T-Site Selective Photocleavage of DNA by Cationic Schiff Base Complex of Manganese(III)

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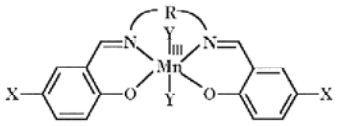
Manganese(III)-Schiff base complexes of cationic salen and salph derivatives were newly prepared and their molecular structures were determined by X-ray crystal structure analysis. The cationic salph complex effectively cleaved T-site of DNA in ca. 88% selectivity upon visible light irradiation.

Of many transition metal reagents which can cleave DNA/RNA in non-enzymatic fashion, a photocleaving regent is of great interest in the development of both new chemical tools and new phototherapeutic agents.[1] The typical examples are Ru(III)-diimines,[2] Co(III)-diimines,[3] Co(III)-BLM systems,[1] Co(II)-peptide,[4] V(V)-diimines,[1] Rh(III)-diimines,[1,5] Re(I)-diimines,[6] and Pt(IV)-POP,[1] the most of which contains diimine ligands and redox-active metal. Thus, it is conceivable that other redox active transition-metal complexes with diimine ligand are likely to be effective as well. Mn(III)-Schiff base complexes are known to be photoredox active,[7,8] so, in this paper we investigated the photochemical DNA scission by Mn(III)-Schiff base complexes **1-4** in Scheme 1.



	R	X	Y
1	$-C_2H_4-$	-H	OH_2
2	$-C_6H_4-$	-H	OH_2
3	$-C_2H_4-$	$-CH_2\overset{+}{N}(CH_3)_3$	OH_2
4	$-C_6H_4-$	$-CH_2\overset{+}{N}(CH_3)_3$	OH_2

Scheme 1. Mn(III)-Schiff base complexes.

Complexes **1** and **2** were prepared by the literature method.[9] Complexes **3** and **4** were newly synthesized in "one pot" by reacting 5-(trimethylammoniomethyl)salicylaldehyde chloride[10] with Mn(III) acetate and diamine at 2 : 1 : 1 molar ratio in water at pH 7, and isolated as perchlorate (**3**) or bromide (**4**) salt.[11]

Slow evaporation of acetone solution (**3**) and methanol solution (**4**) produced crystals suitable for single crystal X-ray structure analysis.[12] The ORTEP drawings of cationic parts are shown in Figure 1. Both complex cations comprise a planar tetradentate Schiff base ligand tightly bound to the Mn(III) center *via* two Mn-N bonds [1.973(8), 1.987(7) Å for **3**; 1.987(10), 1.987(10) Å for **4**] and two Mn-O bonds [1.872(6), 1.893(6) Å for **3**; 1.866(8), 1.866(8) Å for **4**], and by axial water molecules which complete an octahedron around the Mn(III) ion. Longer Mn-O distances to the apical ligands [2.297(6), 2.283(7) Å for **3**; 2.440(8), 2.440(8) Å for **4**] may be partly attributed to the Jahn-Teller effect for the d^4 ion. The *trans*-planar structures of **3** and **4** are very similar to those of **1** and **2**, respectively.[8,13] This fact indicates that the cationic substituent gives little effect on the molecular structure of Mn-Schiff base skeleton. Complex **4** exists as monomer in the crystal, and terminal trimethylammonium groups are *trans*-configuration

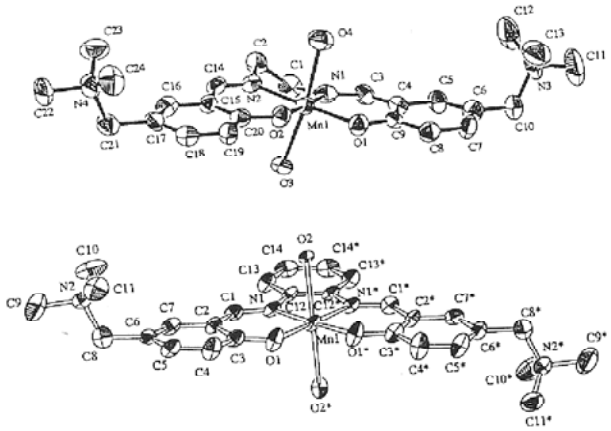


Figure 1. ORTEP drawings of **3(A)** and **4(B)** of cationic parts[10].

