Metal-Ion-Dependent Oxidative DNA Cleavage by Transition Metal Complexes of a New Water-Soluble Salen Derivative

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ABSTRACT

A new water-soluble, salen [salen = bis(salicylidene) ethylenediamine]-based ligand, 3 was developed. Two of the metal complexes of this ligand, i.e., 3a, [Mn(III)] and 3b, [Ni(II)], in the presence of cooxidant magnesium monoperoxyphthalate (MMPP) cleaved plasmid DNA pTZ19R efficiently and rapidly at a concentration ~1 μM. In contrast, under comparable conditions, other metal complexes 3c, [Cu(II)] or 3d, [Cr(III)] could not induce any significant DNA nicking. The findings with Ni(II) complex suggest that the DNA cleavage processes can be modulated by the disposition of charges around the ligand.

INTRODUCTION

The effective clinical use of cis-diammine dichloro platinum(II) complex (DDP) and other platinum complexes [1–3] in the treatment of human cancer has stimulated studies of interactions of nucleic acids with different metal complexes. Vigorous investigation in this area resulted in the development of several new metal-complex systems, e.g., Fe-EDTA, Cu-1,10-Phenanthroline, and Ru-1,10-Phenanthroline, etc., which find important applications in molecular biology, in studying protein–DNA interactions, in determining specific tertiary structural motifs of nucleic acids, etc. [4–11]. Thus, while some metal complexes possess potential antitumor activities, many other metal complexes are very important tools to molecular biologists. However, at the same time, a number of

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metal complexes and metal ions are persistent environmental hazards [12–15]. So, the development of new metal complexes which interact/cleave nucleic acids and the understanding of their precise nature of action with DNA would be crucial for more predictable utilization of metal complexes for diverse purposes such as in pharmacology, in controlling genetic information, in the elucidation of protein–DNA contacts (footprinting) or gene therapy, etc. [16–22].

The DNA scission activities of water-soluble Mn(III)-salen complex, 1 has been recently examined [23]. Preferential cleavages of DNA duplexes at A:T rich region by 1 was observed in the presence of cooxidant, MMPP. Because of their demonstrated utility as a catalyst for olefin epoxidation, Ni(II)-salen complexes would have been a more obvious choice to induce DNA cleavage reactions [24–25]. Unfortunately, however, the corresponding Ni(II)-salen is not water soluble. This prompted Burrows and coworkers [26–27] to develop a water-soluble bis-cationic Ni(II)-salen derivative, 2. Under physiological conditions, however, 2, on reaction with DNA in the presence of cooxidant KHSO₅, produced high molecular weight products, presumably through radical mediated cross-linking and DNA alkylation [26–27]. Importantly, no DNA strand scission was observed.

In this paper, we introduce a new water-soluble, bis-cationic salen-based ligand, 3, which readily forms complexes with different metal ions such as Mn(III), Ni(II), Cu(II), or Cr(III), and the resulting complex interacts with DNA. The present investigation was undertaken to examine the following questions: 1) Do the metal complexes of this ligand, 3, induce DNA cleavage into lower molecular weight fragments under oxidative conditions or lead to the formation of cross-linked products? 2) Is there any metal ion dependence? 3) If active, is there any intrinsic selectivity in such DNA cleavage processes? Upon examination of their DNA modification capacities, we find that the Ni(II) complex in particular and Mn(III) complex of 3 induce rapid, efficient DNA scission activities under oxidative conditions at physiological pH and temperature, while that of Cu(II) or Cr(III) fail to induce any detectable DNA cleavage activity.

EXPERIMENTAL SECTION

Materials

Plasmid pTZ19R was grown within E. Coli cells in 2YT media for 12 hr and purified by the literature procedures [28]. Finally, supercoiled plasmid was obtained by CsCl/ethidium bromide density gradient centrifugations. All aqueous solutions used purified water (millipore) and reagents of the highest available commercial grade. All other chemicals were of reagent quality and used without further purification.

