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CORRELATIONS AMONG THE DNA BINDING/CLEAVING SPECIFICITIES OF SMALL MOLECULES REVEALED BY DOUBLE-STRAND AFFINITY CLEAVING

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Abstract. A DNA double-strand affinity cleaving assay reveals clear positive and negative correlations among the DNA cleavage specificities of a series of minor groove binding/cleaving agents: [SalenMn(III)]+, neocarzinostatin, bleomycin:Fe, and methidiumpropyl-EDTA:Fe.

We recently reported that [SalenMn(III)]$^+$ (**MnS**) mediates the cleavage of right-handed double helical DNA in the presence of terminal oxidants.[1] The combination of [SalenMn(III)]$^+$ and oxidant produces single-strand cleavage, as well as double-strand cleavage through independent, proximal nicks on opposite DNA strands. By simple inspection, the DNA double-strand cleavage patterns produced by [SalenMn]$^+$ could be correlated in a positive sense with those produced by another minor groove binding/cleaving agent, bis(netropsin)succinamide-EDTA:Fe (**BNSE:Fe**). This prompted us to consider to what extent correlations exist among the DNA binding/cleaving specificities of additional small molecules. We now report that double-strand affinity cleaving analysis is a convenient and generally informative tool for the characterization of DNA binding/cleaving agents of nominally limited specificity. Our experiments reveal clear positive correlations in cleavage specificities between [SalenMn(III)]$^+$ and neocarzinostatin (**NCZS**), and negative correlations between the cleavage specificities of these agents and those of bleomycin:Fe (**Blm:Fe**) and methidiumpropyl-EDTA:Fe (**MPE:Fe**). These correlations are also found via high-resolution analysis of DNA cleavage patterns, but are less obvious by simple visual inspection.

DNA double-strand affinity cleaving analysis was carried out on Sty I-linearized pBR322 plasmid DNA which had been independently labeled at either 3'-end using α-[^{32}P]-dATP or α-[^{32}P]-TTP and the Klenow fragment of DNA polymerase. The complementary cleavage patterns produced by **MnS**, **MPE:Fe**, **NCZS**, and **Blm:Fe** on these substrates were resolved by agarose gel electrophoresis and visualized by autoradiography (**Figure 1**). None of these agents produced a uniform smear on the gel, which would have indicated a complete lack of cleavage specificity. **MnS**, **NCZS**, and **Blm:Fe** produced well-defined cleavage patterns, while **MPE:Fe** generated a number of distinguishable cleavage loci against a background of cleavage that varied in intensity. Strikingly, the most intense cleavage loci observed with **MnS** and **NCZS** occur at similar positions and correlate with regions of diminished cleavage intensity observed with **MPE:Fe** and **Blm:Fe**. This can be clearly seen in the overlaid densitometry traces collected in **Figure 1**, where the tallest "peaks" produced by **MnS** and **NCZS** (as well as by **BNSE:Fe**[1]) align with the deepest "valleys" observed with **MPE:Fe** and **Blm:Fe**. These regions of alignment map to positions 3100, 3250, and 4000-4363 of pBR322, and correspond to the most A:T rich segments of the plasmid. Conversely, regions where **MPE:Fe** and **Blm:Fe** produce greatest cleavage intensity correspond to regions of minimal cleavage by **BNSE:Fe**, **MnS** and **NCZS**.

