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Novel Water-Soluble 4,4-Disubstituted Ruthenium(III)-Salen Complexes in DNA Stranded Scission

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Salen-type Ru(III) complexes are found to be capable of reacting with physiologically acceptable oxidants. The water solubility and DNA affinity of these Ru(III)-salen complexes are enhanced by the utilization of a variety of charged groups through the formation of peptide bonds. In the presence of hydrogen peroxide, modified Ru(III)-salen complexes are capable of nicking DNA. In addition, the reactivity in DNA cleavage increases along with the total number of positive charges retaining in Ru(III)-salen complexes and less influence in the electronic effect. Using ^{32}P-end-labeled oligonucleotides and high resolution polyacrylamide gel electrophoresis, Ru(III)-salen complexes are found to randomly cleave DNA regardless of the DNA secondary conformation such as bulge, inter-loop, or double-stranded regions. The possible reactive species of Ru(III)-salen complexes in DNA cleavage is considered as the hydroxyl radical and high valent oxoruthenium(IV) species according to the UV titration, quenching studies, and reaction with varied oxidants.

INTRODUCTION

Ruthenium complexes have been broadly studied in nucleic acids chemistry by means of photo-induction,[1] electrochemical reaction,[2] or oxidation through high valent oxo species.[3] However, only a few types of ruthenium complexes are found to be capable of reacting with physiologically acceptable oxidants such as molecular oxygen or hydrogen peroxide, and Salen-type ruthenium(III) complexes are among them. The complexes have been studied in the C-H bond activation and oxygen up-take reaction;[4] however, their reaction with nucleic acids remain unknown. Since salen and most of its derivatives are insoluble in water,[5] the water-solubility of certain derivatives is considered an important field of study for application to biological systems. Recently, a modified diamine[6] and alkylated salicylidene[7] have been reported to introduce charged species to salen derivatives resulting in the increase of their water-solubilities. Our strategy is to employ the formation of the amide bond adept from the peptide synthesis to accomplish this goal. By way of the coupling reaction of an active ester with a variety of amine derivatives, various salen derivatives will be synthesized, as indicated in Fig. 1, which possess sufficient solubility in water as well as the functionality. Although an increasing number of salen derivatives have been reported, *few examples approach from the 4,4′-positions of salen* moiety linked by the electron-withdrawing groups[8] to attach to the charged species. In this report, the synthesis and characterization of salen derivatives at the 4,4′-positions and their corresponding ruthenium complexes will be reported. The reactivity of ruthenium(III)-salen complexes in DNA stranded scission in the presence of H_2O_2 will be addressed. The preliminary DNA reaction mechanisms will be discussed as well.

EXPERIMENTAL PROCEDURE

Materials and Methods

All reagents and ACS grade solvents were purchased from commercial sources and were used without further purification unless otherwise noted. 6,6′-dicarboxylate salicylaldehyde and salen derivative, **5**, were prepared according to the literature procedure.[9] ^{1}H NMR spectra were determined

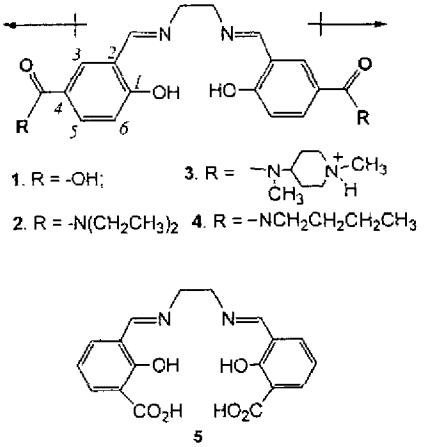


Fig. 1. Novel 4,4-disubstituted salen ligands (**1-4**) for water-soluble ruthenium complexes.

on Bruker ACF-300 NMR spectrometer. IR spectra were recorded on a Perkin-Elmer 290IR spectrometer using KBr pellets. UV-Vis spectra were obtained in H_2O, and 10 mM phosphate buffer on a Hewlett-Packard 8453 diode array spectrometer. Electrochemical measurements were carried out in the 10 mM sodium phosphate buffer (pH 7.06) using ITO glassware as a working electrode on a Bioanalytical System 100B electrochemical analyzer.

