On the Chemical Nature of DNA and RNA Modification by a Hemin Model System†

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ABSTRACT: In order to model the interaction of hemin with DNA and other polynucleotides, we have studied the degradation of DNA, RNA, and polynucleotides of defined structure by [*meso*-tetrakis(*N*-methyl-4-pyridyl)porphinato]manganese(III) (MnTMPP) + $KHSO_5$. The activated porphyrin was shown to release adenine, thymine, and cytosine from DNA; RNA degradation afforded adenine, uracil, and cytosine. The same products were obtained from single- and double-stranded DNA oligonucleotides of defined sequence, and also from single-stranded DNA and RNA homopolymers. The overall yield of bases from the dodecanucleotide d($CGCT_3A_3GCG$) was equal to 14% of the nucleotides present initially, indicating that each porphyrin catalyzed the release of ~4 bases. Although no guanine was detected as a product from any of the substrates studied, the ability of MnTMPP + $KHSO_5$ to degrade guanine nucleotides was verified by the destruction of pGp, and by the appearance of bands corresponding to guanosine cleavage following treatment of ^{32}P end labeled DNA restriction fragments with activated MnTMPP. Inspection of a number of sites of MnTMPP-promoted cleavage indicated that the process was sequence-selective, occurring primarily at G residues that were part of 5′-TG-3′ or 5′-AG-3′ sequences, or at T residues. Also formed in much greater abundance were alkali-labile lesions; these were formed largely at guanosine residues. Also studied was the degradation of a 47-nucleotide RNA molecule containing two hairpins. Degradation of the 5′-^{32}P end labeled RNA substrate afforded no distinct, individual bands, suggesting that multiple modes of degradation may be operative. However, at concentrations of MnTMPP + $KHSO_5$ that led to only limited amounts of RNA substrate degradation, there was enhanced degradation in a single-stranded region between the two hairpins, suggesting that MnTMPP may be a useful probe of RNA conformation.

Several lines of evidence suggest a role for hemin in cellular differentiation (Ishii & Maniatis, 1978; Chen & London, 1981; Lo et al., 1981) and gene regulation (Ross & Sautner, 1976; Bonanou-Tzedaki et al., 1984), possibly including direct chemical alteration of DNA structure. The ability of hemin to accelerate erythroid cell maturation is well documented (Lo et al., 1981; Bonanou-Tzedaki et al., 1984). A selective, hemin-mediated increase in the production of globin mRNAs during rabbit erythroblast maturation (Bonanou-Tzedaki et al., 1981) suggests a role for hemin in regulation of gene expression, as does the requirement for hemin in erythropoietin-mediated induction of globin synthesis (Beru et al., 1983) and its ability to promote transcription in erythroid cells (Charnay & Maniatis, 1983; Bonanou-Tzedaki et al., 1984). That the effects of hemin are not limited to erythroid cells may be appreciated from the reports that hemin also promotes adipocyte differentiation (Chen & London, 1981) and rapid neurite outgrowth in mouse neuroblastoma cells (Ishii & Maniatis, 1978).

While the biochemical mechanisms responsible for these effects are not established, it is interesting to note that hemin has been reported to effect DNA strand scission in vitro (Aft & Mueller, 1983; Sakurai et al., 1986) under conditions that argue for initial binding of hemin to DNA, and that DNA nicking (Terada et al., 1978; Scher & Friend, 1978) and a decrease in the number of topological turns in the DNA (Luchnik & Glaser, 1980) have been observed during Friend cell differentiation. Naturally occurring porphyrins also cause light-mediated DNA and cellular damage (Schothorst et al., 1971; Boye & Moan, 1980), a property that has been exploited for the photodynamic therapy of tumors (Land, 1984). Further, synthetic porphyrins and metalloporphyrins have been shown to interact selectively with double-stranded DNA (Pasternack et al., 1983; Carvlin & Fiel, 1983; Strickland et al., 1987; Gibbs et al., 1988; Dabrowiak et al., 1989) and also to cleave ds DNA[1] following photolytic (Kelly & Murphy, 1985; Praseuth et al., 1986) or chemical (Fiel et al., 1982; Ward et al., 1986) activation.

Given the substantial level of research activity in this area, surprisingly little is known about the underlying chemical mechanisms. To facilitate an understanding of the role of hemin in the aforementioned processes, we have used a hemin model system to study the chemistry of nucleic acid degradation. Presently we demonstrate that this hemin model degrades double-stranded (ds) and single-stranded (ss) DNA; substrate molecules degraded efficiently by MnTMPP + $KHSO_5$ included calf thymus DNA, the tridecanucleotide 5′-d(AGATTCGCATATC) and self-complementary dodecanucleotide 5′-d(CGCTTTAAAGCG), and each of the four DNA nucleotide homopolymers. Analysis of the mode of degradation of ds DNA indicated that the sequence selectivity of DNA cleavage was not limited to AT-rich regions, contrary to what has been reported previously (Ward et al., 1986, 1987; Dabrowiak et al., 1989); when appropriate DNA substrates

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[1] Abbreviations: MnTMPP, [*meso*-tetrakis(*N*-methyl-4-pyridyl)porphinato]manganese(III) pentaacetate; PAGE, polyacrylamide gel electrophoresis; ds DNA, double-stranded DNA; ss DNA, single-stranded DNA; ccc DNA, covalently closed circular DNA.

