Cationic porphyrins as probes of DNA structure

Susan D.Bromley, Brian W.Ward and James C.Dabrowiak*

Department of Chemistry, Syracuse University, Syracuse, NY 13244-1200, USA

Received 22 August 1986; Revised and Accepted 24 October 1986

ABSTRACT

The DNA binding specificity of a group of cationic manganese porphyrin complexes has been examined using DNase I footprinting methodology and by observing the sites of porphyrin-induced DNA strand scission in the presence of potassium superoxide. The compounds, which possess systematic changes in total charge, its distribution on the periphery on the macrocycle and ligand shape, bind in the minor groove of AT rich regions of DNA. While changes in total charge and charge arrangement do not significantly influence specificity, a shape change which blocks close ligand contact with the minor groove relaxes the original AT specificity causing the compound to cleave at both AT and GC sites. The observed changes in binding sequence specificity were interpreted in terms of electrostatic and steric factors associated with both the compounds and DNA.

INTRODUCTION

The study of metal ion-DNA interactions is undergoing a resurgence. While the early work in the area employed simple aquated metal salts in studies with DNA (1) more recent efforts have utilized metal compounds containing a variety of different types of coordinated ligands. For example, cationic complex ions containing coordinated ammonia and various substituted o-phenanthrolines have been found useful in studies of DNA conformation (2-4). With interest stimulated largely by the antitumor antibiotic bleomycin (5), metal complexes have also been designed which can cleave DNA. The Fe-EDTA complex is particularly useful in this regard and in the presence of an activating agent it is capable of DNA strand scission. This compound, when attached to various groups or by itself, has proven useful in identifying the interaction sites of DNA binding ligands and in uncovering certain elements of DNA structure and conformation (3,6,7). DNA strand scission can also be elicited from *bis* (o-phenanthroline) copper (I)(8), and studies with this compound have shown that it may be a useful agent for uncovering sequence dependent changes in DNA structure.

Metalloporphyrins are the most extensively studied type of coordination compound (9). Aside from their importance as prosthetic groups for a host of

naturally occurring enzymes and proteins, certain water-soluble, cationic metalloporphyrins have found use in studies with DNA. Fiel, Pasternack, and Kelly, their coworkers (10, 11, 12), have shown that the metal complexes of *meso*-tetra-(N-methyl-4-pyridyl) porphine, $H_2T4MPyP$ cannot only bind to DNA but that the mode of binding (both outside binding and intercalation have been observed) and base preferences exhibited by the compounds are strongly influenced by the nature of the bound metal ion and porphyrin structure. DNase I footprinting studies involving the porphyrins and a 139-base pair restriction fragment of pBR-322 DNA revealed that Mn^{+3} , Fe^{+3} , Co^{+3} and Zn^{+2} - $T4MPyP$ bind in the minor groove of AT rich sequences of DNA while Cu^{+2} and Ni^{+2} - $T4MPyP$ bind to both AT and GC sites (13). It has also been shown (14) that in the presence of various activators, Mn^{+3} , Co^{+3} , and Fe^{+3} - $T4MPyP$ can cleave DNA.

In an effort to determine if, and to what extent, certain properties of a DNA binding ligand can influence its binding sequence preference, we have examined the DNA cleavage specificity of a series of outside binding cationic porphyrin complexes of manganese, Figure 1. One group of compounds, four isostructural, atropisomeric, porphyrins, have the same total charge, +4, but different distributions of charge within the porphyrin framework. Also part of the study was a second group of isostructural cationic porphyrins having different amounts of total charge, and in the case of a dication, charge arrangement on the macrocyclic ligand. Since the compounds are rigid structures having no or only limited hydrogen bonding capabilities, and they can be activated to produce DNA strand scission, they offer a unique opportunity to ascertain how systematic changes in total charge, charge distribution, and molecular shape influence ligand-DNA recognition.

MATERIALS AND METHODS

Calf thymus DNA and DNase I were purchased from Sigma. Restriction enzymes, phage T4 polynucleotide kinase, bacterial alkaline phosphatase, AMV reverse transcriptase, bovine serum albumin, and plasmid pBR 322 DNA were purchased from Bethesda Research Laboratories and used in accordance with supplier specifications. The 139 base pair, 3' end labeled fragment (*Hind*III site) was prepared as earlier described by Lown, *et al.* (15).