3-Formyl-4-hydroxybenzoic Acid (6)

The introduction of a formyl group is prepared by a modified procedure.[10] A solution containing 4-hydroxybenzoic acid (10.0 g, 72.4 mmol), hexamethylenetetraamine (20.3 g, 144.8 mmol), and glacial acetic acid was allowed to heat to 130 °C, and this temperature was maintained for an additional 2 h. The mixture solution was cooled to 75 °C, hydrolyzed with 33% aqueous H_2SO_4, and then heated to reflux at 110 °C for 60 min. The solution was cooled to room temperature and extracted twice with diethyl ether. The combined organic layer was washed with water, brine, and dried over $MgSO_4$. The solvent was vaporized to obtain a pale-yellow solid. Recrystallization from methanol and water provided a light-yellow solid. Yield: 3.42 g (32%). ^{1}H NMR (DMSO-d_6) δ 12.8 (b, 1H, -CO_2H), 11.46 (b, 1H, -OH), 10.28 (s, 1H, CHO), 8.22 (d, J = 1.8 Hz, 1H), 8.03 (dd, J = 2.0, 8.0 Hz, 1H), 7.07 (d, J = 8.2 Hz, 1H).

N-Succinimidyl 3-Formyl-4-hydroxybenzoate (7)

The preparation was followed by the modified procedure reported by Moriya, et al.[11] 3-Formyl-4-hydroxybenzoic acid (200 mg, 1.2 mmol) was dissolved in a minimum amount of DMF and added with a N-hydroxysuccinimide (140 mg, 1.2 mmol) in a dioxane solution under N_2 at room temperature. To this solution, a dioxane solution (8 mL) of DCC (248 mg, 1.2 mmol) was added and stirred at room temperature for an additional 18 h. The white solid was filtered off, and the solvent was evaporated to provide a clear sticky oil. A pure compound was crystallized by ethanol in a 82% (1.3 g) yield. ^{1}H NMR ($CDCl_3$) δ 11.6 (s, 1H, -OH), 9.97 (s, 1H, CHO), 8.44 (d, J = 2.2 Hz, 1H), 8.26 (dd, J = 2.2, 17 Hz, 1H), 7.12 (d, J = 8.8 Hz, 1H), 2.92 (s, 4H); FAB-MS (m/e) = 264 (M+1).

General Procedure for Salen Derivatives (8)

A solution of an aldehyde and triethyl amine in dioxane was added to 4 equivalents of a proper amine under N_2 at room temperature. The mixture solution was heated to 60 °C for 24 h. A yellow oil was obtained by a sequence of evaporation of solvent, washing with ether, water addition, and extraction with chloroform twice. **N-Diethylamino-3-formyl-4-hydroxybenzoic acid** Yield: 53%. ^{1}H NMR ($CDCl_3$) δ 11.1 (s, 1H), 9.91 (s, 1H), 7.67 (d, J = 2.1 Hz, 1H), 7.57 (dd, J = 2.1, 8.7 Hz, 1H), 7.02 (d, J = 8.6 Hz, 1H), 3.39 (m, 4H), 1.21 (b, 6H). **N-Methyl-N′-4-(1-methylpiperidiyl)amido-3-formyl-4-hydroxybenzoic acid** Yield: 56%. ^{1}H NMR ($CDCl_3$) δ 9.91 (s, 1H), 7.70 (d, J = 0.3 Hz, 1H), 7.57 (dd, J = 0.3, 12 Hz, 1H), 7.03 (d, J = 12.9 Hz, 1H), 2.94-1.77 (b, 15H).