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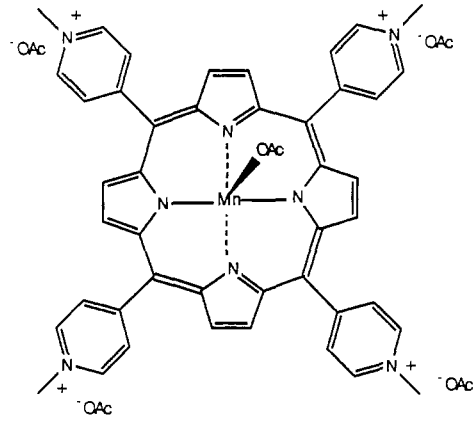
were employed, most of the strand scission events occurred at G residues that were part of 5′-TG-3′ or 5′-AG-3′ sequences. Further, the present studies revealed the existence of a second, previously unrecognized, mode of DNA degradation involving the formation of alkali-labile lesions. The latter lesion, which seems to involve predominantly G residues, clearly constituted the major pathway for MnTMPP-promoted DNA degradation. The chemical products of the reaction included cytosine,



MnTMPP

thymine, and adenine; guanine was not observed as a product of the degradation of any guanosine-containing polynucleotide substrate, even though both pGp and oligo(dG)$_{20}$ were degraded extensively by MnTMPP + $KHSO_5$, and much of the DNA degradation observed on sequencing gels involved guanosine residues. Analysis of a sample of poly(dA) that had been treated with $KHSO_5$-activated MnTMPP revealed the presence of 2-methylene-5-oxo-2*H*,5*H*-furan (Goyne & Sigman, 1987), indicating that at least part of the observed degradation involved oxidation of C-1′ of deoxyribose. The lack of formation of free guanine, which is a known product of polynucleotide degradation by reagents that produce diffusible free radicals, and the ability of the substrate oligonucleotides to protect the activated porphyrin from self-destruction argue strongly that activated MnTMPP bound to its oligo- and polynucleotide substrates prior to effecting their degradation.

Also degraded were RNA substrates containing both double- and single-stranded regions. These included both homopolymers and substrates of mixed sequence, and substrates of substantially different lengths. The chemical products of RNA cleavage by activated MnTMPP were also determined and shown to include cytosine, uracil, and adenine, but not guanine. Also investigated was the degradation of a 47-nucleotide 5′-^{32}P end labeled RNA molecule, under a variety of conditions that ranged from those which produced very little damage, to those which gave essentially complete digestion of the substrate RNA. At the highest concentrations, the lower portion of the gel contained a "smear" of degradation products but no individual bands could be detected, suggesting multiple modes of degradation. Under conditions that produced more limited amounts of cleavage, there was a single-stranded region between two hairpins in which cleavage was significantly enhanced. This finding suggests that activated MnTMPP may recognize a specific polynucleotide conformation.

Experimental Procedures

Materials

Polyribonucleotides and polydeoxyribonucleotides, yeast ribonucleic acid (type XI), calf thymus DNA, acrylamide, and *N*,*N*′-methylenebis(acrylamide) were purchased from Sigma; guanosine 3′,5′-diphosphate, calf intestinal alkaline phosphatase, pBR322 DNA, pSP64 DNA, and *Bcl*I were from Boehringer Mannheim. SP6 RNA polymerase was obtained from Promega. T_4 polynucleotide kinase, SV40 DNA, AMV reverse transcriptase, and restriction endonucleases *Hin*dIII, *Nci*I, and *Eco*RI were obtained from Bethesda Research Labs; [γ^{32}P]ATP and [α^{32}P]dATP were purchased from ICN Radiochemicals. Potassium monopersulfate (Oxone) was from Alfa-Ventron. Enzymes and other reagents required for RNA sequencing were obtained as part of the Bethesda Research Labs RNA sequencing system. Buffers and all other materials were of reagent grade. Oligo(dG)$_{20}$ was prepared by standard phosphoramidite methods on a Biosearch DNA synthesizer. $CGCT_3A_3GCG$ was a gift from Dr. J. van Boom. MnTMPP was prepared as described previously (Fouquet et al., 1987). HPLC analyses were carried out on a Varian HPLC system equipped with a Waters 745 Data Module and a Rainin Short One C_{18} column. Electrophoresis was performed with a Fotodyne Model 4200 power supply.

Methods

Analysis of Nucleic Acid Bases Released from Polynucleotides. Reaction mixtures (50 μL total volume) contained 1 mM final nucleotide concentration (as DNA, RNA, homopolymer, or nucleoside 3′,5′-diphosphate) in 60 mM sodium phosphate, pH 7.2, and 35 μM MnTMPP at 0 °C. Oxone ($KHSO_5$) was added to a final concentration of 3 mM from a concentrated, buffered stock solution to initiate the reactions, which were maintained at 0 °C for 15 min. Variations of these reaction conditions were sometimes employed, as described in the text. Aliquots (20 μL) were analyzed by reverse-phase HPLC on a Rainin Short One C_{18} column (detection at 260 nm). The column was washed with 0.1 M NH_4OAc, pH 6.8, at a flow rate of 1.6 mL/min. Yields were determined by quantitation against authentic standards.