Synthesis of the Compounds

The four atropisomers of *meso*-tetra(6 methyl-N-methyl-2-pyridyl) porphine were synthesized via standard procedures (16) by refluxing 3-methyl-2-pyridine carboxaldehyde (17) with pyrrole in propionic acid. Following workup, the four atropisomers were separated on silica gel using CH_2Cl_2/CH_3OH as an

Figure 1. The diprotonated forms of the cationic porphyrins used in the study are shown.

eluent. The pyridine nitrogens of each isomer were methylated overnight at room temperature using a large excess of methyl iodide in acetonitrile-methanol. Metallation, monitored using absorption spectroscopy, was accomplished in water with an excess of MnCl_2 at room temperature (18). The four atropisomeric Mn^{+3} complexes, Mn-aaaa , $\text{Mn-aaa}\beta$, $\text{Mn-a}\beta\beta$, and $\text{Mn-a}\beta\alpha\beta$ were isolated as their perchlorate salts.

The toluene-pyridinium series of compounds *meso*- α,β,γ -tritolyl- δ -(*N*-methyl-4-pyridiniumyl)porphine, *1+*, *meso*- α,β ditolyl- γ,δ -di(*N*-methyl-4-pyridiniumyl)porphine, *cis*-2+, *meso*- α,γ -ditolyl- β,δ -di(*N*-methyl-4-pyridiniumyl)porphine, *trans*-2+ and *meso*- α -tolyl- β,γ,δ -tri(*N*-methyl-4-pyridiniumyl)porphine, *3+*, were synthesized using the mixed aldehyde method (19). Methylation was conducted at room temperature in CH_2Cl_2 in the presence of excess methyl tosylate. The quaternized porphyrins were metallated in

dimethylformamide to give Mn^{+3} complexes which were isolated as their perchlorate salts, $Mn-1+$, $Mn-cis-2+$, $Mn-trans-2+$, and $Mn-3+$. The Mn^{+3} complex of *meso*-tetra-(*N*-methyl-4-pyridiniumyl) porphine, $Mn-T4MpyP$, was synthesized as earlier described (18). All unmetallated compounds were characterized by proton nmr and optical spectrosopes as well as through reference to their chromatographic R_f values (20).

The 138 Base Pair 5' End Labeled Fragment (*Hind* III site).

The restriction fragment was prepared by cleaving pBR322 DNA with *Hind* III in a total volume of 50 μ L. The digest solution contained: 7 μ L of a 4.5 mM base pair solution of the plasmid; 2 μ L of 0.1M β -mercaptoethanol; 5.0 μ L of *Hind* III (10 units/ μ L); 5.0 μ L of bovine serum albumin (1.0 μ g/ μ L); 5.0 μ L of a buffer containing 500 mM Tris-Cl pH 7.5, 300 mM NaCl, and 100 mM $MgCl_2$; 31 μ L of deionized water. The mixture was allowed to react for 12 hr at 37°C. The protein was removed by phenol extraction as described by Maniatis *et. al.* (21), followed by ethanol precipitation and 70% ethanol wash (to remove excess salts) to recover the linerized plasmid.

The precipitated DNA was dissolved in a solution containing the following: 5.0 μ L of the buffer solution described above, 5 μ L β -mercaptoethanol; 5.0 μ L bovine serum albumin (1.0 μ g/ μ L); 5.0 μ L of bacterial alkaline phosphatase (250 units/ μ L); 30 μ L of deionized water. The solution was allowed to react for 12 hr at 65°C after which time the DNA was recovered as described above. The pelleted DNA with the 5'phosphates removed was dissolved in 16.5 μ L of deionized water. This was subsequently added to 500 μ Ci of lyophilized [γ - ^{32}P]ATP along with 2.5 μ L of the above buffer solution, 1 μ L of phage T4 polynucleotide kinase, 2.5 μ L of β -mercaptoethanol and 2.5 μ L of bovine serum albumin (1 μ g/ μ L). This mixture was allowed to react for 1 hr at 37°C. The labeled DNA was recovered as above.