Preparation of N,N′-Bis(6,6′-disubstitutedamidosalicyldiene)ethylenediimine

The Schiff-base compound was prepared by refluxing 2 equivalents of a proper aldehyde and 1 equivalent of ethylenediamine in ethanol for 2-3 h. The precipitate was collected and washed with cold 95% ethanol. If no precipitate formed, the solution was evaporated to obtain a foamy solid. The crude product was further precipitated by $CHCl_3$/hexane to obtain a yellow solid. **N,N′-Bis(6,6′-dicarboxylatesalicyldiene)ethylenediimine (1)** Yield: 85%. ^{1}H NMR (DMSO-d_6) δ 17.2 (b, 2H), 8.71 and 8.53 (s, 2H), 8.04 (d, J = 7.2 Hz, 2H), 7.83 (dd, J = 8.7, 15.6 Hz, 2H), 6.86 (dd, J = 8.8, 17.0 Hz, 2H), 3.92 (s, 4H); FAB-MS (m/e) = 358 (M+2); IR (KBr) 1635 cm^{-1} (C=N bond). **N,N′-Bis(6,6′-Diethylamidosalicylidene)ethylenediimine (2)** Yield: 93%. ^{1}H NMR (DMSO-d_6) δ 18.4 (b, 2H), 8.63 (s, 2H), 7.46 (s, 2H), 7.30 (d, J = 7.7 Hz, 2H), 6.85 (d, J = 7.7 Hz, 2H), 3.93 (s, 4H), 3.30 (b, 8H), 1.09 (b, 12H); FAB-MS (m/e) = 412 (M+2); IR (KBr) 1630 cm^{-1} (C=N bond); Anal. Calcd. for $C_{26}H_{34}N_4O_4$: C, 66.93; H, 7.35; N, 12.01. Found: C, 67.58; H, 7.95; N, 13.53. **N,N′-Bis(N-methyl-N′-4-(1-methylpiperidiyl)amidosalicylidene)ethylenediimine (3)** Yield: 71%. ^{1}H NMR (DMSO-d_6) δ 18.8 (b, 2H), 8.34 (s, 2H), 7.2-7.3 (m, 4H), 6.93 (d, J = 8.4 Hz, 2H), 3.92 (s, 4H), 1.77-2.94 (m, 30H); FAB-MS (m/e) = 578 (M+2); IR (KBr) 1635 cm^{-1} (C=N bond); Anal. Calcd. for $C_{32}H_{44}N_6O_4$: C, 66.64; H, 7.69; N, 14.57. Found: C, 65.88; H, 7.69; N, 7.59.

Preparation of Ruthenium(III) Complexes

The corresponding ruthenium complexes were prepared by refluxing 1 equivalent ligand and 1 equivalent $K_2[RuCl_5(H_2O)]$ in a n-butanol solution under N_2 or Ar for 27 h. The solution was gradually changed from a green, to a blue, then to a deep blue-green cloudy solution. The solution was filtered to provide a deep blue-green solution. The solution was vaporized to a small amount of solvent. Then, saturated aqueous $NaClO_4$ was added dropwisely to induce the formation of precipitate. The solid was collected in a 40-50% yield. **1-Ru**: IR (KBr) 1607 cm^{-1} (C=N-Ru); FAB-MS (m/e) = 525 (M+2); **2-Ru**: IR (KBr) 1611 cm^{-1} (C=N-Ru); FAB-MS (m/e) = 567 (M+2); **3-Ru**: IR (KBr) 1609 cm^{-1}

(C=N-Ru); FAB-MS (*m/e*) = 748 (M+2).

DNA Preparation

λ-Phage ΦX-174 supercoiled plasmid DNA was purchased from Life Technologies (Gibco BRL). No further purification was needed prior to use. All synthetic oligonucleotides were purchased from Perkin-Elmer Inc. The purification of synthetic oligonucleotides was carried out by gel purification in a 20% denaturing polyacrylamide gel (7 M urea). The DNA bands was visualized with a UV lamp (λ_{max} 254 nm) by placing the gel on a TLC F_{254} plate (20 × 20 cm, Merck). After a successive process of excising the desired visible bands, extracting DNA from gel, and precipitating by EtOH, a pure DNA was obtained as previously described.[12] The DNA concentration was determined using the extinction coefficient (λ_{max} 260 nm) or molecular weight method (1 O.D. = ~ 33 μg and the average molecular weight of one nucleotide = 330 daltons). The 5′-^{32}P-end labeling oligomer was prepared using T4 polynucleotide kinase (New England Biolabs) and deoxyadenosine-5′-[γ-^{32}P]-triphosphate (Amersham). The excess free γ-^{32}P-ATP was removed by filtration with Centricon-10 (Amicon) using an ultracentrifuge (6,000 rpm, Beckmann GS-15R equipped with rotor F0850) at 4 °C for 80 min, followed by an additional centrifuge with Milli-Q water (1 mL) for 60 min. A further dilution to proper radiation intensity with deionized water was performed prior to reacting with metal complexes.

