Specific DNA Cleavage Mediated by [SalenMn(III)]$^+$

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Summary: The combination of [SalenMn(III)]$^+$ and a terminal oxidant affords efficient and specific cleavage of right-handed double-helical DNA in regions rich in A:T base pairs.

Metal complexes of the tetradentate chelating ligand Salen (Salen = *N,N'*-ethylenebis(salicylideneaminato)) have been part of the inorganic chemistry literature for several decades.[1] The cationic manganese(III) complex [SalenMn(III)]$^+$ (1, Figure 1) is an efficient catalyst for the epoxidation of olefins with terminal oxidants such as iodosylbenzene.[2] 1 also catalyzes oxidative C-H bond activation. The flat, crescent shape of 1, its aromatic and cationic nature, and its ability to catalyze hydrocarbon oxidation are features shared in whole or in part by metal complexes which bind to DNA and cleave it via oxidative processes.[3] These similarities prompted us to evaluate the DNA-cleaving properties of 1, and we now report that 1 mediates specific cleavage of right-handed double-helical DNA in a reaction requiring a terminal oxidant.

Supercoiled plasmid pBR322 DNA (4363 base pairs) was incubated with a range of concentrations of 1[4] (100-0.010 μM) in the absence and presence of magnesium

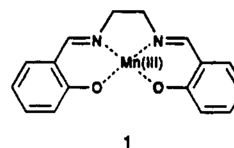

Figure 1. [SalenMn(III)]$^+$, 1.

monoperoxyphthalate (1.0 mM). Products of DNA cleavage were separated by electrophoresis on a 1% agarose gel, visualized by ethidium bromide staining/fluorescence and quantitated by densitometry (Table I). At micromolar concentrations in the presence of oxidant, 1 afforded discernible single-strand DNA cleavage, as evidenced by the conversion of pBR322 from Form I (supercoiled) to Form II (nicked). At higher concentrations of 1 (10-100 μM), the production of Form III (linear) DNA was observed, which is consistent with independent, proximal single-strand cleavage events on opposite strands of the duplex DNA substrate.[8] In these reactions, plasmid was present at a concentration of 2.3×10^{-8} M, significantly below the concentrations of 1 which produced single-strand cleavage. Therefore, the data do not demonstrate catalytic DNA cleavage by 1.[9] This may be a matter of insufficient DNA binding saturation at the low concentrations employed, the use of nonoptimized cleavage conditions, and/or the trapping of activated intermediates by buffer rather than true lack of catalytic potential—1 achieves significant turnover in olefin epoxidation.[2] A small increase in the amount of Form II DNA was noted upon incubation with oxidant alone, but DNA cleavage was not observed with 1 in the absence of oxidant (Table I). In the presence or absence of oxidant, manganous or manganic ions did not produce DNA cleavage (data not shown).

DNA affinity cleaving[3a,13] was used to examine the DNA cleavage specificity of 1. A double-strand cleavage assay was used to determine specificity at a resolution of 25-50 base pairs on plasmid pBR322.[14] pBR322 was linearized with Sty I (which cuts at the nonpalindromic sequence 5'-C/CTTGG-3' in this plasmid) and independently labeled at each of the resulting unique recessed 3' ends by filling them in with either α-^{32}P dATP or α-^{32}P TTP and the Klenow fragment of DNA polymerase. The cleavage

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(4) 1 was synthesized as the acetate salt by heating an equimolar mixture of the ligand SalenH$_2$ and manganese(III) acetate in ethanol. The complex crystallized upon cooling to room temperature and addition of ether. The analytical and spectroscopic data for the complex were consistent with the structure and with data reported in the literature:[2] IR (cm^{-1}, from evaporated EtOH) 1630 (s), 1595 (s), 1560 (s), 1460 (s), 1440 (s), 1385 (s), 1330 (s), 1290 (s), 1200 (m), 1140 (m), 1130 (s), 1080 (m), 1030 (m), 960 (m), 900 (s), 800 (s), 770 (s); UV-vis (EtOH) λ_{max} (ϵ, M^{-1} cm^{-1}) 400 (6100), 350 (8000), 310 (15 500), 283 (21 250), 240 (46 750), 220 (41 000) nm; μ_{eff} 4.89;[5] FAB MS calcd for $C_{16}H_{14}N_2O_2Mn$ ([SalenMn(III)]$^+$) 321.0436, found 321.0448. Anal. Calcd for $C_{18}H_{17}N_2O_4Mn$: C, 56.85; H, 4.50; N, 7.37%. Found: C, 56.82; H, 4.47; N, 7.11.