Methods

Synthesis of the Ligand and Preparation of Metal Complexes. Caution! Although we did not face any difficulty during working, perchlorate salts are potentially explosive! Utmost care should be exercised in handling perchlorate salts.

The synthesis of \(N,N\)-bis-[4-(3-trimethylammoniopropoxy) salicylidene] ethylene diamine, 3 as perchlorate salt could be accomplished in the following
manner. Briefly, 2,4-dihydroxybenzaldehyde was coupled with 1,3-dibromopropane in dry acetone in the presence of KHCO$_3$ under refluxing conditions. This gave 4-[(3-bromopropyl)oxy] salicylaldehyde in about 60% yield which, on reaction with Me$_3$N, afforded the corresponding trimethylammonium bromide salt in quantitative yield. This, on treatment with aq. NaClO$_4$, produced the corresponding perchlorate salt (98%). The perchlorate salt was then treated with ethylenediamine in MeOH to give the bis-cationic Schiff base ligand, 3 (92%) which, on treatment with Mn(OAc)$_3$, NiCl$_2$, Cu(OAc)$_2$, or Cr(OAc)$_3$ in CH$_3$OH, yielded 3a (42%), 3b (50%), 3c (60%), and 3d (43%), respectively. All new compounds were characterized by $^1$H-NMR, IR, and UV spectroscopy, and metal complexes gave spectral (EPR, IR, and UV) and analytical (elemental analysis) characteristics consistent with their given structures (Fig. 1).

**DNA-Cleaving Activity.** Supercoiled plasmid DNA pTZ19R [plasmid DNA pTZ19R (2.9kbp, Pharmacia), contained ~ 30% of nicked circular as well as dimeric DNA forms] 0.25 μg/reaction) was incubated in a reaction mixture (10 μL) containing various concentrations of individual metal complexes of 3 and magnesium monoperoxyphthalate (MMPP) (0.5 mM) in 20 mM Tris.HCl buffer, pH 7.4 at 37°C. Reactions were initiated by the addition of MMPP and stopped by the addition of 5 μL of a terminating agent containing 10 mM β-mercaptoethanol, 20% glycerol, 25 mM EDTA, and 0.05% (1:1 bromophenol blue and xylene cyanol) after 5 min. The samples were loaded on 1% neutral agarose gel, and were subjected to electrophoresis in a horizontal slab gel apparatus (at 100 V for ~ 1.5 hr). The gel was stained with a solution of 0.5 μg/mL ethidium bromide for ~ 60 min. Bands of DNA were detected and photographed (Canon SLR camera with an orange filter) under UV light (Photodyne transilluminator, 312 nm) in a darkroom. Quantitation of cleavage products, form I (supercoiled), form II (nicked), and form III (linear), generated as a result of treatment of reagents 1, 3a–3b with DNA in the presence of MMPP was performed using a LKB Ultroscan XL densitometric scanner.

**Autoradiogram of the Sequencing Gel.** Reaction products of incubation of plasmid pTZ19R with 3a or 3b in the presence or absence of MMPP for 5 min at 37°C in 20 mM Tris.HCl, pH 7.4 were purified by repeated ethanol precipitations and used as templates for primer extension reaction. The primer (a 19-mer oligonucleotide) was 5'-end($^{32}$P)-labeled, and it was extended on the reaction

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**FIGURE 1.** Structures of different metal complexes.
templates until the complex induced a nicked site using DNA polymerase. The extension products were analyzed on 8% polyacrylamide-bisacrylamide, 50% urea denaturing gel, and autoradiogrammed.