Reaction with 5′-γ-^{32}P-Labeling DNA[12]

A total volume 20 μL solution containing the final concentration of ~ 8 nCi of 5′-γ-^{32}P-labeling oligomer, unlabeled DNA (4 μM), and 10 mM sodium phosphate buffer (pH 6.96) were added with desired the amount of ruthenium complex and H_2O_2 (0.001-0.05%) at 25 °C for 30 min. The reaction was quenched by adding sonicated calf thymus DNA (5 μg), 3 M sodium acetate (pH 7, 15 μL), and 95% EtOH (900 μL), then cooled at -78 °C for 20 min, centrifuged (12,000 rpm) at 4 °C for 6 min, and lyophilized to dryness to obtain a pellet. The reaction mixture was subjected to a piperidine treatment achieved by adding 0.7 M piperidine aqueous solution (60 μL) and heating at 90 °C for 30 min. After lyophilizing, washing with deionized H_2O (20 μL), and lyophilizing again to dryness, the DNA sample was resuspended in a gel-loading buffer (5 μL) containing 0.25% bromophenol blue, 0.25% xylene cyanol FF, and 7 M urea. The DNA fragment was analyzed by 20% denaturing polyacrylamide gel (7 M urea) and then visualized using Kodak BioMax MR-1 films with intensifying screens. The optical density of DNA fragments was quantified using image programs from NIH image (free shareware) and UVP Inc. (GelBase/GelBlot™ Pro) equipped with an Vista S-12 scanner (UMAX).

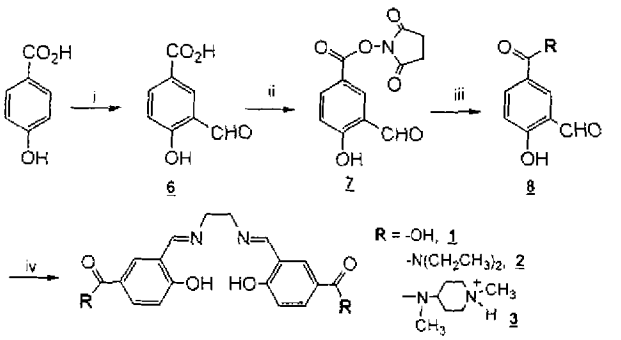
Modified Maxam-Gilbert G Lane

A modified Maxam-Gilbert G lane was performed by the following procedure: A DNA solution containing ~ 10 nCi ^{32}P-labeling oligomer and deionized H_2O in a total volume of 20 μL was cooled to 0-4 °C prior to use, followed by successive steps of adding DMS (< 1 μL) to the DNA solution, vortexing the tube (< 1 sec), immediately adding 2-mercaptoethanol (10 μL), and vortexing for an additional 30 sec to quench the reaction. After adding sonicated calf thymus DNA (4 μg) and 3.0 M sodium acetate (pH 6.3, 15 μL), DNA solution was precipitated with 95% EtOH (1 mL) at -30 °C for 30 min and centrifuged to obtain a DNA pellet which was then treated with piperidine as mentioned previously before loading on gels.