The double 5' end labeled linerized plasmid DNA was dissolved in the following reaction solution: 5.0 μ L of a buffer solution containing 100 mM Tris-Cl, 50 mM NaCl, and 100 mM $MgCl_2$; 5.0 μ L of *Nci* I (10 units/ μ L); 2.0 μ L of β -mercaptoethanol; 5.0 μ L of bovine serum albumin (1 μ g/ μ L); 38 μ L of deionized water. After reacting at 37°C for 8 hr, 50 μ L of the 70% glycerol solution described above was added and the mixture was loaded into a non-denaturing 8% polyacrylamide (29:1 acrylamide: *bis*-acrylamide) gel and electrophoresed for 6 hr at 600 V. Following electrophoresis, the gel was subjected to autoradiography and the appropriate band corresponding to the 138 base pair fragment was excised from the gel. The DNA was removed from the gel by agitation of a shaker-bath over a 48 hr period in the presence of 20 mM Tris-Cl buffer, pH 8.0. The DNA was collected in the supernatant upon

filtration through a glass wool plug (21), and was subsequently precipitated with ethanol, centrifuged, lyophilized, and resuspended in 50 mM Tris-Cl, 1 mM EDTA buffer, pH 7.5. The labeled fragment was stored at 5°C until needed.

Porphyrin-Induced DNA Cleavage Reactions.

The porphyrin cleavage reactions were carried out in a 50 mM Tris-Cl, 0.1 mM EDTA, pH 7.5 buffer in the manner described by Ward *et. al.* (14). The reactions were carried out in a total volume of 8 μ L which contained, as final concentrations, 192 μ M sonicated calf thymus DNA (base pairs), $\sim$ 2 μ M fragment (3' or 5' end labeled) and 5mM freshly prepared aqueous potassium superoxide. The values of r_t employed, where r_t is the ratio of porphyrin to DNA base pairs, were in the range $0.001 \leq r_t \leq 0.5$. In the case of Mn-1+ and Mn-*trans*-2+, their low solubility in water required predissolution of the compounds in aqueous methanol. The amounts of methanol in the various experiments with these compounds was < 15%.

The concentrations of Mn⁺³-porphyrin complexes were determined spectrophotometrically using the following optical data, $\lambda_{\max}(\epsilon, \text{mM}^{-1}\text{cm}^{-1})$: Mn-T4MPyP, 463(92.0); Mn-3+, 464(89); Mn-*cis*-2+, 467(118); Mn-*trans*-2+, 466(127); Mn-1+, 467(103); Mn-all atropisomers, 452(119).

The porphyrin-DNA solutions were allowed to equilibrate for 30 min prior to the addition of the activating agent, aqueous KO₂, after which time they were incubated for an additional 20 min at 37°C. To the reaction was then added 10 μ L of a solution containing 70% aqueous urea, 20 mM EDTA and 0.025% each of bromophenol blue and xylene cyanol and loading buffer followed by heating to 90°C for 5 min. Electrophoresis, autoradiography, and microdensitometry were carried out as earlier described (13, 14, 22). The size of the "bar" on the histograms presented showing the site of cleavage is proportional to the maximum optical density derived from the linear scanning through the band (nucleotide) in question. Establishment of sequence was accomplished by guanine specific cleavage of DNA according to Maxam and Gilbert (23) and by reference to the cleavage pattern of the fragment in the presence of DNase I (15).

DNase I Footprinting Experiments

The DNase I footprinting experiments were carried out in the manner previously described by Ward *et. al.* (13).

The footprinting experiments were carried out in a total volume of 8 μ L which contained, as final concentrations, 192 μ M sonicated calf thymus DNA (base pairs), $\sim$ 2 μ M fragment (3' labeled) and porphyrin at the porphyrin to DNA base pair ratios, r_t , indicated below. After addition of porphyrin, the mixture was allowed to incubate for approximately 30 min at room temperature,

Figure 2. Densitometric scans of autoradiographic data showing the sites of strand scission by the Mn^{+3} -atropisomers on the 139 base pair restriction fragment of PBR-322 DNA. The data were obtained using a DNA concentration of 770 μM (BP) and an r_t of 0.5. The absorbance scale is the same for all experiments shown.

