Cleavage of DNA by electrochemically activated manganese(III) and iron(III) complexes of meso-tetrakis(N-methyl-4-pyridiniumyl)porphine

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Cleavage of DNA by Electrochemically Activated Mn^{III} and Fe^{III} Complexes of *meso*-Tetrakis(*N*-methyl-4-pyridiniumyl)porphine

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Electrochemical methods were used to activate Mn^{III} and Fe^{III} complexes of *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)porphine (H_2TMPyP) to cause cleavage of pBR322 DNA and to study their interaction with sonicated calf thymus DNA. Electrochemical reduction of Mn^{III}TMPyP and Fe^{III}TMPyP (at low concentrations) in the presence of O_2 was required to activate these complexes. However, Fe^{III}TMPyP at 1×10^{-6} M produced DNA strand breakage without being electrochemically reduced. At low concentrations, Fe^{II}TMPyP was more efficient at cleaving DNA than Mn^{II}TMPyP. Reduction of O_2 at a platinum electrode also produced some cleavage but to a much smaller extent. The oxidized form of Mn^{III}TMPyP (charge 5+) has higher affinity for sonicated calf thymus (CT) DNA than the reduced form (charge 4+), as determined by the negative shift in $E^{\circ\prime}$ for the voltammetric wave in the presence of DNA. Both forms of Fe^{III}TMPyP (charge 4+) interact with DNA to about the same extent. Differential pulse voltammetry was used to determine binding constants (K) and binding-site sizes (s) of the interaction of these metalloporphyrins with sonicated CT DNA. The data were analyzed assuming both mobile and static equilibria. Mn^{III}TMPyP binds to DNA (5 mM Tris, 50 mM NaCl, pH 7) with $K = 5\ (\pm 2) \times 10^6\ M^{-1}$, $s = 3$ bp (mobile) or $K = 3.6\ (\pm 0.3) \times 10^6\ M^{-1}$, $s = 4$ bp (static). Fe^{III}TMPyP at that ionic strength caused DNA precipitation. At higher ionic strength (0.1 M Tris, 0.1 M NaCl, pH 7), Fe^{III}TMPyP associates to DNA with $K = 4.4\ (\pm 0.2) \times 10^4\ M^{-1}$, $s = 5$ bp (mobile) or $K = 1.9\ (\pm 0.1) \times 10^4\ M^{-1}$, $s = 6$ bp (static).

INTRODUCTION

In the present work, we report cleavage of pBR322 DNA by electrochemical activation of manganese(III) and iron(III) complexes of *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)porphine in presence of molecular oxygen. We also describe differential pulse voltammetric studies of the interaction of these metalloporphyrins with sonicated calf thymus DNA.

Several species, including metal ions and metal complexes, have been attached to DNA-interactive groups to produce DNA chain scission. For example Fe^{II}–EDTA covalently bound to methidium as an intercalator (*1*, *2*), to oligonucleotides (*3–6*), and to oligopeptides/proteins (*7–9*) in the presence of O_2 and a reducing agent efficiently cleaves DNA. In a similar fashion some Fe^{III}–porphyrins associated with intercalators (*10–13*) and oligonucleotides (*14*, *15*) produce DNA cleavage. A number of metal ions such as Fe^{II}, Cu^{I}, Co^{III}, and Mn^{II} are cofactors of bleomycin and induce DNA degradation in vitro (*16–24*). The DNA cleaving ability of $Cu(phen)_2^+$ and other polypyridyl complexes of Co^{III}, Ru^{II}, Rh^{III}, Pt^{II}, Fe^{III}, Cr^{III}, and Mn^{II} have also been reported (*25–35*). Some of these metal complexes are activated by photoirradiation rather than by added chemical agents. Nuclease activity has been observed for Cu^{II} attached to peptides (*36*, *37*), adriamycin (*38*), and camptothecin (*39*) and for various metal ions like Fe^{III}, Mn^{III}, Co^{III}, and Cu^{II} complexed to porphyrins (*40–44*). Similarly acridine orange, a DNA intercalator, linked to (diaminoethane)dichloroplatinum(II) can be photoactivated to nick DNA (*45*).