RESULTS AND DISCUSSION

The synthesis of salen derivatives with a diversity of charged groups is prepared using 4-hydroxylbenzoic acid as starting material as illustrated in Scheme I, comprising sequential steps of modified Reimer-Tiemann type formylation[13] with hexamethylenetetramine in acetic acid[10] in a yield of 30-35%, active-ester formation with DCC and N-hydroxylsuccimide[10,11] in a yield of 80-85%, substitution with a suitable amine, and condensation with ethylenediamine to generate the salen derivative. Thus, these salen derivatives are readily introduced to the negative, neutral, and positive charge moiety to the frame in a similar manner. Ligand **4** is prepared by a similar manner to give a 70% yield, which is different from the multistep synthesis via the formation of a benzofurane derivative.[8b] The preparation of ruthenium complexes are achieved by refluxing with $Ru(III)Cl_3 \cdot 5H_2O$ and a variety of salen derivatives in *n*-butanol under N_2 or Ar atmosphere. The UV-VIS absorption of **1**-Ru showed at 338 (ε 10,640 $M^{-1}cm^{-1}$) and 607 nm (ε 2,810 $M^{-1}cm^{-1}$), IR vibration of the Schiff base[14] bond shifted from 1635 (free salen ligand) to 1609 cm^{-1}, and redox potential around -0.25 and -0.40 V ($Ru^{2+/3+}$ and $Ru^{3+/4+}$ vs. Ag/AgCl), which agreed with the characteristics of ruthenium(III)-salen complexes reported by Khan and coworkers.[4] Furthermore, these modified ruthenium(III)-salen complexes with the amide groups at the 4,4′-position are capable of reacting with ambient molecular oxygen in aqueous solution, monitored by UV-VIS spectra, but very slowly.

Scheme I



Reagents and conditions: i) hexamethylenetetraamine, 4-hydroxybenzoic acid, and glacial acetic acid, 130 °C, 2 hrs, 32%; ii) DCC, N-hydroxysucciniamide, DMF/dioxane, N_2, r. t., 18 hrs, recrystallization with EtOH 82%; iii) a proper amine (4 eq.), CH_2Cl_2, N_2, 60 °C, 24 h, 53-65%; iv) aldehyde (2 eq.) and ethylenediamine (1 eq.), EtOH, N_2, reflux, 2-3 hrs, 71-93%.

When 6,6′-dicarboxylate salen, **5**,[9] is reacted with $RuCl_3$, a green solid of ruthenium complex is obtained with the UV absorption at 332 and 458 nm (in EtOH) but with an unchanged IR absorption of the Schiff-base bond at 1635 cm^{-1}. Even reacted with two equivalents of $RuCl_3$ or $Ru(DMSO)_4Cl_2$, the same result was obtained. Ruthenium(III) ion was found to preferably coordinate to the anionic carboxylate group instead of the Schiff base. Due to the limited cavity size of the salen, it is difficult for ruthenium ions to have two molecules to get in the salen cavity, which is unlike nickel or copper ions.[15] In addition, this green ruthenium complex quickly decomposed once dissolved in H_2O, and turned to a black solid with the disappearance of the IR absorption of the Schiff base bond.

In the reaction with supercoiled plasmid DNA, these modified ruthenium(III)-salen complexes showed negative results in the use of ambient molecular oxygen as oxidant. In fact, in the reaction of the C-H bond insertion, ruthenium(III)-salen complexes usually react with moderate pressure (> 1 atm) molecular oxygen.[4] Thus, these ruthenium(III)-salen complexes may reduce their reactivities in DNA cleavage due to low efficiency in the oxygen up-take in ambient condition. However, in the presence of hydrogen peroxide, these ruthenium(III)-salen complexes are capable of nicking DNA in agarose gel electrophoresis. The reactivity of Ru(III)-salen complexes containing the electron-withdrawing groups in DNA cleavage was illustrated in Fig. 2. Under the same condition (10 mM), the reactivity of **3**-Ru (lane 4) in nicking DNA (70 mM per nucleotide) has been found to be more reactive by a factor of six than that of **2**-Ru while **2**-Ru is two times more reactive than **1**-Ru. The oxidation state of ruthenium ion in ruthenium-salen complexes has been determined to be a trivalent species according to the measurement of magnetic susceptibility in a range of 1.97-2.08 μ_B and EPR study with a g_{av} around 2.105-2.332.[16] Thus, the total charge of ruthenium salen complexes of **1**, **2**, and **3** are represented as -3, -1, and +1 species in the neutral buffer, respectively, owing to the introduction of the different charged groups. Since DNA is a polyanion species, the positively charged species provide ruthenium(II)-salen with better affinity toward DNA. As expected, an increasing intensity in DNA cleavage was observed in reacting **3**-Ru complex and H_2O_2.