Figure 3. Histograms representing the strand scission activity of the Mn^{+3} -atropisomers on a portion of the restriction fragment are shown.

followed by adding, 2 μ L of stock DNase I solution in 50 mM Tris-Cl, 32 mM $MgCl_2$, 8 mM $CaCl_2$, in pH 7.0 buffer. The stock enzyme solution consisted of 2 μ L of enzyme, 12.1 units/ μ L, in 50% aqueous glycerol in 398 μ L of buffer. The 8 μ L solutions were allowed to react for 10 min at 37°C. Digestion was terminated upon addition of the loading buffer as described for the porphyrin cleavage reactions. The values of r_t studied were in the range $0.001 \leq r_t \leq 0.5$. All digests, footprinting and porphyrin strand scission, were terminated so as to leave approximately 70% buffer as ($\pm 5\%$) of full length fragment uncleaved (24). Electrophoresis, autoradiography, microdensitometry, and establishment of sequence was as described above.

RESULTS

In the presence of the activating agent, potassium superoxide, the porphyrin complexes $Mn-\alpha\alpha\alpha\alpha$, $Mn-\alpha\alpha\beta\beta$, $Mn-\alpha\beta\alpha\beta$, and $Mn-\alpha\alpha\alpha\beta$ were able to induce DNA strand scission with specificity which appeared to be independent of the charge arrangement on the porphyrin framework, Figures 2 and 3. The relative amounts of cleavage at each nucleotide position, as measured by densitometry, appeared to be the same for all four isomers. However, the total extent of digest induced by the porphyrins varied somewhat and was in the order $Mn-\alpha\alpha\alpha\alpha > Mn-\alpha\alpha\alpha\beta > Mn-\alpha\beta\alpha\beta \approx Mn-\alpha\alpha\beta\beta$.

From Figures 2-5 it is apparent that while the atropisomers exhibit the same cleavage pattern, they possess reduced specificity over that of $Mn-T4MPyP$, (14) and the toluene-pyridinium series of compounds. Although the atropisomers cleave at all possible analyzed sites of the fragment, positions 45-114, the regions of highest cleavage occur at nucleotide numbers, 50-45 (5'-ATAAAC-3'), 66-54 (5'-TTAGCAATTTAACT-3') and 99-84

Figure 4. Densitometric scans of autoradiographic data showing the sites of strand scission of the toluene-pyridinium series of compounds on the 139-mer, positions 45-99. The values of r_t are: Mn-1+, 0.5; Mn-*trans*-2+, 0.5; Mn-*cis*-2+, 0.5; Mn-3+, 0.02. Although the densitometry is not shown, the extent of digest in the case of Mn-T4MPyP at r_t of 0.02 was ~ 3 times that of Mn-3+ at 0.02. The absorbance scales are the same for all scans presented.

(5'-TTGTTAGATTTCATAC-3'). A fourth site is partially uncovered at nucleotide numbers 114-111 (5'-ATGA-3').

For a given cleavage region the atropisomers exhibit asymmetric cleavage

Figure 5. Histograms representing the strand scission activity of the toluene-pyridinium series of compounds.

patterns similar to Mn-T4MPyP (14). On one side of the most intense cleavage event for a given site, most commonly the 3' side, there is a gradual decrease in the amount of strand scission with increasing distance away from the site. However, on the opposite side of the site there is a sharp decrease in cleavage intensity with increasing distance from the site.