Thus the interactions of porphyrins and metalloporphyrins with DNA, especially those of cationic porphyrins, such as *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)-porphine (H_2TMPyP) and its metal complexes (*46–58*), are of interest. Especially important is the observed tendency of some porphyrins to accumulate in malignant tumors and to cause tumor destruction after light irradiation (*59–73*). The water-soluble, tetracationic porphyrin H_2TMPyP is known to bind to DNA by intercalation with GC specificity (*46–50*) and to damage DNA upon photoillumination (*44*).

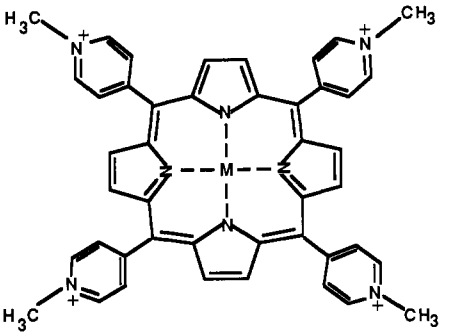

Figure 1. Structure of M^{III}TMPyP, M = Fe, Mn.

Studies have shown that metal complexes of TMPyP (Figure 1) also bind to DNA with binding modes dependent on the coordination number of the metal atom. The Mn^{III}, Fe^{III}, and Co^{III} complexes of TMPyP bind electrostatically in the minor groove at AT base pairs (*47*, *48*, *54*, *55*). These metalloderivatives of TMPyP cleave DNA in the presence of O_2 and reducing and oxidizing agents, such as ascorbate, superoxide, and iodosobenzene (*40*). However, Fiel et al. (*41*) reported that Fe^{III}-TMPyP can nick DNA without any added activating agent. Praseuth et al. (*44*) reported that photoactivation of Co^{III}-TMPyP (but not Mn^{III}TMPyP) in the presence of O_2 can induce DNA cleavage. They showed that Fe^{III}-TMPyP can degrade DNA in a few minutes without light irradiation, but that the extent of degradation increased with exposure to light.

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The mechanism of the chemically activated cleavage process includes the generation of a reduced form of oxygen produced in a redox reaction between the metal ion and oxygen. Several iron and manganese porphyrins in the presence of oxygen donor molecules such as iodobenzene, hypochloride, or alkyl hydroperoxides can catalyze oxidation of hydrocarbons through a high valent metal–oxo intermediate (*74–78*). The chemical activation of Mn^{III}TMPyP and Fe^{III}TMPyP has been suggested to occur through a similar mechanism. These activated metalloporphyrins are able to cause oxidative cleavage of the deoxyribose moiety and of DNA strands at the site of binding (*40*). Activated Fe^{III}TMPyP has been found to cause base modification of the dinucleotide dTdA (*79*).

It is thus well-established that these and other porphyrins and metalloporphyrins are very useful tools for probing nucleic acid structure, studying drug–DNA interactions, and detecting and destroying tumor and neoplastic tissue. Mn^{III}TMPyP has been used in quantitative footprinting studies of neptrosin bound to a 139 base pair restriction fragment (*80, 81*) and as a selective magnetic resonance contrast agent for human tumors in mice (*82*).

In previous reports (*83, 84*) we showed that electrochemical methods can be used to study the interaction of small electroactive molecules with DNA. In those studies we used voltammetric data to determine the binding constant and binding-site size of tris-1,10-phenanthroline (phen) and tris-2,2′-bipyridine (bpy) complexes of Co^{III} and Fe^{II} with calf thymus (CT) DNA. In this report we show that electrochemical methods can be used to activate Mn^{III}TMPyP and Fe^{III}TMPyP in the presence of O_2 to cause DNA breakage. Differential pulse voltammetric data were used to obtain quantitative information about the interaction of these metalloporphyrins with sonicated CT DNA. We have chosen these metalloporphyrins for our initial studies, because they are water-soluble and easy to synthesize and their electrochemical behavior is well-understood (*85–92*). While this work was under way, degradation of oligodeoxynucleotides by electrochemically reduced Fe^{III}–bleomycin was reported in a brief communication by Van Atta and co-workers (*93*).