Furthermore, since the amide bond is an electron-withdrawing group, the electronic effect of ruthenium(III)-salen complexes in DNA stranded scission was also studied as shown in Fig. 3. Ru(III)-salen complex with electron-withdrawing groups, lane 6 in Fig. 3, showed less DNA cleavage compared to those with the electron-donating group such as the hydroxyl and methoxy groups in lane 3 and 4, respectively. Thus, the reactivities of the *para*-substituted salen derivatives (neutral species) in nicking DNA are in a decreasing order: -OMe ~ -OH > $-CO_2N(CH_3)_2$. However, in

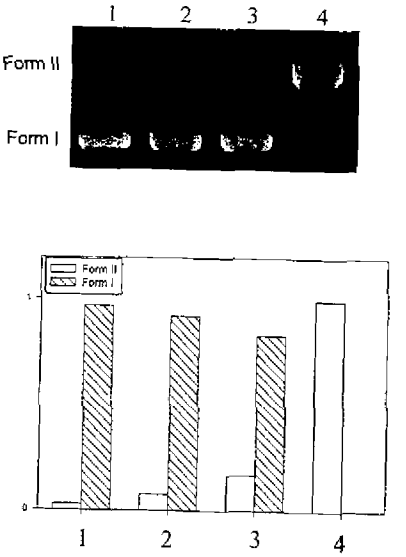


Fig. 2. Comparison of modified ruthenium(III)-salen complexes under the same concentration (10 μM) in nicking ΦX-174 plasmid DNA (70 μM per nucleotide) with 0.01% hydrogen peroxide in 10 mM sodium phosphate buffer (pH 6.9) at 25 °C for 30 min (the top one). Lane 1, DNA control.; lane 2-4, **1**-Ru, **2**-Ru, and **3**-Ru, respectively. Quantification of DNA cleavage (the bottom) was obtained from the above figure. The Y-axes represent the ratio of relative intensity after normalization. The X-axes represent lane 1, DNA control.; lane 2-4, **1**-Ru, **2**-Ru, and **3**-Ru, respectively.

lane 7, the concentration of circular-DNA displays the highest abundance induced by 3-Ru, for which a molar concentration of ruthenium complex one-tenth smaller was used. Complex 3-Ru has the electron-withdrawing group linkage but also with more positive charge-species. This result showed the electronic effect is able to enhance the reactivity of ruthenium(III)-salen in nicking DNA; however, the major dominating factor in DNA cleavage is the charge effect, namely, the electrostatic interaction between DNA and ruthenium(III)-salen complexes.

In order to examine the selectivity and reactivity of ruthenium(III)-salen complexes in DNA cleavage, a ^{32}P-5′-end-labeled hairpin oligonucleotides (5′-GCAGATCTGA-GCCTGGGAGC-TCTCTGC-3′) and polyacrylamide gel electrophoresis were used. The designed DNA sequence was based on the RNA hairpin from the *trans*-activation response element (TAR-RNA), but it is not necessary to refer to a model study of TAR-RNA.[17] After the piperidine treatment, as depicted in Fig. 4, ruthenium(III)-salen complexes display equally random cleavage with a slight favor at the guanine site regardless of the conformation of bulge, intra-loop, or double stranded regions in DNA. These similar phenomena are also observed when ruthenium(III) complexes containing pyridyl-schiff bases are used in the DNA stranded scission study.[18] In addition, the more positive charged ruthenium complex is found to have a much greater reactivity in DNA cleavage without altering the selectivity in DNA sequence, which is consistent with the observation in the plasmid DNA study.