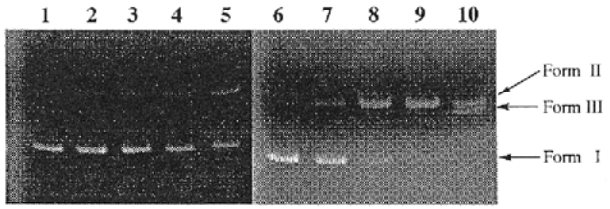


Figure 2. Cleavage of ϕX-174 phage DNA by Schiff base complexes **1-4** upon visible light irradiation (lanes 7, 8, 9 and 10) and under dark (lanes 2, 3, 4 and 5).
lanes 1 and 6: control, lanes 2 and 7: complex 1, lanes 3 and 8 : complex 2, lanes 4 and 9 : complex 3, lanes 5 and 10 : complex 4; DNA(b.p.) = 7 μ M(1 M=1 mol dm^{-3}); complex : 20 μ M; light source : 150 W tungsten halogen lamp (UV-cut filter, SL-39); reaction temperature : 25 °C; reaction time : 24 h.

with each other. Whereas, **3** exists as loosely associated dimer in the crystal, and terminal trimethylammonium groups are *cis* to avoid the steric repulsion between them.

Figure 2 shows the agarose gel electrophoresis pattern of ϕX-174 phage DNA in the presence or absence of **1 - 4** upon visible light irradiation. All of the complexes gave the form II DNA as scission product, and **4** produced the form III as well. On the other hand, under dark no DNA scission was observed for **1 - 3** or it was very slow for **4**. Of course, no DNA scission occurred in the absence of complexes. These facts clearly indicate that the photoreaction of the complexes results in the DNA scission and that **4** is most active.

The time-course of the decrease of form I DNA obeyed the first-order kinetics. So, apparent rate constant, k_{app}(DNA) for the reaction was estimated and listed in Table 1, together with the k_{app}(Complex) for the photolysis of the complexes in the absence of DNA. The table indicates that the increasing order of the

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Table 1. Apparent rate constants for DNA photoscission and complex photolysis at 25 °C under light [a]

Complex	k_{app}/h^{-1} for DNA Photoscission[b]	k_{app}/h^{-1} for Complex Photolysis[c]
1	1.5×10^{-2}	1.7×10^{-2}
2	2.8×10^{-2}	2.2×10^{-2}
3	2.9×10^{-2}	2.9×10^{-2}
4	2.0×10^{-1}	1.7×10^{-1}

[a] 150 W tungsten halogen lamp (UV-cut filter, SL-39), complex = 20 μ M; buffer = Tris-HCl (10 mM, pH 7.4) + NaCl (20 mM). [b] DNA(b.p.) = 7 μ M. [c] spectrophotometric method (λ = 330 nm); the time course of the decrease of **4** followed the 1st order kinetics.

DNA scission rate, **1**<**2**≦**3**<**4**, is parallel with that of the photoreactivity of the complexes. Since the DNA photoscission rate is consistent with the photolysis rate of the complexes in the employed experimental conditions, the rate determining step of the DNA scission can be ascribed to the photolysis process of the complexes. In the photolysis of **2** and **4**, the formation of Mn(II) and transient organic radical have been reported.[8] Similarly, the formation of Mn(II) was observed for the photolysis of **1** and **3**. The Mn(II) species is inactive for the DNA scission, so it is suggested that the DNA is cleaved by the organic radical.