EXPERIMENTAL PROCEDURES

Materials. The manganese(III) and iron(III) complexes of H_2TMPyP were synthesized according to the procedure of Pasternack et al. (*47*) and their concentrations were determined spectrophotometrically with $\epsilon_{424} = 1.02 \times 10^5$ M^{-1} cm^{-1} for Fe^{III}TMPyP (*55*) and $\epsilon_{463} = 0.92 \times 10^5$ M^{-1} cm^{-1} for Mn^{III}TMPyP (*94*). Stock solutions were prepared with 5 mM Tris–HCl, 50 mM NaCl, pH 7 (buffer 1), or 0.1 M Tris–HCl, 0.1 M NaCl, pH 7 (buffer 2), and were stored in a refrigerator (4 °C) in the dark for no more than 3 days.

Calf thymus DNA was purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification (*95*). DNA stock solutions were prepared by dissolution overnight in the buffer. Calf thymus DNA was sonicated for 1 h at 0–5 °C with the sonicator on for 1 min and off for the same amount of time. The average size of fragments (600 bp) was obtained by gel electrophoresis using the *Hae* III digest of *φX174* RF DNA as the standard. DNA concentration per nucleotide phosphate was determined by UV absorbance at 260 nm with $\epsilon_{260} = 6600$ M^{-1} cm^{-1} (*96*). Stock solutions were stored at 4 °C and discarded after no more than 3 days.

pBR322 DNA was isolated from the *Escherichia coli* strain HB101 and purified according to the procedure of Maniatis (*97*). pBR322 was dissolved in 10 mM Tris–HCl, 1 mM EDTA buffer, pH 7.4, and stored at −20 °C.

All other chemicals were of reagent grade and used as received. Solutions were prepared with water that was purified by passage through a Millipore Milli-Q system.

Instrumentation. Voltammetric [cyclic voltammetric (CV) and differential pulse voltammetric (DPV)] studies and controlled-potential bulk electrolysis (BE) experiments were done with a Bioanalytical Systems (BAS) (West Lafayette, IN) Model BAS-100 electrochemical analyzer. DPV experiments were performed under the following conditions: pulse amplitude (PA), −50 mV; pulse width, 50 ms; sweep rate (υ), 4 mV/s; sample width, 17 ms; and pulse period, 1 s.

Ultraviolet–visible absorption spectra were obtained on a Hewlett-Packard 8451 A diode-array spectrophotometer. The agarose gels were photographed with a Polaroid MP-4 camera and quantified by scanning densitometry.

CV and DPV Experiments. Voltammetric studies were carried out in a one-compartment cell, containing a Pt disk (area, 0.023 cm^2) working electrode, a Pt flag counter electrode, and a saturated calomel electrode (SCE) reference electrode. The working electrode surface was highly polished with 0.3 μm alumina paste (Buehler, Lake Bluff, IL) prior to each experiment.

Solutions were thoroughly deoxygenated, unless otherwise indicated, by bubbling with argon that had been previously saturated with water. During the data acquisition, an argon atmosphere was maintained over the solution in the cell. Before use, all glassware was silanized in 5% trimethylchlorosilane (Petrach Systems, Bristol, PA) in toluene. All the measurements were performed at 25 °C.