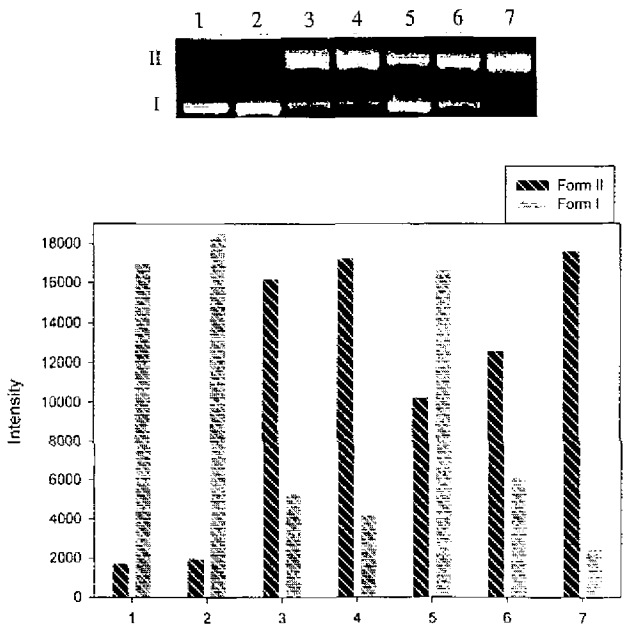


Fig. 3. Demonstration of DNA Cleavage by modified ruthenium(III)-salen complexes. Lane 1, DNA control; lane 2, DNA with H_2O_2 (0.01%); lane 3, Ru-4-OH salen (100 μM); lane 4, Ru-4-OMe salen (100 μM); lane 5, 1-Ru (100 μM); lane 6, 2-Ru (100 μM); lane 7, 3-Ru (10 μM).

The reaction mechanism of ruthenium(III)-salen complexes in DNA cleavage has been preliminarily studied in this report. Due to the co-planner structure of salen ligand, ruthenium(III)-salen complexes may intercalate with DNA. However, no intercalation is observed for these ruthenium(III)-salen complexes in the titration study with calf-thymus DNA monitored by UV-VIS spectroscopy. In addition, in the absence of H_2O_2, no high molecular weight band was observed in the gel-mobility shift experiment. It suggests that ruthenium(III)-salen complexes are neither a good intercalator to DNA nor an alkylating reagent to nucleobases to generate the covalent bonds. In the reaction of 1-Ru with hydrogen peroxide, three isosbestic points at 304, 373, and 541 nm in phosphate buffer (pH 6.9) were observed along with the increasing time as illustrated in Fig. 5, sug-

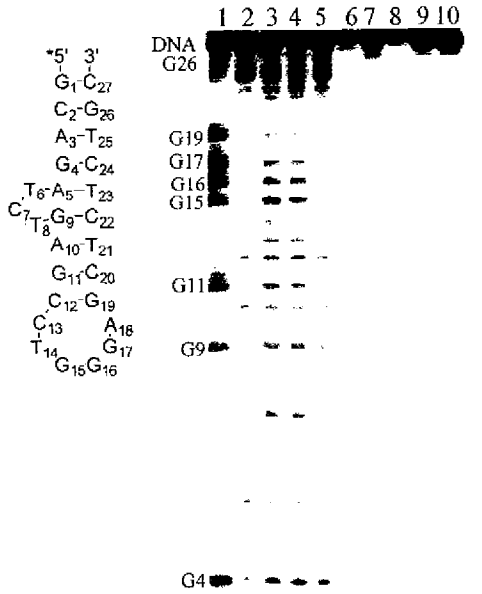


Fig. 4. Autoradiography of a 20% denaturing polyacrylamide gel (7 M urea) showing the results of oxidation of a 5′-end-labeling hairpin DNA (4 μM) with 3-Ru(III) activated by H_2O_2 (0.005%) in 10 mM sodium phosphate buffer (pH 6.9) at 25 °C for 30 min reaction time. The piperidine treatment was allowed to heat with 0.7 M piperidine at 90 °C for 30 min. Lane 1, Maxam-Gilbert G-lane; lane 2, DNA with piperidine treatment; lane 3-5, [3-Ru] ~ 200, 100, and 50 μM and piperidine treatment, respectively; lane 6, [3-Ru] = 100 μM with heat 90 °C without piperidine; lane 7, DNA with heat only; lane 8, 1 with heat; lane 9, 1 only; lane 10, DNA only.