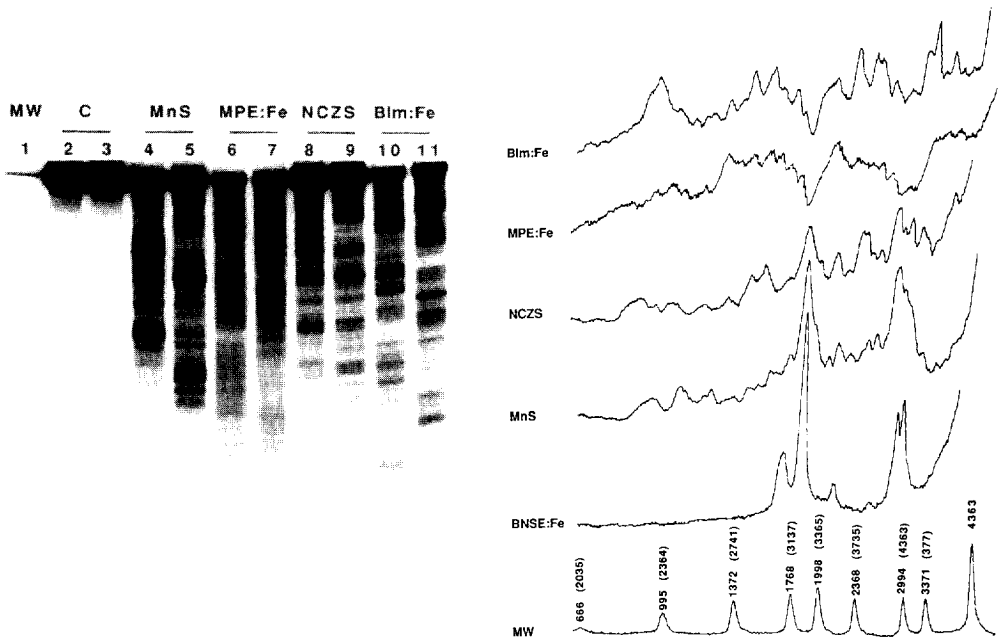

Figure 1. Left: Autoradiograph of DNA double-strand cleavage patterns produced by **MnS, MPE:Fe, NCZS,** and **Blm:Fe(II)** on Sty I-linearized, 3'-^{32}P end-labeled pBR322 DNA. Cleavage patterns were resolved by electrophoresis on a 1% agarose gel. Lanes 2,4,6,8,10 contain DNA labeled at one end with ^{32}P dATP; lanes 3,5,7,9,11 contain DNA labeled at the other end with ^{32}P TTP. Lane 1, molecular weight markers; lanes 2 and 3, uncleaved DNA; lanes 4 and 5, DNA cleavage by **MnS** (20 μM) in the presence of hydrogen peroxide (1.0 mM); lanes 6 and 7, DNA cleavage by **MPE:Fe** (0.50 μM) in the presence of dithiothreitol (5.0 mM); lanes 8 and 9, DNA cleavage by **NCZS** (0.125 units) in the presence of mercaptoethanol (10 mM); lanes 10 and 11, DNA cleavage by **Blm:Fe(II)** (0.50 μM). **Right:** Optical densitometry of DNA double-strand cleavage produced by **Blm:Fe, MPE:Fe, NCZS, MnS,** and **BNSE:Fe** on ^{32}P dATP labeled substrate. At bottom is the densitometry trace of the molecular weight marker lane. Lengths of the double-stranded DNA fragments (and corresponding positions of cleavage on pBR322) are given.

The DNA cleavage specificities of **MnS, MPE:Fe, NCZS, Blm:Fe,** and distamycin-EDTA:Fe (**DE:Fe,** which like **BNSE:Fe** binds specifically and promotes cleavage at sites of multiple contiguous A:T base pairs)[2] were then probed at nucleotide resolution using 169 base pair Dde I restriction fragments, which had been independently labeled at either 3'-end using α-[^{32}P]-dGTP or α-[^{32}P]-dCTP. These fragments, spanning pBR322 positions 3159-3327, contain the most intense double-strand cleavage locus observed with **MnS** and **BNSE:Fe,** and the region of lowest cleavage intensity observed with **MPE:Fe** and **Blm:Fe.** This cleavage locus maps to the most A:T-rich segment of pBR322, where 28 out of 31 base pairs are A:T. Cleavage patterns produced by these agents were resolved by denaturing polyacrylamide gel electrophoresis and visualized by autoradiography (**Figure 2**). **DE:Fe** produces intense cleavage within the A:T-rich tract. **MnS** and **NCZS** also produce intense cleavage in this region, where cleavage by **MPE:Fe** and **Blm:Fe** is relatively weak. These results are consistent with those obtained by double-strand affinity cleaving and with the known DNA cleavage