Self-Inactivation of $KHSO_5$-Treated MnTMPP. Reaction mixtures (50 μL total volume) contained 35 μM MnTMPP + 0.9 mM $KHSO_5$ in 60 mM sodium cacodylate, pH 7.2. Where present, d($CGCT_3A_3GCG$) was used at a final concentration of 4.0 mM. The reaction mixtures were incubated at 0 °C for 15 min, then diluted to 1.0 mL, and analyzed on a Perkin-Elmer 3840 diode-array UV–visible spectrophotometer.

Analysis of the Cleavage of ^{32}P End Labeled DNAs by MnTMPP + $KHSO_5$. The 5′- and 3′-^{32}P end labeled DNA fragments were prepared as described (Sugiyama et al., 1985; Ward et al., 1986, 1987). The reaction mixtures (20 μL total volume) contained 2.0 μM MnTMPP, 40 μM calf thymus DNA, and 10^5 cpm of ^{32}P end labeled DNA in 6 mM sodium phosphate, pH 7.2, at 0 °C. The reactions were initiated by the addition of $KHSO_5$ from a concentrated stock solution and maintained at 0 °C for 1 h. The reactions were quenched with 2 μL of 50 mM NaHepes buffer, pH 7.2; where noted, alkali treatment consisted of adding 2 μL of 1.0 N NaOH to the quenched reactions and heating to 90 °C for 30 min. The samples were precipitated with ethanol three times, dried as pellets, redissolved in 80% formamide loading buffer (0.5% xylene cyanol, bromophenol blue), and run on 10% denaturing polyacrylamide gels (8 M urea, 90 mM Tris–borate–2 mM EDTA buffer, pH 8.0).

Analysis of the Cleavage of a ^{32}P End Labeled RNA by MnTMPP + $KHSO_5$. The unlabeled RNA was prepared by transcription from a linearized pSP64 plasmid DNA with SP6 RNA polymerase. The purified RNA was treated successively with calf intestinal phosphatase, and with T4 polynucleotide kinase + [γ-^{32}P]ATP. The 5′-^{32}P end labeled RNA was re-

purified on a 20% denaturing polyacrylamide gel. Reaction mixtures (20 μL total volume) contained 2 μM MnTMPP, 75 μM (nucleotide concentration) carrier tRNA, and 5×10^4 cpm of 5′-^{32}P end labeled RNA in 5.5 mM sodium phosphate, pH 7.2, at 0 °C. Reactions were initiated by the addition of $KHSO_5$ from a concentrated stock solution and maintained at 0 °C for 1 h. The reactions were then treated with 2 μg of tRNA, ethanol precipitated, and dried in pellet form prior to 20% PAGE analysis as described above.

Results and Discussion

The dearth of information concerning the actual chemistry of porphyrin-mediated DNA degradation prompted us to investigate the properties of MnTMPP following chemical activation with the single oxygen donor $KHSO_5$. This species was chosen on the basis of the high affinity of positively charged porphyrins for DNA (Pasternack et al., 1983; Carvlin & Fiel, 1983; Strickland et al., 1987; Gibbs et al., 1988; Dabrowiak et al., 1989), as well as the ability of $KHSO_5$-activated MnTMPP to nick covalently closed circular (ccc) φX174 DNA (Fouquet et al., 1987) and mediate the oxidation and oxygenation of low molecular weight substrates (Meunier, 1986).

Initially, $KHSO_5$-activated MnTMPP was incubated with calf thymus DNA, and with the self-complementary dodecanucleotide 5′-d(CGCTTTAAAGCG). HPLC analysis indicated that both incubations resulted in the release of free nucleoside bases, and more polar products not readily characterized. For d($CGCT_3A_3GCG$), adenine, cytosine, and thymine were released in approximately equal amounts (Figure 1, upper panel). While calf thymus DNA afforded adenine in greatest abundance, the actual facility of production of free bases was C ≃ A > T when the (39%) GC content of calf thymus DNA was considered. Interestingly, no guanine was detected from either substrate, although control experiments established that guanine was stable under the reaction conditions. The overall yield of bases from the dodecanucleotide was equivalent to 14% of the nucleotides present initially or one lesion per 3.6 base pairs, indicating that each porphyrin catalyzed the release of ~4 bases.

Axially ligated cationic metalloporphyrins are known to interact with ds DNA through an outer binding mode (Pasternack et al., 1983; Strickland et al., 1987). The possible binding of such species to single-stranded polynucleotides has not been demonstrated and would not be predicted on the basis of the current understanding of the nature of porphyrin–DNA interactions. To determine whether $KHSO_5$-activated MnTMPP was also capable of degrading ss DNA, we used as a substrate the tridecanucleotide d(AGATTCGCATATC), which was expected to exist predominantly as a single-stranded species based on the lack of Watson–Crick complementarity within the sequence. Surprisingly, under conditions identical with those employed for the other substrates in Figure 1, the yields of free bases from this putative single-stranded substrate were similar to those observed for the ds DNAs tested (92 μM adenine, 41 μM cytosine, 50 μM thymine). Thus, degradation of both ds DNA and ss DNA was found to proceed to a comparable extent, especially when relative base contents were considered.