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(7) S, the average number of single-strand breaks per plasmid molecule, was calculated from $P_n = [S^n/n!]e^{-S}$, a Poisson distribution in which P_n is the fraction of molecules possessing n breaks.[6,8] When only Forms I and II are present, this equation simplifies to $S = -\ln f_1$, where f_1 is the fraction of Form I molecules. When Forms I, II, and III are present, S is calculated from $f_1 + f_2 = [1 - S(2h + 1)/2L]^{S/2}$, where f_1 and f_2 are the fractions of Form I and Form II molecules present, respectively, h is the maximum distance between opposite-strand breaks which will result in the production of Form III DNA (16 bp), and L is the size of pBR322 in phosphodiester bonds per strand (4363). This analysis assumes that single-strand breaks are produced at random among the population of plasmid molecules. In cases where Form III DNA is produced, it is further assumed that breaks are produced randomly along each individual plasmid molecule. As the data presented in Figures 2 and 3 clearly show that [SalenMn(III)]$^+$-mediated cleavage of pBR322 is not random, the values for S calculated for reaction mixtures containing Form III DNA may overestimate the extent of DNA cleavage.

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(9) Catalytic cleavage of DNA (i.e., the production of more than one DNA lesion per molecule of DNA cleaving agent) has been explicitly demonstrated for EDTA:Fe-based affinity cleaving agents,[6b,10] bleomycin: Fe,[11] and a manganese-porphyrin complex.[12]

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Table I. Cleavage of Supercoiled Plasmid pBR322[a]

[1] (μM)	[MgMPPA] (mM)	% Form			S^b
		I	II	III	
0	0	91	9	0	0.09
0	1.0	81	19	0	0.21
100	0	89	11	0	0.12
10	0	90	10	0	0.11
1	0	89	11	0	0.12
0.1	0	92	8	0	0.08
0.01	0	95	5	0	0.05
100	1.0	0	66	34	15
10	1.0	0	98	2	3.3
1	1.0	50	50	0	0.69
0.1	1.0	79	21	0	0.24
0.01	1.0	80	20	0	0.22

[a] Combinations of plasmid pBR322 (1 μg per reaction, 10^{-4} M bp in 15 μL total volume), 1, and magnesium monoperoxyphthalate (MgMPPA) were incubated for 30 min at 37 °C in 20 mM tris acetate buffer, pH 7.4. The data were corrected for the reduced stainability of Form I pBR322 DNA using the factor of 1.22 determined by Hertzberg and Dervan.[6] [b] Calculated average number of single-strand breaks per plasmid molecule.[6-8]