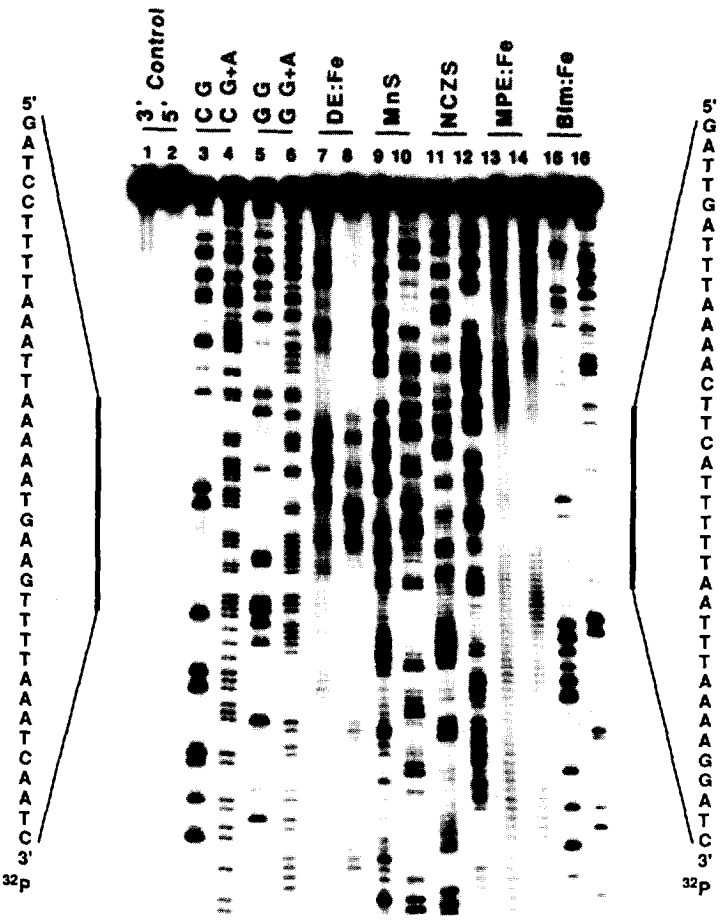

Figure 2. Autoradiograph of DNA cleavage patterns produced by **DE:Fe, MnS, NCZS, MPE:Fe,** and **Bim:Fe** on 3'-^{32}P end-labeled 169 bp Dde I restriction fragments from pBR322. Cleavage patterns were resolved on a 1:20 cross-linked 8% polyacrylamide, 45% urea denaturing gel. Lanes 1,3,4,7,9,11,13,15, DNA labeled at one 3' end with ^{32}P dCTP. Lanes 2,5,6,8,10,12,14,16, DNA labeled on the opposite strand at the 3' end with ^{32}P dGTP. Lanes 1 and 2, uncleaved DNA; lanes 3,5 and 4,6, Maxam-Gilbert chemical sequencing G and G + A reactions, respectively; lanes 7 and 8, DNA cleavage by **DE:Fe** (10 μM) in the presence of dithiothreitol (5.0 mM); lanes 9 and 10, DNA cleavage by **MnS** (20 μM) in the presence of magnesium monoperoxyphthalate (2.0 mM); lanes 11 and 12, DNA cleavage by **NCZS** (0.25 units) in the presence of mercaptoethanol (10 mM); lanes 13 and 14, DNA cleavage by **MPE:Fe** (1.0 μM) in the presence of dithiothreitol (5.0 mM); lanes 15 and 16, DNA cleavage by **Bim:Fe(II)** (2.0 μM). The position and sequence of the $(A:T)_{15}$ tract and the A:T rich flanking sequences on the dCTP- and dGTP-labeled substrates are shown to the left and right of the autoradiograph, respectively.

specificities of these agents. **NCZS** produces direct strand breaks preferentially at thymidine and adenine residues,[3] and its cleavage patterns correlate in a positive sense with those produced by the A:T specific agents **MnS, BNSE:Fe,** and **DE:Fe. MPE:Fe** exhibits low overall sequence specificity with some preference for G:C base pairs versus A:T base pairs;[4] its cleavage patterns correlate in a negative sense with those of the A:T specific agents, but in a positive sense with those of **Bim:Fe,** which cleaves preferentially at the pyrimidine in 5'-GC or 5'-GT sequences.[3a,5]

Double-strand cleavage assays are routinely employed to study highly specific DNA binding/cleaving agents, including restriction enzymes and their synthetic mimics.[6] Such assays have been little used in the study of less specific agents,[7] perhaps because it is presumed that specific cleavage will not be observed or that complex patterns of cleavage will be intractable. However, our results indicate that almost all DNA binding/cleaving small molecules will produce distinct patterns of cleavage when one utilizes substrates that have been selectively labeled on one end of one strand. Further, the complexities of the cleavage patterns are information-rich and may be used quite powerfully to determine "global"[8] DNA binding/cleaving specificities and their relationships among DNA binding/cleaving agents, as well as to serve as a guide to subsequent studies at higher resolution.

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