and 7. The mobility shift induced by the reaction with the complex, especially under the irradiation, suggests an intercalative binding or a covalent binding of the ruthenium-smc complex with DNA and conversion of the closed circular form DNA to the relaxed form.[14-16] Initial non-covalent binding is probable. However, spectroscopic and mobility-shift changes caused by the irradiation correlate to the covalent binding with coordinate bond between the ruthenium complex and heterocyclic bases of DNA by photo-activated ligand substitution.[12] Ruthenium complexes have high affinity for the N7 guanine of DNA,[17] which is accessible to the major groove. To check the covalent binding with the coordinate bond , the mixture of the photo-reaction product obtained as lane 7 was treated with NaCN for 48 h. The CN^- ion, which has strong affinity for the ruthenium(II), replaced bases of DNA for the coordination and liberated the closed circular and nicked form DNA as shown in lane 9. The formation of the nicked form DNA by NaCN treatment indicates that the Ru complex has DNA cleaving activity under the irradiation and cross-links the nicked DNA by bifunctional coordination. The covalent binding is also confirmed by extensive dialysis of the photo-reaction mixture of the ruthenium complex with DNA. The dialysis study showed that the ratio of the bound ruthenium to nucleotide residue of DNA was 0.072 which is larger than that in the case of *cis*-Ru(phen)$_2Cl_2$.[14]

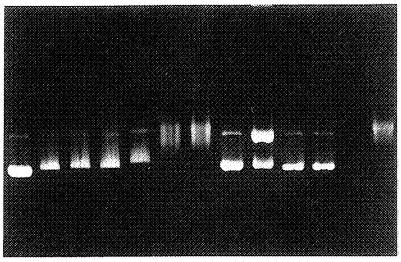


Figure 2. Covalent binding and strand scission of pBR322 plasmid DNA with Ru(smc)Cl_2. Agarose gel electrophoresis of the plasmid DNA. Lane 1, control DNA (c. c. form); lane 2-4, after reaction with 0.2 mM of Ru(smc)Cl_2 in the dark for 1, 3 and 6 h, respectively; lane 5-7, after reaction with 0.2 mM of Ru(smc)Cl_2 with irradiation of UV light (>310 nm) for 1, 3 and 5 h, respectively; lane 8, after irradiation with 0.2 mM of Ru(smc)Cl_2 in the presence of 10 mM NaCN for 6 h; lane 9, after reaction in the same manner as lane 7 followed by treatment with 10 mM NaCN for 48 h at dark; lane 10 and 11, after irradiation without Ru(smc)Cl_2 for 3 and 6 h, respectively; lane 12, after reaction in the same manner as lane 9 but without NaCN treatment. Reactions were performed at 25 °C with 0.3 μg plasmid DNA in the assay buffer.

In conclusion, Ru(smc)Cl_2 binds to DNA covalently, especially by photo-activation with stronger binding activity than the corresponding ruthenium-phenanthroline complex. The ligand smc has a planar structure and only the *trans*-dichlroro ruthenium complex is feasible for Ru(smc)Cl_2. On the other hand, the ruthenium-phenanthroline complex possesses only *cis* structure. The planar smc and the *trans* coordinated chloro ion in the ruthenium complex differentiate the binding mode to DNA from the phenanthroline complex and may enhance the ligand substitution with the base part of DNA. Further studies of the structure of the ruthenium complex and its photo-activated reaction with DNA are in progress.

References and Notes

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11 [Ru(smc)Cl2]: m.p, 395 °C (decompose); MS (FAB) m/e, 521; IR(KBr) (cm^{-1}), 1558, 1474, 1332 and 1134; Anal. Found:C, 48.67; H, 1.58; N, 9.22 %. Calcd for C24H12N4S2RuCl2: C, 48.65; H, 2.02; N, 9.46 %.
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