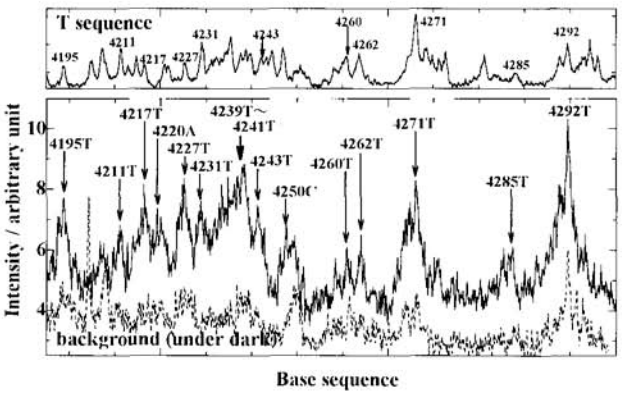

Figure 3. Histgram of DNA photocleavage site by complex **4**. DNA(b.p.) : ca. 7 μ M; complex 4 : ca. 6 μ M; Tris-HCl : 10 mM(pH7.4); NaCl : 20 mM; Reaction temperature : 25 °C; Reaction time : 22 h; Light source : 150 W tungsten lamp with UV-cut filter.

Figure 3 shows the histogram for photocleavage site of DNA by **4**. In the experiment, we used ^{32}P labeled pBR322 plasmid DNA, which was prepared by ^{32}P labeled pBR322 primer B1 (= (d)TGGAGCCACTAT). The labeled primer was prepared by 5'-terminal labeling method with [γ -^{32}P]ATP and T4 poly-nucleotide kinase. The labeled DNA was incubated under the conditions in Figure 3 with or without complex. After heat denaturation, the DNA was resolved by electrophoresis on a 6% polyacrylamide gel with dideoxy sequencing A, C, G and T. The radioactivity was measured by Bio Image Analyzer "BAS2000" made by Fujifilm Co., Ltd. The analyzed region contains 102 bases constituting of 26A, 20C, 20G and 36T. As is seen in Figure 3, the scission site is highly limited to T-site, and the base-site selectivity of **4** reaches to ca. 88% for T. In the case of **3**, however, no site selectivity was observed. Contrast to these, in the DNA scission using potassium peroxodisulfate as terminal oxidant, the site selectivity was about 70% (GC) for **4** and 65%(AT) for **3**. The similar AT-site selectivity to **3** has been reported for **1** by Griffin *et al.*[14] These facts clearly indicate that the site selectivity largely depends on both the *N, N'*-bridging group of employed complexes and scission method. The latter should come from the difference of active species which attacks DNA, because Mn(IV)=O is presumed as the active species in the scission with terminal oxidant, however, organic radical is presumed in the photocleavage treated here. Therefore, the high T-site selectivity for **4** suggests that cationic organic radical formed by photolysis of **4** selectively attacks thymine or thymidine rather than that **4** favors T-site. A study for the reaction mechanism is under way.

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11 Found for **3** : C, 34.69; H, 4.79; N, 6.76; Mn, 6.53%. Calcd for $C_{24}H_{40}N_4O_{17}Cl_3Mn$: C, 35.25; H, 4.93; N, 6.85; Mn, 6.72%. Found for **4** : C, 41.05; H, 4.99; N, 6.94; Mn, 6.55%. Calcd. for $C_{28}H_{42}N_4O_6Br_3Mn$: C, 40.75; H, 5.13; N, 6.79; Mn, 6.66%.

12 Crystal Data for **3**: $C_{24}H_{38}N_4O_{16}Cl_3Mn\cdot H_2O$, M = 817.89, monoclinic, $P2_1/c$, a = 10.427(2), b = 19.169(4), c = 17.872(2) Å, β = 97.01(1)°, V = 3545.4(10) Å^3, Z = 4, Dc = 1.566 gcm^{-3}, Mo-Kα, R = 0.082 and R_w = 0.085 for 8410 unique reflections with $I > 3\sigma(I)$.
Crystal data for **4**: $C_{28}H_{38}N_4O_4Br_3Mn\cdot 2H_2O$, M = 825.32, monoclinic, C2/c, a = 15.28(1), b = 17.546(9), c = 15.72(1) Å, β = 119.11(5)°, V = 3682(4) Å^3, Z = 4, Dc = 1.423 gcm^{-3}, Mo-Kα, R = 0.088 and R_w = 0.087 for 4391 unique reflections with $I > 3\sigma(I)$.

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