Similar treatment of poly(dA), poly(dT), and poly(dC) with $KHSO_5$-activated MnTMPP also effected extensive release of free bases, as shown in Figure 1. Analogous treatment of yeast RNA and ss RNA homopolymers gave the same products, albeit in somewhat lower yield. At least for poly(dA), oxidative degradation must involve C-1′ of deoxyribose, since subsequent heating released significant quantities of 2-

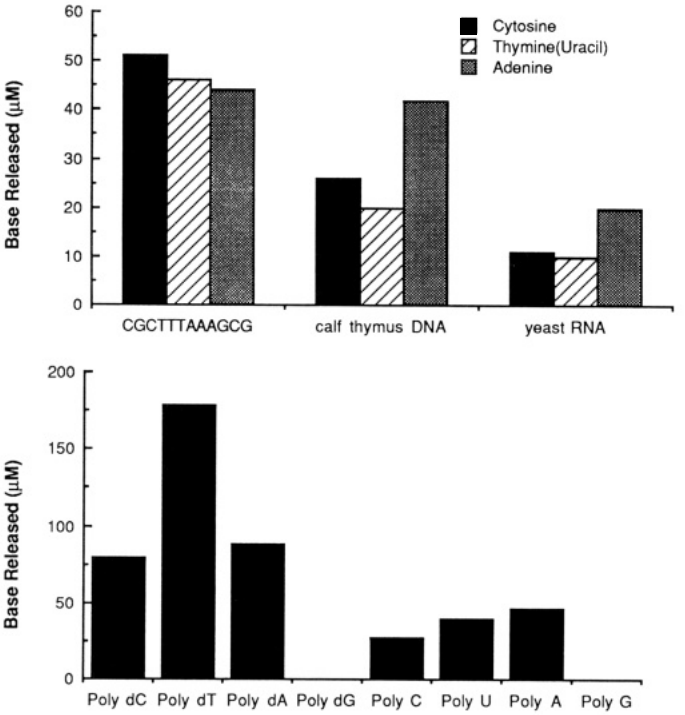


FIGURE 1: Analysis of nucleic acid bases released from polynucleotides following treatment with 35 μM MnTMPP + $KHSO_5$. The upper panel illustrates base release from DNA and RNA; the lower panel summarizes the results obtained with synthetic homopolymers and substrates. No significant base release was observed unless both MnTMPP and $KHSO_5$ were present.

methylene-5-oxo-2*H*,5*H*-furan (Goyne & Sigman, 1987), identified by comparison with an authentic sample. Although no guanine was released from poly (dG), poly (rG), or any other tested substrate, MnTMPP treatment of radiolabeled oligo$(dG)_{20}$ (PAGE analysis) revealed extensive degradation. Given the well-documented release of guanine and other free bases that attends polynucleotide degradation by reagents believed to generate diffusible oxygen radicals (Hertzberg & Dervan, 1984; Sigman, 1986), and the lack of degradation of guanine by $KHSO_5$-activated MnTMPP, these data argue that MnTMPP-mediated polynucleotide degradation involves binding of the porphyrin to its substrates prior to degradation. Also consistent with this view was the finding that the substrates protected the activated porphyrin from self-destruction: the intensity of the Soret band at 462 nM was unchanged under reaction conditions [35 μM MnTMPP, 0.9 mM $KHSO_5$, 4 mM d($CGCT_3A_3GCG$)] similar to those in Figure 1, but diminished >90% in the absence of polynucleotide (Figure 2).

Spectroscopic studies (Pasternack et al., 1985) have revealed little interaction between MnTMPP and mononucleotides, thereby suggesting that electrostatic interactions might not be of prime importance in cationic porphyrin–nucleotide complexation. We have found, however, that two nucleoside 3′,5′-diphosphates, pGp and pAp, were degraded extensively under conditions identical with those employed for the oligo- and polynucleotide substrates in Figure 1. HPLC analysis revealed the presence of several products, which could not be identified readily, but the absence of any guanine from pGp and the presence of only minor amounts of adenine from pAp. While the absence of guanine would have been predicted on the basis of results obtained with oligo- and polynucleotide substrates (vide supra), the fact that adenine was not a major degradation product formed from pAp suggests that MnTMPP-mediated degradation of the nucleoside diphosphates may proceed in a fashion different from that of

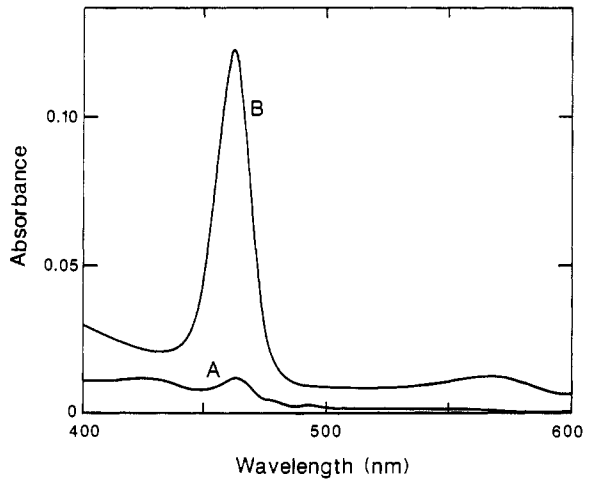


FIGURE 2: Visible spectrum of MnTMPP after activation with Oxone in the presence and absence of a DNA oligonucleotide. Individual reaction mixtures contained 35 μM MnTMPP and 0.9 mM $KHSO_5$ in the absence (A) or presence (B) of 4.0 mM d($CGCT_3A_3GCG$).