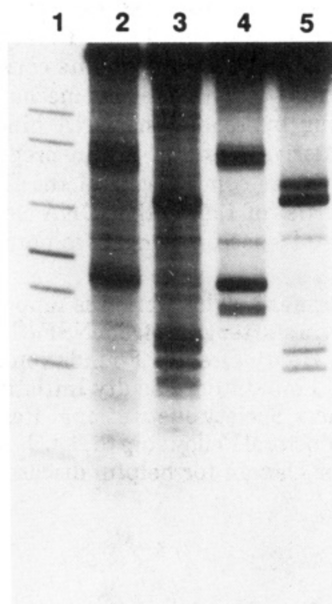

Figure 2. Autoradiograph of DNA double-strand cleavage patterns produced by 1 and **BNSE:Fe** on Sty I-linearized, 3′-^{32}P end-labeled pBR322 plasmid DNA and resolved by electrophoresis on a 1% agarose gel. Each reaction contained approximately 20 000 dpm of end-labeled substrate and calf thymus carrier DNA (100 μM in base pairs). Reactions were carried out in a total volume of 15 μL at 37 °C for 30 min in 20 mM tris acetate buffer, pH 7.4: lane 1, ^{32}P-end-labeled restriction fragment molecular weight markers from pBR322 4365, 3371, 2994, 2368, 1998, 1768, 1372, 995, and 666 bp in length; lane 2, DNA labeled at one 3′ end with ^{32}P dATP and incubated with 10 μM 1 and 1.0 mM magnesium monoperoxyphthalate; lane 3, DNA labeled at the other 3′ end with ^{32}P TTP and incubated with 10 μM 1 and 1.0 mM magnesium monoperoxyphthalate; lane 4, ^{32}P dATP-labeled DNA incubated with 0.05 μM **BNSE:Fe** and 5.0 mM dithiothreitol; lane 5, ^{32}P TTP-labeled DNA incubated with 0.05 μM **BNSE:Fe** and 5.0 mM dithiothreitol.

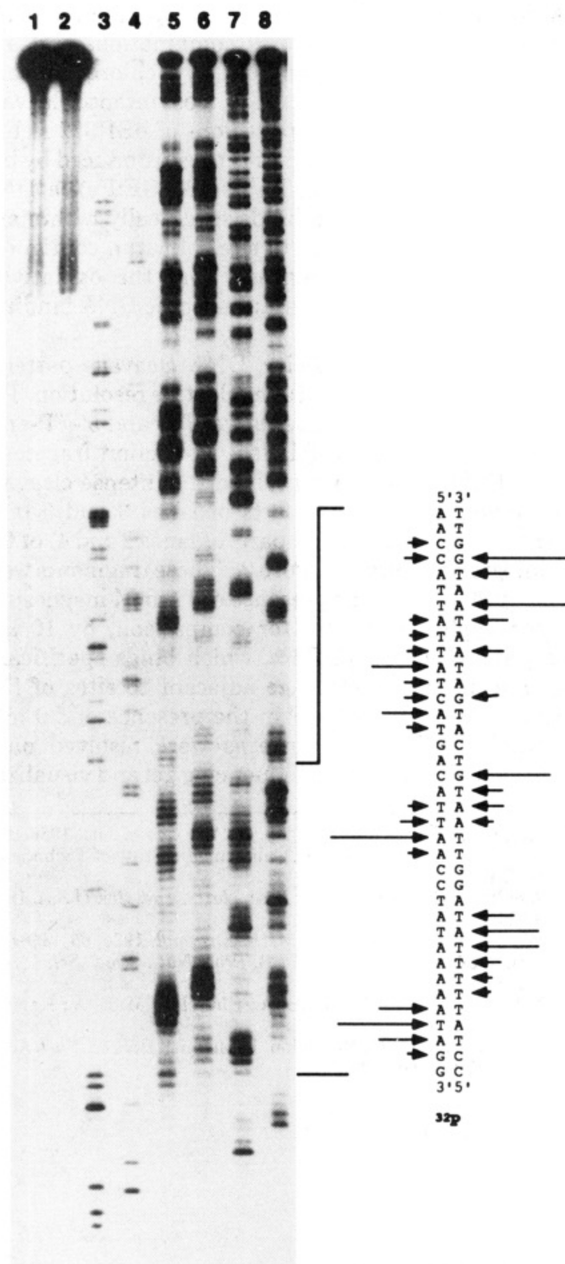

Figure 3. Left: Autoradiograph of DNA cleavage patterns produced by **DE:Fe** and 1 on 3′- and 5′-^{32}P end-labeled 517 bp Eco RI/Rsa I restriction fragments from plasmid pBR322 and resolved on a 1:20 cross-linked 8% polyacrylamide, 45% urea denaturing gel. Reactions were carried out as described in Figure 2 and then evaporated, resuspended in 80% formamide loading buffer, heat denatured, and electrophoresed: odd-numbered lanes, 3′ end-labeled DNA; even-numbered lanes, 5′ end-labeled DNA; lanes 1 and 2, uncleaved DNA; lanes 3 and 4, Maxam–Gilbert chemical sequencing G reactions;[17] lanes 5 and 6, DNA incubated with 10 μM **DE:Fe** and 5.0 mM dithiothreitol; lanes 7 and 8, DNA incubated with 20 μM 1 and 1.0 mM magnesium monoperoxyphthalate. Right: Histogram of DNA cleavage produced by the combination of 1 and magnesium monoperoxyphthalate and observed in indicated lower portion of the autoradiogram.