RESULTS AND DISCUSSION

DNA Cleavage Experiments and Agarose Gel Assay

The interactions of the metal complexes 3a, 3b, 3c, and 3d with DNA in the presence or absence of a cooxidant were investigated using supercoiled plasmid pTZ19R. Figure 2 shows strand scission of plasmid pTZ19R induced by 1, 3a, or 3b in the presence of MMPP (magnesium monoperoxyphthalate) at pH 7.4 (Tris.HCl, 20 mM) and 37°C. Cleavage products generated as a result of treatment of reagents 1, 3a, and 3b with DNA in the presence of MMPP were also quantitated by densitometry (Table 1). Based on the time-course studies at 37°C, pH 7.4, we found that a mere 2–5 min incubation with 3a or 3b suffices to cause appreciable DNA cleavage in the presence of cooxidant, MMPP (0.5 mM). As shown in lane 3 in Figure 2, with 0.5 mM MMPP, there was practically undetectable background DNA cleavage. Use of other cooxidants, e.g., oxone (KHSO₅), afforded somewhat more background DNA cleavage under comparable conditions (data not shown). Complexes 3c (up to 500 μM) and 3d (up to 200 μM) in the presence of 0.5 mM MMPP at pH 7.4, 20 mM Tris.HCl and 37°C even after > 30 min incubation did not induce any discernible DNA scission (data not shown). As a result, these complexes were not studied further.

Concentration-dependent studies revealed that, with concentration ranges from 1–50 μM of either monocationic 1 or tricationic 3a and 0.5 mM MMPP, nearly comparable single-strand DNA cutting was observed. This was evidenced

![Figure 2](image)

**FIGURE 2.** Plasmid DNA pTZ19R (0.25 μg/reaction) was incubated for 5 min at 37°C, 20 mM Tris HCl, pH 7.4 and analyzed by 1% agarose gel-electrophoresis (ethidium bromide stain). Lane 1: MW marker; lane 2: DNA alone; lane 3: 0.5 mM MMPP; lane 4: 1 μM, 1; lane 5: 10 μM, 1; lane 6: 50 μM, 1; lane 7: 1 μM, 3a; lane 8: 10 μM, 3a; lane 9: 50 μM, 3a; lane 10: 1 μM, 3b; lane 11: 10 μM, 3b; lane 12: 50 μM, 3b. Lanes 4–12 also contain 0.5 mM MMPP.
TABLE 1. Cleavages of Supercoiled DNA by Reagents 1, 3a, and 3b

<table>
<thead>
<tr>
<th>Run No.</th>
<th>2</th>
<th>3b</th>
<th>4c</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
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<tbody>
<tr>
<td>Reagent</td>
<td>--</td>
<td>--</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3a</td>
<td>3a</td>
<td>3a</td>
<td>3b</td>
<td>3b</td>
</tr>
<tr>
<td>Conc. (μM)</td>
<td>--</td>
<td>--</td>
<td>1</td>
<td>10</td>
<td>50</td>
<td>1</td>
<td>10</td>
<td>50</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Supercoiled (%)</td>
<td>70</td>
<td>69</td>
<td>30</td>
<td>4</td>
<td>3</td>
<td>46</td>
<td>7</td>
<td>g</td>
<td>49</td>
<td>g</td>
</tr>
<tr>
<td>Nicked Circle (%)</td>
<td>30</td>
<td>31</td>
<td>70</td>
<td>66</td>
<td>68</td>
<td>54</td>
<td>59</td>
<td>56</td>
<td>51</td>
<td>100</td>
</tr>
<tr>
<td>Linear (%)</td>
<td>g</td>
<td>g</td>
<td>g</td>
<td>30</td>
<td>29</td>
<td>g</td>
<td>34</td>
<td>44</td>
<td>g</td>
<td>g</td>
</tr>
</tbody>
</table>

a See legend for Figure 1(a) for conditions.
b Run 2 is DNA control.
c Run 3 is DNA with 0.5 mM MMPP.
d Form I + I'.
e Form II + II'.
f Form III + III'.
g Not detectable.

by the conversion of the circular, supercoiled (Form I) pTZ19R to nicked-circle (Form II) DNA (lanes 4–9, Fig. 2, respectively). With [3a] > 10 μM, however, the propensity to form linearized DNA (Form III) was greater compared to that of 1. It is interesting to note that, although 3a is much more soluble than 1, the cleavage efficiencies of the two are almost comparable. This suggests that greater water solubility alone does not provide additional DNA strand scission abilities (see below).