The cleavage pattern of Mn-*cis*-2+, Mn-*trans*-2+, Mn-3+ and Mn-T4MPyP are shown in Figures 4 and 5. Using the conditions described, no DNA cleavage by Mn-1+ was observed. As is shown in the figures, the toluene-pyridinium series of compounds exhibit nearly identical cleavage patterns which are different than the atropisomers. These compounds produce strong cleavages at positions 66-56 (5'-TAGCAATTAA-3') and 99-84 (5'-TTGTTAGATTTCATAC-3') with weaker cutting occurring at positions 50-45 (5'-ATAAAC-3') and 72-70 (5'-CTG-3'). Also, the total amount of cleavage produced by each compound was significantly different under identical experimental conditions and was in the order Mn-T4MPyP > Mn-3+ > Mn-*cis*-2+ > Mn-*trans*-2+. Inspection of the apparent electrophoretic mobilities of the smallest porphyrin produced 3' end labeled products (14-16 mers) using both series of porphyrins revealed that they co-migrate with their DNase I produced counterparts and with the products obtained by cleaving the fragment using the Maxam and Gilbert sequencing protocols (23).

The opposite strand (5' end labeled) cleavage events for Mn- $\alpha\alpha\beta$ are shown in Figure 6. Cleavage occurs at the following nucleotide positions: 45-69 (5'-GTTTATCACAGTTAAATTGCTAACG-3'), 74-76 (5'-CAG-3'), and 86-101 (5'-ATGAAATCTAACAATG-3'). Within these cleavage regions there are variations in cleavage intensity, with maxima occurring at positions 50 (T), 60 (A), 68 (C), 87-88 (5'-TG-3'), 92 (T), 97 (C), and 101 (G). These sites of maximum cleavage are displaced from their corresponding sites on the opposing strand

Figure 6. The strand scission activity of the atropisomer, Mn- $\alpha\alpha\alpha\beta$ (r_t , 0.5) on both strands of the 139 base pair Hind III/Nci I restriction fragment of PBR-322 DNA is shown.

Figure 7. Densitometric scans of autoradiographic data of a DNase I footprinting experiment involving Mn-3+ at r_t of 0.5 are shown. A site exhibiting a $> 50\%$ decrease in the amount of cleavage is considered strongly inhibited while one with a $< 50\%$ decrease in cleavage is weakly inhibited. Sites experiencing an increase in cleavage in the presence of the compound are enhancements.

by 2-3 base pairs; but always to the 3' end of the fragment. Unlike the 3' end labeled experiments the apparent electrophoretic mobilities of the smallest 5' end labeled oligomer (14-16 mers) were approximately one nucleotide faster than their DNase I produced counterparts but identical to those obtained by cleaving the fragment using the Maxam and Gilbert sequencing protocols (23). However, as the fragment length increased, > ~ 30 mers, the electrophoretic mobilities of fragments from both DNase I and porphyrin digests appeared to be the same.

Densitometric scans of autoradiographic data obtained from DNase I footprinting experiments involving Mn-3+ are shown in Figure 7 and the sites of altered enzyme cleavage are indicated on the sequence of the fragment in Figure 8. As is evident from the latter Figure, Mn-3+ affected enzymatic cleavage in a manner nearly identical to Mn-T4MPyP. Sites of strong inhibition, i.e. > 50% decrease in the concentration of an oligomer at $r_t = 0.5$, were at position 59-56 (5'-TTAA-3'), 91-83 (5'-TTTCATACA-3') and 97-94 (5'-GTTA-3') with weak inhibition occurring at the outer 1-2 nucleotides of these sites. For this compound, a region of enhanced cleavage at 81-68, (5'-GGTGCCTGACTGCG-3') was also observed. Where solubility permitted, increasing the value of r_t to 30-50 did not result in site specific inhibition for any of the atropisomers or the toluene-pyridinium series of compounds with charge < +3.

DISCUSSION

The manganese porphyrin complexes shown in Figure 1 possess different total charge, charge distribution, and shape. The four atropisomers, Mn- $\alpha\alpha\alpha\alpha$, Mn- $\alpha\alpha\alpha\beta$, Mn- $\alpha\beta\alpha\beta$ and Mn- $\alpha\beta\beta\beta$ are isostructural and have axial dimensions of $\sim 9\text{\AA}$. Consideration of the disposition of the pyridinium groups in the compounds reveals that the presence of the two methyl substituents block free

rotation about the carbon-carbon bond between the porphyrin *meso* carbon and the C2 carbon of the porphyrin ring. Thus, the pyridinium groups are perpendicular to the plane of the porphyrin ring giving the compounds a broad axial dimension. This structural feature contrasts with that present in the toluene-pyridinium series of compounds. As with the atropisomers these compounds are isostructural (the shapes of the toluene and pyridinium groups are similar) but since they lack substituents in positions ortho to the porphyrin-aryl single bond, they possess a reduced axial dimension, $\sim 6\text{\AA}$. Although a rotational barrier about the bond joining the pyridinium (toluene) group to the porphyrin ring exists, it is expected to be considerably lower than that of the atropisomers.