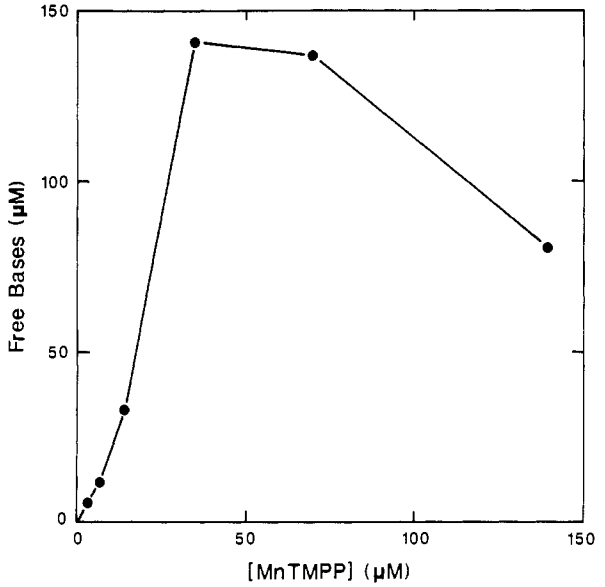


FIGURE 3: Effect of MnTMPP concentration on free base release from d($CGCT_3A_3GCG$). All other conditions were the same as those used for the experiments summarized in Figure 1 (see Experimental Procedures for details).

the substrates summarized in Figure 1.

For several of the substrates listed in Figure 1, studies of the time course of degradation indicated that the reactions were complete within 5 min. The effect of varying the concentration of MnTMPP was also studied. As shown in Figure 3, the yield of free bases increased with increasing MnTMPP, with maximum degradation occurring at 35 μM MnTMPP. At 140 μM MnTMPP, the yield of free bases diminished to ~60% of the maximum value. The reasons for this decrease seem likely to include (bimolecular) self-inactivation of the activated species at higher concentrations of MnTMPP, analogous to what is demonstrated in Figure 2. A similar effect was noted when Oxone concentration was varied; free base yields increased as the concentration of Oxone was raised to 3 mM and decreased at higher Oxone concentrations (data not shown).

Also studied was the effect of ionic strength on the ability of MnTMPP + $KHSO_5$ to degrade DNA. Relaxation of supercoiled ccc DNA occurred with greatest efficiency in the presence of 80–150 mM NaCl (data not shown). When the degradation of d($CGCT_3A_3GCG$) was carried out in 20 mM

Table I: Effect of Buffer and Salt Concentration on the Degradation of d($CGCT_3A_3GCG$) by MnTMPP + Oxone

sodium phosphate buffer, pH 7.2 (mM)	NaCl (mM)	free bases (μM)
20[a]		117
50[a]		178
100[a]		36
20[b]		94
20[b]	200	47
20[b]	400	18

[a] Carried out as indicated under Experimental Procedures with a 5-min incubation period. [b] Carried out with a 2-min incubation period.

sodium phosphate (pH 7.2), the total yield of free bases was insensitive to ionic strength at moderate NaCl concentrations, but the ratios of individual bases varied significantly at NaCl concentrations of <50 mM (not shown). As illustrated in Table I, higher salt concentrations actually decreased the overall extent of oligonucleotide degradation, consistent with the diminished DNA binding constant demonstrated for cationic porphyrins under such conditions (Strickland et al., 1988). The effect of increased buffer concentrations was even more pronounced; e.g., the total yield of free bases in 100 mM sodium phosphate (pH 7.2) was about one-fifth of that obtained in 50 mM sodium phosphate at the same pH. The efficiency of free base formation was determined in each of several buffers. The total yields of free bases were comparable in sodium phosphate, sodium arsenate, and sodium cacodylate buffers; the overall yield of products was somewhat lower in Tris-HCl. No release of free bases was observed in reactions buffered with sodium Hepes or sodium Pipes, consistent with the report that such tertiary amines are susceptible to oxidation by metal complexes (Wang & Sayre, 1989).

To determine possible sequence selectivity in the degradation of DNA by activated MnTMPP, and better define G-site reactivity, a 5′-^{32}P end labeled 127 base pair (bp) restriction fragment from SV40 DNA was incubated in the presence of MnTMPP and $KHSO_5$. As shown in Figure 4, admixture of MnTMPP and $KHSO_5$ resulted in DNA breakage at several sites; over a 10-fold concentration range, the extent of strand scission was in proportion to the amount of $KHSO_5$ present. Inspection of the sites of cleavage for this DNA duplex indicated that most occurred at G residues that were part of 5′-TG-3′ or 5′-AG-3′ sequences, although a few T and C residues produced bands of comparable intensity. Because the apparent specificity for cleavage at guanosine was inconsistent with recent reports from the Dabrowiak laboratory (Ward et al., 1986, 1987; Dabrowiak et al., 1989), we also investigated a 3′-^{32}P end labeled 139-bp fragment derived from pBR322 DNA that contained a greater proportion of AT-rich regions. As shown in Figure 5, most of the strand breaks for this DNA duplex occurred at T residues, although damage at other sites was also evident (Ward et al., 1986, 1987). It may be noted that these DNAs were not heated to effect denaturation prior to application to the polyacrylamide gels because of the discovery of an abundant latent, but chemically labile lesion (vide infra); accordingly, some of the strand breaks actually present in the above experiments could have been (partially) obscured.