Bulk Electrolysis. To conserve the DNA it was necessary to do the bulk electrolysis experiments on a very small scale. Thus a microcell assembly (BAS Type MCA), consisting of an outer working electrode compartment and an inner compartment with a porous Vycor plug containing the reference and counter electrodes was used to electrolyze different amounts of the metalloporphyrins with pBR322 levels of 2 μg. The reaction mixture was contained in the working electrode compartment and the other compartment held supporting electrolyte. The working solution compartment volume was 100 μL.

The electrolysis was carried out in the dark for 12 h at −0.4 V for Mn^{III}TMPyP and at −0.3 V for Fe^{III}TMPyP. During the electrolysis O_2 was bubbled into the supporting electrolyte. The working electrode was a Pt foil (area, 1 cm^2), the counter electrode was a Pt wire, and the reference was a SCE.

Gel Electrophoresis. A fraction (20 μL) of the reaction mixture and 5 μL of the loading buffer (bromophenyl blue dye and sucrose) were loaded into the wells of a 0.8% horizontal agarose gel containing 1 μg/mL ethidium bromide. The gel was electrophoresed at 5 V/cm for 5 h in 89 mM Tris, 89 mM boric acid, 2 mM EDTA buffer, pH 8. Samples containing pBR322 and metalloporphyrin that were not electrolyzed were incubated for a period of 2 h. The same results were obtained when they were incubated for 12 h. After electrophoresis, the DNA bands were visualized and photographed under ultraviolet light. Scanning densitometry of the photographic negatives was used to quantitate the amount of supercoiled circular (form I), open circular (form II), and linear (form III) species.

RESULTS

CV of Mn^{III}TMPyP and Fe^{III}TMPyP. Figure 2 shows typical cyclic voltammograms of 0.50 mM Mn^{III}TMPyP and 0.78 mM Fe^{III}TMPyP in the absence and in the presence of molecular oxygen. In the absence of O_2 (Figure

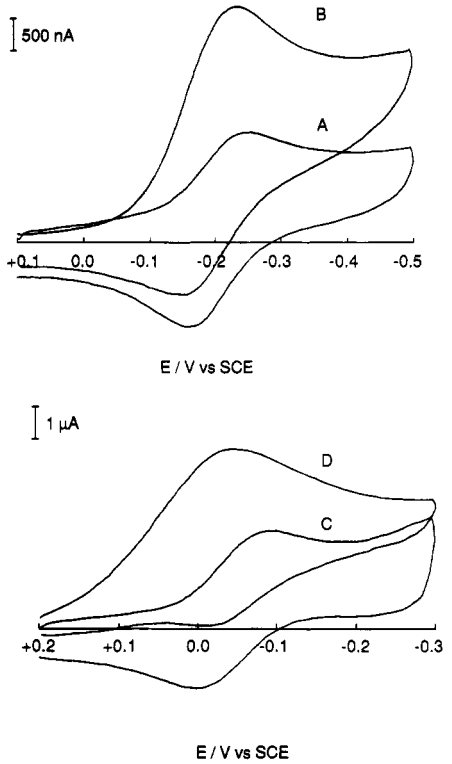

Figure 2. Cyclic voltammograms of (A, B) 0.50 mM Mn^{III}TMPyP and of (C, D) 0.78 mM Fe^{III}TMPyP, (A, C) in the absence and (B, D) in the presence of air (oxygen): sweep rate, 100 mV/s; supporting electrolyte, buffer 1.

Table I. Cyclic Voltammetric Data of Mn^{III}TMPyP in the Absence of Oxygen[a]

v, mV s^{-1}	E_{pc}, mV[b]	E_{pa}, mV	ΔE, mV	$E^{\circ\prime}$, mV	i_{pa}/i_{pc}
50	-249 (4)	-156 (5)	93	-203	0.9
100	-247 (5)	-159 (3)	88	-203	1.0
200	-250 (4)	-153 (3)	97	-202	1.0
500	-259 (3)	-146 (2)	113	-203	1.0

[a] [Mn^{III}TMPyP] = 0.50 mM; supporting electrolyte, buffer 1. [b] Numbers in parentheses are standard deviations for three measurements.

