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SIMILARITIES AND DIFFERENCES IN THE DNA BINDING/CLEAVING SPECIFICITIES AND MECHANISMS OF [SALENMn(III)]$^{+}$ AND [TMPPMn(III)]$^{5+}$

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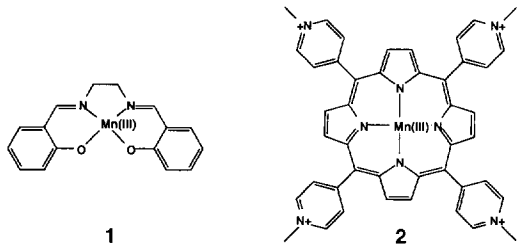
Abstract. Affinity cleaving analysis reveals that [SalenMn(III)]$^{+}$ (**1**) and [TMPPMn(III)]$^{5+}$ (**2**) exhibit nearly indistinguishable DNA double strand binding/cleaving specificities. At nucleotide resolution, the complexes generate distinct patterns of cleavage within shared A:T rich target sequences. DNA end product analysis indicates that **1** and **2** produce oxidative cleavage through both common and different mechanisms.

The manganese complex [SalenMn(III)]$^{+}$ (**1**, Salen = *N,N'*-ethylenebis(salicylideneaminato)) catalyzes alkene epoxidation and activates carbon-hydrogen bonds in the presence of terminal oxidants.[1] We have discovered that DNA is a substrate for this oxidative chemistry—the combination of **1** and oxidants yields efficient cleavage of DNA at sequences rich in A:T base pairs.[2] The manganese porphyrin [TMPPMn(III)]$^{5+}$ (**2**, TMPP = *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrinato) exhibits similar activity, cleaving DNA at sites containing ≥ 3 contiguous A:T base pairs in the presence of oxidants.[3] To further define and understand the basis for DNA recognition and cleavage by metal complexes and other small molecules,[2b] we have compared the DNA binding/cleaving specificities and end products produced by **1** and **2**. Our experiments reveal both similarities and differences in the cleavage specificities and mechanisms exhibited by these agents.



1 **2**

Figure 1. Complexes [SalenMn(III)]$^{+}$ (**1**) and [TMPPMn(III)]$^{5+}$ (**2**).

The DNA binding/cleaving specificities of **1** and **2** were determined through a two stage affinity cleaving approach.[2,4] "Global" specificities were mapped through analysis of cleavage patterns produced on Sty I linearized pBR322 plasmid DNA, which had been labeled with ^{32}P at one 3' end. Patterns of double strand cleavage produced on this substrate were resolved by agarose gel electrophoresis and visualized by autoradiography (Figure 2, Left). Strikingly, **1** and **2** produced nearly identical patterns of cleavage at the resolution of this assay (± 25-50 base pairs). While we have previously reported notable positive correlations among the double strand cleavage patterns produced by **1**, neocarzinostatin, and netropsin-like affinity cleaving agents,[2b] the correspondences in position and relative intensity of cleavage loci produced by **1** and **2**, which map to the most A:T rich sequences of pBR322, are far more pronounced.