Agents that mediate oxidative transformation of DNA sugars at C-1′ or C-4′ can create lesions that lead to depurination/depyrimidination and the formation of alkali-labile lesions, but not to immediate DNA strand scission (Hertzberg & Dervan, 1984; Hecht, 1986; Kappen et al., 1987). Accordingly, samples of DNA that had been treated with MnTMPP + $KHSO_5$ were subsequently subjected to 0.1 N NaOH. As shown in Figure 4, the number of DNA breaks

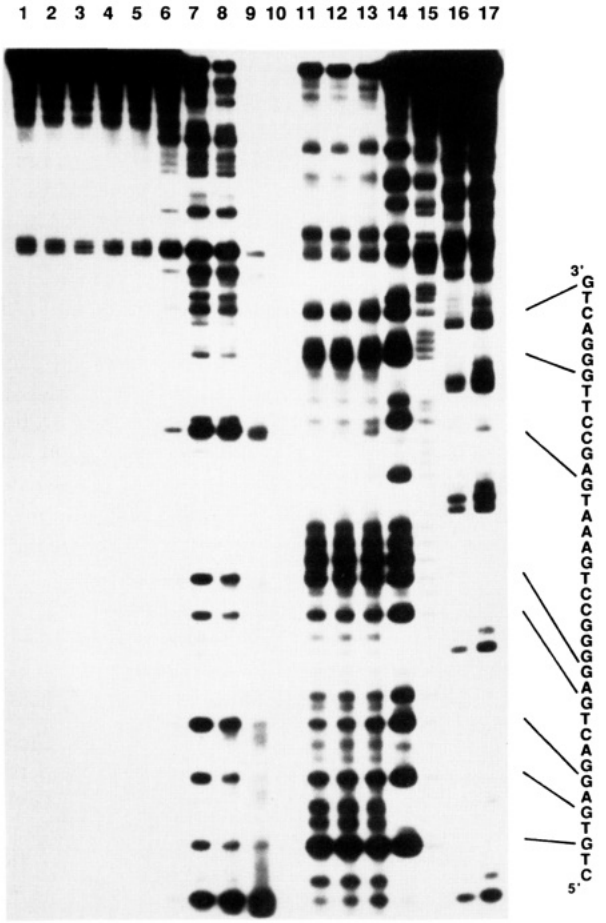


FIGURE 4: Polyacrylamide gel electrophoretic analysis of a 5′-^{32}P end labeled 127-bp SV40 DNA restriction fragment following treatment with 2.0 μM MnTMPP + $KHSO_5$. Lane 1, DNA alone; lane 2, MnTMPP; lane 3, 100 μM $KHSO_5$; lane 4, control alkali treatment; lane 5, 10 μM $KHSO_5$ followed by alkali treatment; lanes 6–10, MnTMPP plus 10, 20, 50, 75, and 100 μM $KHSO_5$, respectively; lanes 11–13, MnTMPP plus 2, 5, and 10 μM $KHSO_5$, respectively, followed by alkali treatment; lanes 14–17, Maxam–Gilbert sequencing reactions (Maxam & Gilbert, 1980) (G, G + A, C, C + T, respectively). Densitometric analysis indicated that the extraneous bands in the control lane constituted <5% of the DNA present.

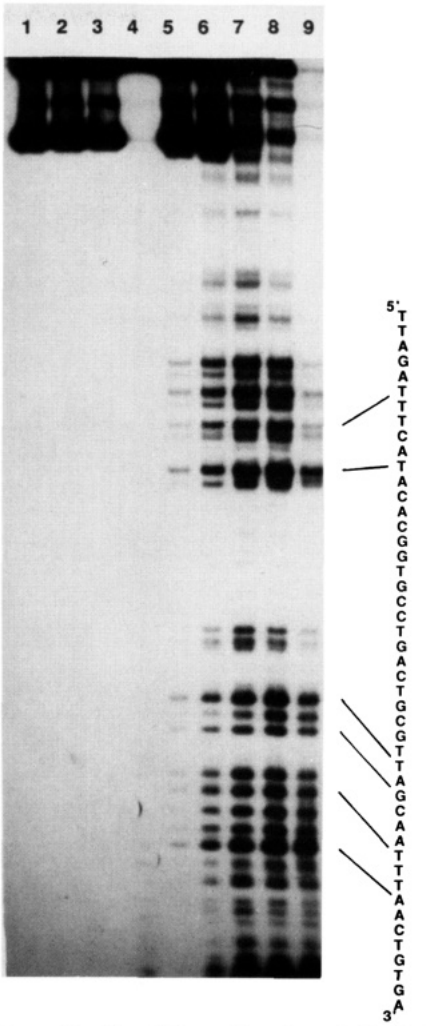


FIGURE 5: Polyacrylamide gel electrophoretic analysis of a MnTMPP + $KHSO_5$ treated 139-bp, 3′-^{32}P end labeled DNA restriction fragment from pBR322. Lane 1, DNA alone; lane 2, 2 μM MnTMPP; lane 3, 100 μM $KHSO_5$; lane 4, control alkali treatment; lanes 5–9, 2 μM MnTMPP plus 5, 10, 25, 50, and 100 μM $KHSO_5$, respectively. Reaction mixtures in which the DNA was treated successively with MnTMPP + $KHSO_5$, and then with alkali, failed to provide any precipitate upon treatment with ethanol, presumably reflecting extensive DNA degradation.