The total charge on the porphyrin ring of the atropisomers is +4, but the distribution of charge within the cyclic framework for each isomer varies. The most polar isomer, Mn- $\alpha\alpha\alpha\alpha$, has all of the charged nitrogen atoms located on one side of the porphyrin plane while Mn- $\alpha\alpha\alpha\beta$ has only three of its four charges on one side of the plane. On the other hand, the atropisomers, Mn- $\alpha\beta\alpha\beta$ and Mn- $\alpha\alpha\beta\beta$, have symmetrically displaced charges (*cis* and *trans*) on both sides of the plane. Based on their structure the latter two atropisomers isomers as well as Mn-T4MPyP and Mn-*trans*-2+ in the toluene-pyridinium series are expected to have no net dipole moment.

Due to their cationic charge, all of the compounds studied are expected to bind to DNA. However, the "thickness" of the ligand framework (the atropisomers) and the presence of the axial substituents on the Mn⁺³ ion (both series) block porphyrin intercalation between the base pairs of DNA. Molecular models indicate that "end-on" binding, wherein the porphyrin contacts the surface of the the minor groove of DNA, is possible for the toluene-pyridinium series of compounds, but, without distortion of DNA, is not possible for the atropisomers.

Earlier studies with the parent complex, Mn-T4MPyP, revealed that the compounds can be activated with potassium superoxide to produce DNA strand scission (14). Kinetic studies employing closed circular PM2-DNA and optical measurements monitoring porphyrin autodestruction in the presence of superoxide revealed that the porphyrin remains largely intact during the strand scission process and thus is the species inducing DNA breakage. Analysis of the cleavage sites on a 139 base pair segment of pBR-322 DNA (14) indicated that the activated complex attacks all four possible nucleotides of DNA but that the preferred site is (A·T)₃. Gel electrophoretic studies strongly suggest that the group on DNA adjacent to the site of porphyrin-mediated strand scission is 5'-phosphate (14).

The sites of DNA strand scission produced by the atropisomers and the toluene-pyridinium series of compounds are shown in Figures 2-6. In the case of Mn- $\alpha\alpha\alpha\beta$, analysis of the cleavage events on both strands of the restriction fragment shows that sites of maximum cleavage on one strand relative to those on the opposing strand are shifted 2-3 base pairs in the direction of the 3' end of the fragment. This type of strand scission pattern, which has been previously observed for other minor groove binding ligands equipped with EDTA-Fe(II) (3), is consistent with Mn- $\alpha\alpha\alpha\beta$ interacting with the minor groove of DNA. Since Mn-T4MPyP shows similar skewing in its DNase I protection patterns (13), all of the compounds shown in Figure 1 likely bind in the minor groove of DNA.