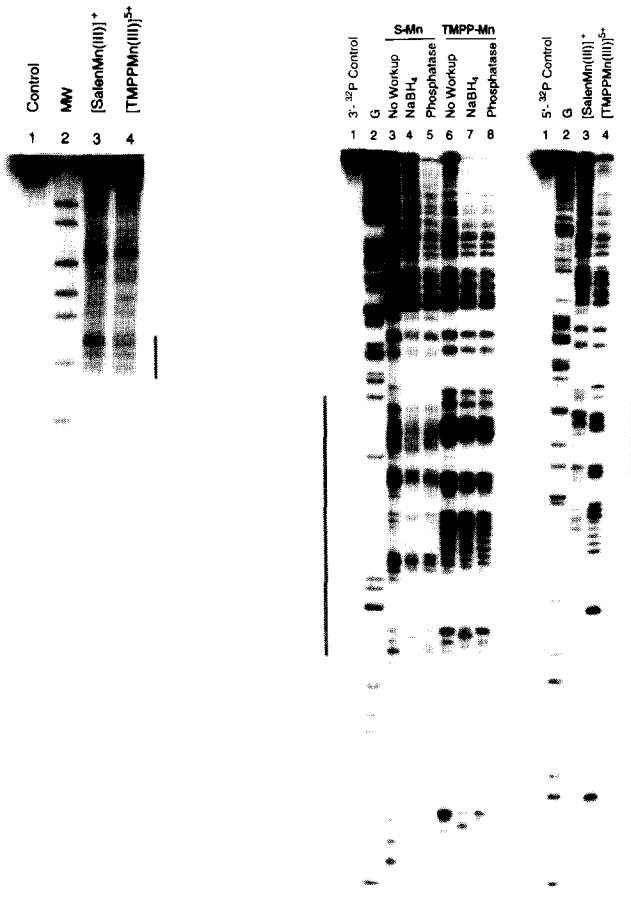


Figure 2. Autoradiographs of DNA double and single strand cleavage patterns produced by **1** and **2** on ^{32}P end labeled pBR322 plasmid DNA. **Left**: Double strand cleavage patterns produced on Sty I linearized, 3' ^{32}P TTP end labeled pBR322 and resolved by electrophoresis on a 1% agarose gel. Each reaction contained approximately 25,000 dpm of end labeled substrate and calf thymus carrier DNA (100 μM in base pairs, bp). Reactions were carried out in a total volume of 15 μL at room temperature in 40 mM Tris·HCl, pH 7.0. Lane 1, uncleaved DNA; lane 2, molecular weight markers from pBR322 4365, 3371, 2994, 2368, 1998, 1768, 1372, 995, and 666 bp in length; lane 3, DNA incubated with 20 μM **1** and 1 mM magnesium monoperoxyphthlate (MgMPP) for 30 minutes; lane 4, DNA incubated with 0.1 μM **2** and 1 mM MgMPP for 8 minutes. The bar to the right of the autoradiogram indicates the cleavage loci which were studied at higher resolution. **Right**: Single strand cleavage patterns produced by **1** and **2** on 3' and 5' ^{32}P end labeled 517 base pair restriction fragments (Eco RI/Rsa I) from pBR322 and resolved on a 1:20 cross-linked 15% polyacrylamide, 45% urea denaturing gel. Cleavage reactions were carried out as described for the double strand cleavage experiments. Post treatment with $NaBH_4$ or phosphatase was as described.[3f,5] Lanes 1-8 contain 3' ^{32}P end labeled DNA. Lane 1, uncleaved DNA; lane 2, Maxam-Gilbert chemical sequencing G reaction;[6] lane 3, 20 μM **1** and 1 mM MgMPP; lane 4, 20 μM **1** and 1 mM MgMPP, followed by sodium borohydride ($NaBH_4$) treatment; lane 5, 20 μM **1** and 1 mM MgMPP, followed by phosphatase treatment; lane 6, 0.1 μM **2** and 1 mM MgMPP; lane 7, 0.1 μM **2** and 1 mM MgMPP, followed by $NaBH_4$ treatment; lane 8, 0.1 μM **2** and 1 mM MgMPP, followed by phosphatase treatment. The adjacent set of lanes 1-4 contain 5' ^{32}P end labeled DNA. Lane 1, uncleaved DNA; lane 2, G reaction; lane 3, 20 μM **1** and 1 mM MgMPP; lane 4, 0.1 μM **2** and 1 mM MgMPP. The dark bars indicate the sequences presented in the histogram shown in **Figure 3**.

While it is difficult to distinguish **1** and **2** on the basis of their DNA double strand binding/cleaving specificities, the complexes do exhibit substantial differences in DNA binding/cleaving efficiency. To achieve similar levels of double strand scission by complexes **1** and **2**, **1** must be used in higher concentrations and for longer times, e.g., 20 μM **1** for 30 minutes versus 0.1 μM **2** for 8 minutes. We believe that the observed differences in reactivity derive from two primary sources. First, the pentacation **2** should have substantially greater affinity for the DNA polyanion than does the monocationic complex **1**. It has been shown that substitution of three of the four *N*-methylpyridinium groups of **2** with toluene moieties yields a dicationic complex which does not cleave DNA.[3b] Second, **2** is probably the more robust oxidant. This complex, but not **1**, is capable of producing catalytic cleavage of DNA.[2a,3c]