increased dramatically upon alkali treatment (cf. lanes 6 and 13). Also altered substantially was the pattern of DNA strand scission; while some breaks were seen at virtually every position, exposure of the gels for shorter periods of time made it clear that strand scission occurred overwhelmingly at G residues. Thus, contrary to earlier reports (Ward et al., 1986, 1987), the cleavage noted in AT-rich regions would seem to obtain only in substrates that are intrinsically AT rich and are far less abundant than the predominant lesions that appear to be different chemically and occur primarily at guanosine residues.

Activated manganese porphyrins have been shown previously to effect the oxidation and oxygenation of alkenes and alkanes (Meunier, 1986; Groves & Stern, 1988); the reactive species is believed to be a high-valent oxo manganese porphyrin complex, similar to the "ferryl" intermediate implicated in the cytochrome P-450 cycle (White & Coon, 1980). Therefore, while the predominant reactivity at G residues was not anticipated based on the initial HPLC results (Figure 1) or earlier studies with metalloporphyrins that omitted alkali treatment, it is logical in view of the substantially lower oxidation potential of guanosine in comparison with other DNA nucleotides (Brabec, 1980) as well as the report of some base oxidation by metalloporphyrins tethered to oligonucleotide probes (Le Doan et al., 1987). Presumably, the greater sequence selectivity of DNA cleavage observed by the use of sequencing gels as compared with that indicated by HPLC analysis of free base release (cf. Figures 4 and 5 and Figure 1) reflects the more limited extent of DNA degradation employed in experiments involving PAGE sequence analysis. It may be noted that for both 5′ and 3′ end labeled DNAs, the products created by activated MnTMPP comigrated with the DNA sequencing bands, suggesting that 3′- and 5′-phosphate termini had been formed at the sites of the breaks; this type of product is characteristic of a number of DNA damaging agents that function by oxidative mechanisms (Kappen & Goldberg, 1978; Hertzberg & Dervan, 1984; Kuwabara et al., 1986; Hecht, 1986).

In order to assess the possible effects of hemin and related species on cellular RNAs, we also investigated the effects of $KHSO_5$-activated MnTMPP on a 5′-^{32}P end labeled RNA molecule 47 nucleotides in length and containing two potential hairpins. As shown in Figure 6, the RNA substrate was degraded extensively at the higher concentrations employed but distinct, individual bands could not be detected, suggesting that multiple modes of RNA degradation may be operative.

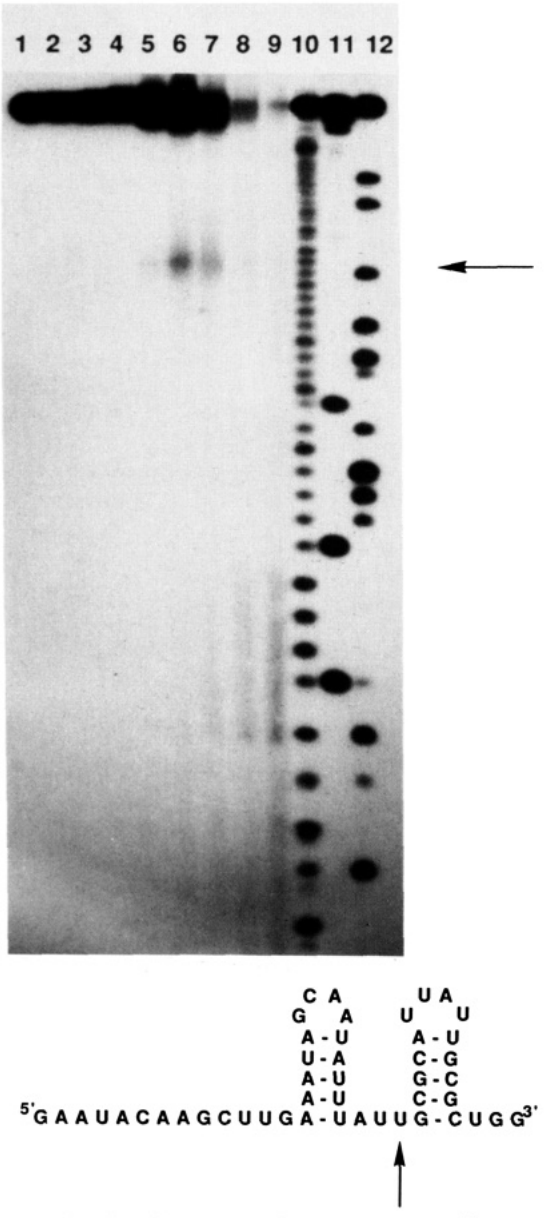


FIGURE 6: (Top) PAGE analysis of a 47-nucleotide 5′-^{32}P end labeled RNA molecule. Lane 1, RNA alone; lane 2, 2.0 μM MnTMPP, lane 3, 500 μM $KHSO_5$; lanes 4–9, 2.0 μM MnTMPP + 10, 25, 50, 100, 200, and 500 μM $KHSO_5$, respectively; lanes 10–12, sequencing reactions (alkaline hydrolysis, RNase T_1 digestion (G) and RNase U_2 digestion (A > G), respectively). Densitometric analysis of lane 6 indicated no loss of total RNA concomitant with production of the observed band centered at U_{30}. (Bottom) Structure of RNA molecule and approximate position of enhanced cleavage (↑) by MnTMPP + $KHSO_5$.