In the absence of hydrogen bonding to DNA and with intercalation blocked due to steric effects, the ability of these compounds to select AT rich regions of the polymer is apparently electrostatic/steric in origin. Theoretical calculations by Kollman and Pullman and their coworkers (25, 26) have shown that although many factors can influence the outcome of the calculations, the surface of the minor groove of AT rich regions of DNA possesses a high negative potential. Thus, the AT specificity observed for all of the compounds studied may be related to strong electrostatic attractive forces between them and the dipoles located on adenine and thymine in the minor groove of DNA. However, consideration of the structures of both DNA and the compounds strongly suggests that steric effects may also influence the preference of the compounds for AT sites. X-ray crystallographic studies have revealed that the 2-amino group of guanine extends above the floor of the minor groove of DNA (27). In the absence of appropriately located hydrogen bonding acceptor sites on the ligand (as is the case with the porphyrins) the 2-amino group of guanine, discourages ligand binding through steric effects. It is this factor which is in part responsible for the high degree of AT specificity exhibited by the minor groove binding agent netropsin (27). Thus, for those porphyrins which can easily end on bind within the minor groove of DNA, i.e. the toluene-pyridinium series of compounds, both electrostatic and steric effects encourage binding at AT sequences of the polymer. However, as is the case with the atropisomers if the shape of the molecule is modified in such a manner so as to prevent its close contact with the surface of the minor groove, thereby reducing its ability to "read" the dipoles located on the heterocycles bases in the groove, specificity is reduced, Figures 2 and 3. Reduced accessibility to the groove also lessens potential steric interactions between the 2 amino group of guanine and the porphyrins. This is probably the factor which allows a G-C base pair(s) to be present in the cleavage sequence

of the atropisomers, Figures 2 and 3. Interestingly, neither the arrangement of charge on the porphyrin nor its total magnitude significantly affects specificity. As is evident in Figures 2-5 all of the compounds studied produced nearly identical cleavage patterns within their respective series.

Figures 4 and 5 show that reduction of the total charge on the metal complex has the primary effect of decreasing the amount of cleavage produced by the compound. While a full explanation of this observation must await further study, particularly with regards to porphyrin activation and the mechanism of the strand scission process itself, the differences in cleavage appear to be due to differences in the DNA affinity constants of the compounds. Although binding constants have not been measured, electrostatic arguments indicate that the constants should decrease in the order $\text{Mn-T4MPyP} > \text{Mn-3+} > \text{Mn-cis-2+} > \text{Mn-trans-2+} > \text{Mn-1+}$. As is evident from Figures 4 and 5, this order is the same as that obtained by considering the total amounts of digest produced by each compound using identical experimental conditions. Similarly, the smaller differences in the extents of digest between the atropisomers parallels the magnitudes of their permanent net dipoles.

Comparison of the electrophoretic mobilities of oligomers produced by $\text{Mn-}\alpha\alpha\alpha\beta$ with those obtained through DNase I digest of the fragment and via cleavage using the sequencing reactions indicates that the groups on DNA immediately adjacent to the site of porphyrin strand scission are 3' and 5' phosphates. However, as is evident from studies of the cleavage chemistry of Fe-MPE, careful analysis of the shortest oligonucleotide products using enzymes sensitive to DNA terminal chemistry and high percentage crosslinked polyacrylamide gels would be necessary to fully characterize the groups remaining on DNA (3). Parallel studies in the case of the porphyrins are currently in progress.

DNase I footprinting studies with the compounds revealed that only Mn-T4MPyP(13) and Mn-3+ exhibit site specific enzymatic inhibition, Figures 7 and 8. The tri-cation inhibits the enzyme in generally the same regions of the fragment where porphyrin-induced DNA cleavage is observed. A notable exception is the cleavage site at position 72-70, having the sequence 5'-CTG-3', which is not observed in the footprinting experiment. Since this site would be expected to possess reduced affinity when compared to (A·T)₃, the large amount of porphyrin which must be present to populate the site produces DNA precipitation (13) before DNase I inhibition at the site is observed. In light of the observation that remaining porphyrins are likely to have binding constants which are lower than Mn-T4MPyP and Mn-3+ , they would be more difficult to detect than the former in the footprinting experiments

and no site specific DNase I inhibition for them was observed. As with Mn-T4MPyP, Mn-3+ exhibits enhanced cleavage at positions 68-81 of the fragment, Figures 7 and 8. Although DNA structural changes induced by porphyrin binding to DNA, and, which are sensed by the enzyme cannot be ruled out, the enhancements at these positions are probably due to an increase in the available substrate DNA for the enzyme as sites on the fragment load with porphyrin.

In summary we have shown that changes in total charge and the location of charge on the framework of the ligand have little or no effect on the DNA binding specificity of a group of cationic metalloporphyrin complexes. However, if the shape of the porphyrin is altered in such a way so as to prevent its close contact with the minor groove of DNA, the original AT specificity is relaxed and binding/cleavage can be observed at both AT and GC sequences of DNA. The study shows the importance of ligand charge and shape on DNA sequence recognition.