The DNA binding/cleaving specificities of **1** and **2** were studied at high resolution with 517 base pair, ^{32}P end labeled Eco RI/Rsa I restriction fragments. These substrates contained a cluster of the most intense binding/cleaving loci observed in the double strand analysis. The single strand cleavage patterns produced on these substrates were resolved using DNA sequencing gels, visualized by autoradiography (Figure 2, Right), subjected to densitometry, and converted to histogram form (Figure 3). As expected from the double-strand cleavage results, **1** and **2** produced cleavage within and adjacent to sites containing multiple contiguous A:T base pairs. However, the precise patterns of cleavage within these common target sites are clearly distinct, indicating that the factors which control interactions of oxidatively activated forms of **1** and **2** (**1*** and **2***, probably the corresponding manganese(V) oxo species[1-3]) with DNA, while very similar, are not identical. For both complexes, cleavage patterns are shifted to the 3' side on one DNA strand relative to the other. Single positions of cleavage are observed at some sites, while at others cleavage extends symmetrically or unsymmetrically over many nucleotides. These and other results[2,3] support a model for minor groove DNA recognition and cleavage by **1*** and **2*** in which the common A:T selectivity of these cationic complexes derives from their affinity for the electron rich, relatively narrow minor groove found in A:T tracts.[7,8] Here the complexes engage in stabilizing van der Waals interactions. Differences in the observed cleavage patterns arise from differences in the relative rates at which **1*** and **2*** initiate DNA cleavage at each nucleotide within A:T rich target sites, which may reflect differences in the relative binding affinities of **1*** and **2*** for individual subsites and/or the rate constants for DNA oxidation at those subsites.

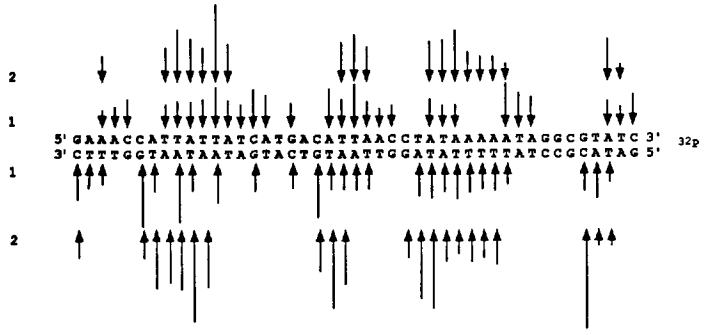


Figure 3. Histograms of single strand DNA cleavage produced by **1** and **2** in the presence of 1.0 mM magnesium monoperoxyphthlate on the 517 bp restriction fragments (Eco RI/Rsa I) from pBR322 plasmid DNA. Histograms were derived from densitometric analysis of the autoradiographs shown in Figure 2, Right. Lengths of arrows correspond to the relative amounts of cleavage as determined by optical densitometry. Sequence positions of cleavage were determined by comparing the electrophoretic mobilities of DNA cleavage fragments to bands in Maxam-Gilbert chemical sequencing lanes.

It has been established that cleavage of DNA by **2** in A:T rich regions is initiated by hydroxylation at $C_{5'}$ of the deoxyribose ring, with a minor contribution from hydroxylation at $C_{1'}$.[3d] Subsequent fragmentation of a $C_{5'}$ hydroxylated nucleotide generates a fragment terminating in a 3' phosphate group and a fragment terminating in a 5' aldehyde group.[3d-g] The dominance of 5' aldehydes in the cleavage products generated by **2** was confirmed by reduction with sodium borohydride, which led to nearly quantitative conversion of all cleavage fragments to species with enhanced electrophoretic mobility (compare lanes 6 and 7 in Figure 2, Right). In contrast, post treatment with borohydride increased the electrophoretic mobilities of only a subset of the cleavage products generated by **1** (compare lanes 3 and 4 in Figure 2, Right). Another subset of the cleavage products exhibited reduced electrophoretic mobility upon treatment with alkaline phosphatase, which hydrolyzes 5' phosphate groups. Fragments bearing 5' phosphate groups could arise through strand scission at abasic sites (formed via oxidation of the nucleobase),[9] or by hydroxylation of the deoxyribose moiety at $C_{1'}$ or $C_{4'}$.[10,11] Thus, it appears that **1** is capable of producing a greater variety of oxidative lesions in DNA than is **2**, leading to the formation of 5' aldehyde, 5' phosphate, and other end products. This suggests that **1*** is able to gain access to more reactive sites on the double helix, which is consistent with the smaller size of the parent complex.

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