Lanes 10–12 show the DNA cleavage reactions induced by Ni complex 3b at 1, 10, 50 μM concentrations, respectively, on the plasmid in the presence of 0.5 mM MMPP. Strikingly, 3b afforded quantitative conversion of Form I to Form II through single-strand nicking, even at 10 μM concentration (run 11, Table 1). However, neither the Form II nor the Form III could withstand higher concentrations of 3b. Thus, at 50 μM [3b] in the presence of 0.5 mM MMPP, a smear rather than a band was seen (lane 12), and at 200 μM [3b], most likely due to complete degradation of DNA, no ethidium bromide staining was detectable (data not shown). The finding that the present set of Ni complex is highly efficient in inducing DNA cleavage into smaller DNA fragments is significant. This is particularly striking in the light of the previous observations of Burrows and coworkers [26] who also utilized a bis-cationic Ni-salen derivative, 2, although of different chemical structure, cf. Figure 1. We believe that the disposition of positive charges around the ligand plays an important role in the modulation of the metal-complex mediated reactions towards DNA.

Primer Extension Assay and Autoradiogram

We also examined the DNA nicking by performing a primer extension assay [29]. In this experiment, the primer is extended (by Klenow DNA polymerase) until the nick in the phosphodiester backbone. The products of this reaction were analyzed on high-resolution sequencing gel. Figure 3 shows the nicking patterns produced by 5 or 20 μM of either of the reagents 3a or 3b (lanes 1, 3, 5, and 6). Control experiments verified that neither of the metal complexes 3a or 3b generated any cleaved products from plasmid DNA without the addition of any cooxidants (Fig. 3, lanes 2 and 4). We found that Mn(III) complex, 3a induced nicks to an extent of ~60% at the A:T regions of DNA duplexes. In contrast,
Ni(II) complex, 3b preferred to cut at the G:C region (~70%). Without attachment to DNA binding vectors, however, it is hardly surprising that the selectivity observed in the present study is modest. These intrinsic selectivities, although modest, might point toward prior coordination to preferred base regions of the duplexes by the metal ions of 3a or 3b. The sequence of a portion of DNA is shown along with Figure 3.

The advantages of the newly described reagents are several. First, because of the presence of two quarternary ammonium functionalities, all metal complexes are highly water soluble irrespective of the metal ion and their oxidation states. As different metal complexes have different efficiency and selectivity towards DNA cleavage, the nature of the DNA modification can be readily modulated by an appropriate choice of central metal ion. DNA nicking modes (double strand cut vs. single strand cut) and the extents of cuts can also be controlled by the
selection of adequate concentration of the reagents. Under the conditions of the present study, we do not see, however, any significant difference between 1 and 3a in eliciting DNA scission. But the fact that 3a is more soluble may be advantageous, especially under less favorable conditions of ionic strength and pH values where DNA has to be kept for studying DNA–protein interactions, etc. The reagents with limited solubility might precipitate under such conditions.

The findings reported herein suggest that the mode of the interaction of the metal complexes with Ni(II) as the central metal ion can be controlled by the choice of an appropriate ligand structure. While Ni(II) complex developed by Burrows and coworkers, i.e., 2, induces DNA cross-linking, the complex, 3b leads to efficient DNA strand scission. The results of 3b-induced DNA cleavage processes are therefore more interesting. It should also be pointed out that while other Ni complexes based on 13–14 membered tetradeptate macrocycles show DNA modification activities, their oxidative scission could only be effected by treatment with piperidine [27]. The reagent described herein, i.e., 3b, does not, however, require this additional step. In addition, with this new reagent 3b, now we have achieved the primary goal of exploiting the oxidative chemistry of Ni-salen complexes in water to induce DNA nicking.

In conclusion, the new complex 3b showed high DNA cleavage activity with modest selectivity towards G:C regions. Under the same ligand environment, while Mn(III) also confers strong DNA scission activity, the corresponding Cu(II) and Cr(III) complexes do not induce any DNA nicking under comparable conditions. We are now planning to use the “active” metal complexes as putative auxiliaries to attach them to DNA-binding matrices to achieve nicking at directed sites in DNA.

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REFERENCES


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