ACKNOWLEDGEMENT

The work was supported by a grant from the National Institutes of Health (GM 31895).

*To whom correspondence should be addressed

REFERENCES

1. Eichhorn, G.L. (1973) in *Inorganic Biochemistry*, Eichhorn, G.L. Ed, Vol.II, pp. 1191-1243.
2. Barton, J. K. (1985) *Comments Inorg. Chem.*, **3**, 321-348.
3. Dervan, P. (1986) *Science*, **232**, 464-471.
4. Behe, M. and Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 1619-1623.
5. Dabrowiak, J. C. (1982) *Adv. Inorg Biochem.* **4**, 69-113.
6. Tullius, T. D. and Dombroski, B. (1985) *Science*, **230**, 679-681.
7. Tullius, T. D. and Dombroski, B. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 5469-5473.
8. Spassky, A., and Sigman, D. S. (1985) *Biochemistry*, **24**, 8050-8056.
9. Dolphin, D. (1978), *The Porphyrins*, Academic Press, New York.
10. Fiel, R. J., Carvlin, M. J., Byrnes, R. W., and Mark, E. H. (1985) in *Molecular Basis of Cancer, Part B: Macromolecular Recognition, Chemotherapy, and Immunology*, Rein, R. Ed. pp. 215-226, Alan R. Liss, New York.
11. Pasternack, R. T., Autebi, A., Ehrlich, B., and Sidney, D. (1984) *J. Mol. Catalysis*, **23**, 235-242.
12. Kelly, J. M., Murphy, M. J., McConnell, D. J. and OhUigin, C. (1985) *Nucleic Acids Res.*, **13b**, 167-184.
13. Ward, B., Skorobogaty, A., and Dabrowiak, J. C., (1986), *Biochemistry*, **25**, 0000.

14. Ward, B., Skorobogaty, A., and Dabrowiak, J. C., (1986), *Biochemistry*, **25**, 0000.
15. Lown, J. W., Sondhi, S. M., Org, C. W., Skorobogaty, A., Kishikawa, H., and Dabrowiak, J. C., (1986), *Biochemistry*, **25**, 5111-5117.
16. Ginsburg, S. and Wilson, J. B. (1975). *J. Am. Chem. Soc.*, **79**, 481-485.
17. Longo, F. R., Finarelli, M. G. and Kim, J. B. (1969) *J. Heterocyclic Chem.*, **6**, 927.
18. Pasternack, R. F., Gibbs, E. J. and Villafranca, J. J. (1983) *Biochemistry*, **22**, 5409-5417.
19. Franco, C. and McLendon, G. (1984) *Inorg. Chem.*, **23**, 2370-2372.
20. Collman, J. P., Gagne, R. R., Reed, C. A., Halbert, T. R., Lang, G. and Robinson, W. T. (1975) *J. Am. Chem. Soc.*, **97**, 1427-1438.
21. Maniatis, T., Fritsch, E. T., and Sambrook, J., (1982), *Molecular Cloning-A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor.
22. Dabrowiak, J. C., Skorobogaty, A., Rich, N., Vary, C. P. H., and Vournakis, J. N., (1986), *Nucleic Acids Res.*, **14**, 489-499.
23. Maxam, A., and Gilbert, W., (1980), *Meth. Enzymology*, **65**, 499-559.
24. Goodisman, J., and Dabrowiak, J. C., (1985), *J. Biomol. Struct. Dyn.*, **2**, 967-979.
25. Lavery, R. and Pullman, B. (1985) *J. Biomol. Struct. Dynamics*, **2**, 1021-1032.
26. Weiner, P.K., Langeridge, R., Blaney, J.M., Schaefer, R., and Kollman, P.A. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 3754-3758.
27. Kopka, M. L., Yoon, C., Goodsell, D., Pjura, P., and Dickerson, R. E. (1985), *Proc. Natl. Acad. Sci. USA*, **82**, 1376-1380.