Table II. Cyclic Voltammetric Data of Fe^{III}TMPyP in the Absence of Oxygen[a]

v, mV s^{-1}	E_{pc}, mV[b]	E_{pa}, mV	ΔE, mV	$E^{\circ\prime}$, mV	i_{pa}/i_{pc}
50	-91 (4)	3 (2)	94	-44	0.9
100	-90 (4)	3 (2)	93	-44	1.0
200	-92 (2)	2 (4)	94	-45	0.9
500	-97 (4)	5 (5)	102	-46	0.8

[a] [Fe^{III}TMPyP] = 0.78 mM; supporting electrolyte, buffer 1. [b] Numbers in parentheses are standard deviations for three measurements.

2, parts A and C), reduction of M^{III} to M^{II} (M = Mn or Fe) occurs during the forward scan while the reoxidation of M^{II} occurs in the reverse scan. The cathodic and anodic peak potentials for the reduction and oxidation processes of Mn^{III}TMPyP were observed at E_{pc} = -245 mV and E_{pa} = -161 mV and of Fe^{III}TMPyP at E_{pc} = -90 mV and E_{pc} = 1 mV, respectively.

Voltammetric data obtained for both metalloporphyrins at different scan rates in the absence of oxygen are given in Tables I and II. For both compounds the cathodic peak potentials (E_{pc}) were slightly dependent on scan rate (v) and the separation of the anodic peak potential (E_{pa}) and E_{pc} (ΔE) was between 88 and 113 mV, suggesting quasireversible one-electron transfer reactions. The ratio of anodic to cathodic peak current ($i_{pa}/i_{pc} \cong 1$) indicated the absence of following reaction kinetic complications. The formal potential ($E^{\circ\prime}$), calculated as the average of E_{pc} and E_{pa}, was essentially independent of v, and for 50 mV/$s \leq v \leq$ 500 mV/s, $E^{\circ\prime}$ = -202 (±1) mV for Mn^{III}TMPyP and $E^{\circ\prime}$ = -44 (±2) mV for Fe^{III}TMPyP.

In the presence of O_2 (Figure 2, parts B and D) $i_{pa}/i_{pc} < 1$, characteristic of an electrochemical catalytic mechanism (EC′) in which the parent molecule M^{III}TMPyP is regenerated by reaction of the M^{II} form with O_2 as shown by the following formal overall reactions (*85–89*) (the actual species undergoing reaction and their charges depend upon the pH and the state of protonation, as discussed briefly in the discussion section):

$$\text{E step} \quad M^{III}TMPyP + e^- \rightleftarrows M^{II}TMPyP$$

$$\text{C′ step} \quad 2M^{II}TMPyP + O_2 + 2H^+ \rightarrow 2M^{III}TMPyP + H_2O_2$$

where M = Mn or Fe.

For Fe^{III}TMPyP, the reoxidation of the reduced form by oxygen-producing H_2O_2 occurs via an oxygen adduct in which the oxygen is bound axially to the metal (*87, 88*).

$$Fe^{II}TMPyP + O_2 \rightarrow Fe^{III}TMPyP\cdot(O_2^-)$$

This adduct is then further reduced by another reduced metalloporphyrin or at the electrode.

$$Fe^{III}TMPyP\cdot(O_2^-) + 2H^+ + Fe^{II}TMPyP \rightleftarrows 2Fe^{III}TMPyP + H_2O_2$$

or

$$Fe^{III}TMPyP\cdot(O_2^-) + 2H^+ + e^- \rightleftarrows Fe^{III}TMPyP + H_2O_2$$

Furthermore, H_2O_2 can be converted to H_2O.

$$2Fe^{II}TMPyP + H_2O_2 + 2H^+ \rightarrow 2Fe^{III}TMPyP + H_2O$$

The final product depends on the electrode material as well as on the concentration ratio of the metalloporphyrin to oxygen. At a glassy carbon electrode, oxygen is reduced primarily to H_2O_2, when the oxygen concentration is equal or higher than that of the metalloporphyrin (*88, 90*).