Also apparent in the figure (lanes 6 and 7) is substantially enhanced degradation in the single-stranded region connecting the two hairpins under conditions of limited RNA degradation. Both of these findings were verified in an independent series of experiments (data not shown). The observation that the RNA substrate was cleaved preferentially between the potential hairpins suggests that MnTMPP may also be a useful probe of nucleic acid *conformation* (Barton & Raphael, 1985) and could constitute a possible molecular basis for selectivity of porphyrin interaction with nucleic acids. Although the generality of this process is unclear at present, the potential biological implications are obvious and of considerable interest.

The accumulated data extend our understanding of the way in which metalloporphyrins interact with polynucleotides. Past studies have focused nearly exclusively on the binding and degradation of double-stranded DNA by metalloporphyrins. For metalloporphyrins having axial ligands, interaction has been suggested to result primarily from binding in the minor groove of DNA (Pasternack et al., 1983). Clearly, when single-stranded DNAs or RNAs are employed as substrates, binding must occur in some fashion other than by mechanisms such as intercalation or groove binding. Nonetheless, the observation that both single- and double-stranded substrates exhibit comparable degrees of reactivity toward MnTMPP + $KHSO_5$ suggests that the interactions between metalloporphyrins and single-stranded substrates can be quite substantial.

One possible binding mode between MnTMPP and single-stranded substrates could involve electrostatic interaction of the positively charged *N*-methylpyridinium groups with the negatively charged DNA phosphate oxygens. This could result in juxtaposition of the porphyrin metal center close to the DNA sugars. The fact that DNA degradation is diminished substantially at moderate concentrations of phosphate buffer (Table I) is consistent with this suggestion, in that it could reflect the ability of the phosphate buffer to compete with DNA phosphates for MnTMPP binding.

Although they do not possess the rigid conformation of ds DNA, π interactions between adjacent nucleotide bases in single-stranded polynucleotides are known to result in base stacking in such species as well (Cantor et al., 1966; Mellema et al., 1984). Since porphyrins have been shown to participate in π interactions with molecules containing aromatic rings (Fulton & LaMar, 1976a,b; Williamson & Hill, 1987), it seems reasonable to anticipate that such porphyrins may also interact with ss polynucleotides via (partial) intercalation. Clearly, binding studies are needed to determine the strength of the interaction between cationic porphyrins and single-stranded polynucleotide substrates. Also needed to define the nature of the binding to such substrates are studies of the sequence selectivity of cleavage of single-stranded oligonucleotides in direct comparison with duplexes in which one of the two strands has the same sequence as the single-stranded substrate.

It is clear from the HPLC results and PAGE analysis that polynucleotide degradation by MnTMPP + $KHSO_5$ involves the production of more than one type of lesion. Indeed, the observed release of free bases could result from oxidative transformation of the DNA sugars at more than one position (Pfitzner & Moffatt, 1965; Uesugi et al., 1982; Charnas & Goldberg, 1984; Hecht, 1986; Goyne & Sigman, 1987). That 2-methylene-5-oxo-2*H*,5*H*-furan (Goyne & Sigman, 1987) was observed as a degradation product resulting from treatment of poly(dA) with MnTMPP + $KHSO_5$ suggests strongly that oxidative attack at C-1′ of deoxyribose represents at least one route by which MnTMPP degrades DNA. Additionally, the analyses of DNA degradation carried out by polyacrylamide gel electrophoresis (Figures 4 and 5) indicate that at least one degradative process leads directly to DNA strand scission, a type of oxidative transformation demonstrated for reagents that effect oxygenation of C-4′ or C-5′ of deoxyribose (Charnas & Goldberg, 1984; Hecht, 1986).

At least under the conditions employed for degradation of 5′-^{32}P end labeled DNA restriction fragments (Figure 4), the majority of DNA lesions resulted in the production of alkali-labile sites; these occurred primarily at G residues. This observation, and the lack of formation of free guanine as judged by HPLC analysis, suggest that oxidative modification of guanine residues may constitute the major pathway of DNA degradation by $KHSO_5$-activated MnTMPP. Loss of the

oxidized guanine residues (Subramanian et al., 1987) via solvolysis could afford apurinic lesions sensitive to subsequent treatment with alkali.

Although the HPLC profiles of bases released following treatment of DNA and RNA substrates with MnTMPP + $KHSO_5$ were not dissimilar, the lack of distinct bands on polyacrylamide gels following RNA degradation suggests that RNA cleavage may be more complicated mechanistically than that of DNA. Presumably, any differences in the chemistry of degradation of DNA and RNA are determined at the level of individual nucleotides, although the possibility that RNA secondary structure also plays a role cannot be excluded.

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