gesting no intermediate is generated during the formation of Ru(III)-OOH adduct. Furthermore, in the reaction of ruthenium(III)-salen complexes and DNA, the concentration of circular DNA (Form II) is increased along with a decreasing pH from 8.0 to 4.0. In addition, when other oxidants such as MMPP or oxone are used instead of H_2O_2, no DNA cleavage was observed in polyacrylamide gel electrophoresis, which confirms that the formation of Ru-OOH species is important to DNA cleavage. Actually, a similar intermediate, Ru(IV)-superoxo species, has been proposed in the C-H bond activation of ruthenium(III)-salen complexes with molecular oxygen (1 atm) in DMF by Taqui Khan and co-workers.[4]

The addition of a proper inhibitor in DNA reaction is commonly done to support the determination of the reactive species. When mannitol was added to act as a hydroxyl radical scavenger[19] in the DNA quenching assay, the concentration of circular DNA (form II) was found to be reduced by two-fold compared to that of the control reaction. Meanwhile, no significant change in DNA cleavage was observed using superoxide dismutase or histamine as an inhibitor, which is used to detect the formation of superoxide species and singlet oxygen, respectively. These data suggest that hydroxyl radical serves as one of the reactive species in DNA cleavage induced by the reaction of ruthenium(III)-salen complexes with H_2O_2. The formation of hydroxyl radical may be generated from a Fenton-type transformation via an intermediate metal-peroxide species, Ru(III)-OOH, as depicted in Eq. 1. Thus, by this transformation, other possible reactive species are considered such as the high valent oxoruthenium(IV) species which is known to undergo 1′-H abstraction of ribose and guanine oxidation resulting in DNA damage.[20] As a matter of fact, more guanine oxidation can be observed in polyacrylamide gel after the piperidine treatment in the DNA cleavage with ruthenium(III)-salen and H_2O_2. Overall, the reactive species generated by ruthenium-salen and H_2O_2 in DNA cleavage are complicated since at least three kinds of reactive species were involved. A detailed mechanism is under investigation.

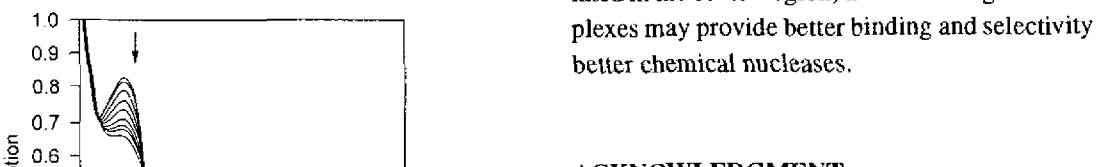
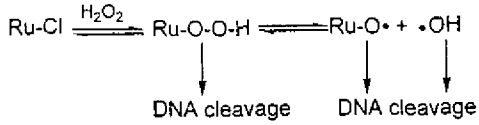


Fig. 5. Electronic spectra of the time-dependent study of 1-Ru reacted with H_2O_2 (0.01%) in 10 mM phosphate buffer (pH 6.9) at 25 °C.



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In summary, we have successfully developed novel water-soluble salen derivatives with various functional groups at the 4,4′-position with the amide group linkage. Using the advantage of the amide bond formation, salen derivatives are capable of enhancing water-solubility and carrying different functionalities such as negative and positive charge species. Even with the electron-withdrawing group linkage, these modified ruthenium(III)-salen complexes are found to be capable of nicking supercoiled plasmid DNA and synthetic DNA in the presence of hydrogen peroxide. The electronic effect of functional groups was found to slightly increase the reactivity of ruthenium(III)-salen complexes. The major dominating factor of ruthenium(III)-salen complexes in DNA cleavage is the charge effect. In the mechanism study of DNA damage, the reactive species involves at least three reactive species, i.e. ruthenium-peroxide, hydroxyl radicals, and high valent oxoruthenium(IV) species. In prospect, when DNA-binding peptides such as HMG-1[21] are introduced to these ruthenium complexes at the 4,4′-position of the salen moiety, ruthenium complexes will saddle in the center region of whole molecule instead of attaching at the end of the recognition moiety. Most of metalloenzymes in biological systems have metal ions encapsulated in the center region, thus this designed ruthenium complexes may provide better binding and selectivity to serve as better chemical nucleases.

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Key Words

Ruthenium; Salen; DNA cleavage; Hairpin.

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