DPV of Mn^{III}TMPyP and Fe^{III}TMPyP in the Presence of Sonicated CT DNA. To study the interaction of the porphyrins with DNA, voltammetric studies of mixtures of the Fe^{III} and Mn^{III} species with different amounts of DNA were undertaken (*83, 84*). To conserve the pBR322 DNA, these studies were carried out with CT DNA. However under the solution conditions needed for the electrochemical studies, the addition of unmodified CT DNA caused precipitation of the porphyrins. Thus sonicated CT DNA, consisting of fragments with an average of 600 bp, were used, with low (0.1 mM) concentrations of the metal porphyrins. Typical differential pulse voltammograms of 0.1 mM Mn^{III}TMPyP and 0.1 mM Fe^{III}TMPyP in the absence of oxygen with and without sonicated CT DNA are shown in Figure 3. The average value of the peak potential (E_p^{av}) obtained by DPV in absence of CT DNA for 0.1 mM Mn^{III}TMPyP was -173 (±7) mV and for 0.1 mM Fe^{III}TMPyP was -108 (±8) mV, where the values in parentheses represent $\pm 2\sigma$ for five measurements.

The DPV peak potential (E_p) of a reversible reaction can be related to the formal potential ($E^{\circ\prime}$) by the following equation (*98*):

$$E^{\circ\prime} = E_p + PA/2 \qquad (1)$$

where PA is the pulse amplitude. The $E^{\circ\prime}$ for Mn^{III}-

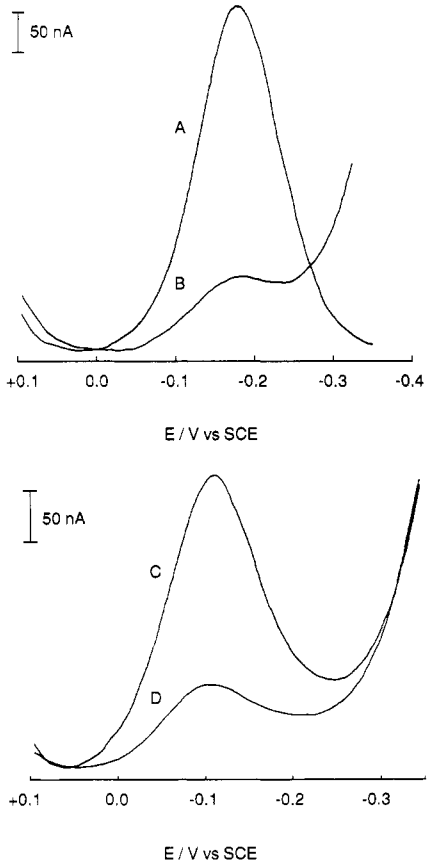

Figure 3. Differential pulse voltammograms of (A, B), 0.1 mM $Mn^{III}TMPyP$ (buffer 1) and of (C, D) 0.1 mM $Fe^{III}TMPyP$ (buffer 2), (A, C) in the absence of DNA and in the presence of sonicated CT DNA with (B) 0.8 mM NP, (D) 4.8 mM NP. Solutions were deoxygenated.

TMPyP calculated from this equation is −198 mV and for $Fe^{III}TMPyP$ is −133 mV. The $E^{\circ\prime}$ obtained from DPV for $Mn^{III}TMPyP$ is essentially the same as that found by CV, however that obtained by DPV for $Fe^{III}TMPyP$ was significantly more negative. To explain this behavior we have to consider the different characteristic times (τ) of CV and DPV experiments. The time scale for a CV experiment (at υ = 100 mV/s) is $\tau \approx RT/\upsilon F \approx 257$ ms [F is the faraday constant (96,485 C/eq) and R is the molar gas constant (8.314 J/mol^{-1} K^{-1})] while for a DPV experiment under the conditions here τ = 17 ms. Therefore, for a system with heterogeneous electron transfer kinetic limitations as is the case for $Fe^{III}TMPyP$, more reversible behavior might be found with CV than with DPV.