patterns produced on these substrates by the combination of 1 (10 μM) and magnesium monoperoxyphthalate (1.0 mM) were resolved by electrophoresis on a 1% agarose gel and visualized by autoradiography (Figure 2). Strikingly, 1 afforded not a smear but a distinct pattern of double-strand cleavage including several strong cleavage loci on this substrate. Identical cleavage patterns were observed with different concentrations of 1 or with the combination of 1 and hydrogen peroxide, iodosyl benzene, *m*-chloroperoxybenzoic acid, or potassium peroxymonosulfate. This indicates that the combination of 1 and these oxidants produces either the same activated intermediate (such as the [SalenMn(V)O]$^+$ cation proposed by Kochi to be the active intermediate in olefin epoxidation and hydrocarbon oxidation[2]) or different intermediates which exhibit similar

DNA double-strand binding/cleaving specificity. DNA cleavage was not observed with combinations of 1 and *tert*-butyl hydroperoxide, sodium hypochlorite, dithiothreitol, or sodium ascorbate. The most intense cleavage loci produced by 1 mapped to regions of pBR322 rich in A:T base pairs and correlated with those produced by bis-(netropsin)succinamide–EDTA:Fe (**BNSE:Fe**), a DNA minor groove ligand which binds specifically within and promotes cleavage adjacent to sites of seven contiguous A:T base pairs.[15] This suggests that the oxidatively activated derivative of 1 is an A:T specific DNA binding/cleaving agent.

To further probe specificity, DNA cleavage patterns produced by 1 were analyzed at nucleotide resolution. The substrates for these experiments were 3'- and 5'-^{32}P-end-labeled 517 base pair EcoRI/RsaI restriction fragments from pBR322[13b,16] which contained the intense cleavage loci observed in the lower parts of lanes 3 and 5 (and correspondingly in the upper parts of lanes 2 and 4) of the autoradiograph shown in Figure 2. These fragments were cleaved by 20 μM 1 in the presence of 1.0 mM magnesium monoperoxyphthalate and, for comparison, by 10 μM distamycin–EDTA:Fe (**DE:Fe**, which binds specifically within and promotes cleavage adjacent to sites of five contiguous A:T base pairs)[13] in the presence of 5.0 mM dithiothreitol. Cleavage patterns were resolved on a denaturing polyacrylamide sequencing gel and visualized by autoradiography (Figure 3). An inexact but notable correlation was observed between the cleavage patterns produced by 1 and **DE:Fe**. In the region of these substrates which appears in the lower part of the autoradiogram, **DE:Fe** produced cleavage flanking two binding sites, 5'-TTATT-3' and 5'-AAAAA-3'.[18] From the histogram of cleavage produced by 1 in this region, it may be seen that 1 afforded cleavage within and adjacent to these and other, shorter sequences of contiguous A:T base pairs. Additional observations and conclusions may be drawn from the sequencing gel analysis. First, 1 afforded cleavage patterns that were shifted to the 3'-side on one DNA strand relative to the other, indicating that cleavage occurred from the minor groove of right-handed double-helical DNA.[3a,13b] Second, 1 did not produce uniform cleavage patterns—single cleavage bands were observed at some sites, while at other sites DNA cleavage extended symmetrically or unsymmetrically over many nucleotides. These patterns are consistent with a cleavage process involving a specifically bound rather than a freely diffusible reactive species such as hydroxyl radical.[6,19]

These experiments define 1 as a DNA minor groove binding/cleaving agent which exhibits considerable A:T specificity. Studies of the modes and mechanisms of DNA binding/cleaving by 1, and the DNA binding/cleaving properties of derivatives of 1 are in progress. Also in progress are further comparisons of the DNA binding/cleaving properties of 1 and other DNA cleaving agents, including the A:T specific manganese porphyrins.[3e,f,20]

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