The E_p^{av} of 0.1 mM $Mn^{III}TMPyP$ in presence of sonicated CT DNA at 0.8 mM nucleotide phosphate (NP) was −177 (±8) mV. At the same supporting electrolyte concentration, $Fe^{III}TMPyP$ caused DNA precipitation. To decrease its strong interaction with CT DNA, the salt concentration was increased and experiments were done in 0.1 M Tris–HCl, 0.1 M NaCl, pH 7, where no precipitation was observed. The E_p^{av} of 0.1 mM $Fe^{III}TMPyP$ in presence of 4.8 mM NP was −100 (±4) mV. The effect of R, defined as [NP]/[$M^{III}TMPyP$], on the DPV peak potential (E_p) of $Mn^{III}TMPyP$ and $Fe^{III}TMPyP$ is shown in Figure 4, parts A and B, respectively. The E_p^{av}, and therefore $E^{\circ\prime}_{DPV}$, of $Mn^{III}TMPyP$ was practically unaffected by the addition of sonicated CT DNA for $0 < NP \leq 1$ mM, but at higher DNA concentration (e.g. [NP] = 2.1 mM), the E_p^{av} shifted to more negative values by 25 mV. This implies that the interaction of the oxidized Mn^{III} form with CT DNA is stronger than that of the

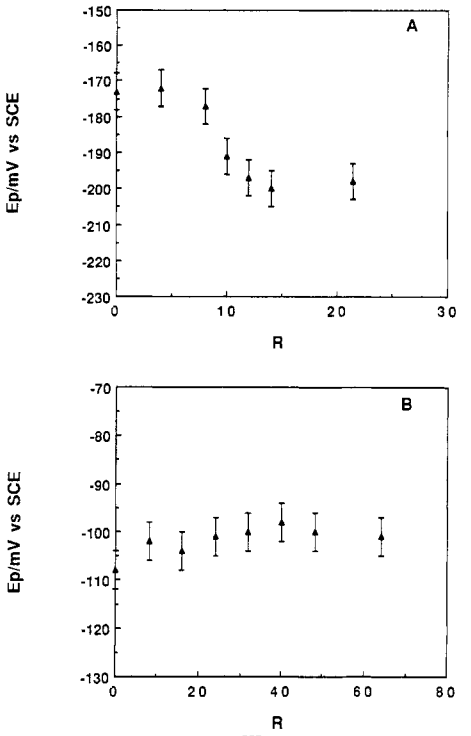

Figure 4. Effect of R ([NP]/[$M^{III}TMPyP$]) on the DPV peak potential (E_p) of (A) 0.1 mM $Mn^{III}TMPyP$ (buffer 1) and (B) 0.1 mM $Fe^{III}TMPyP$ (buffer 2).

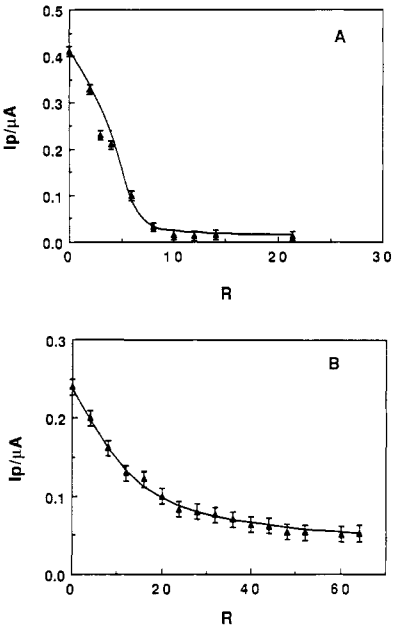

Figure 5. Titration curve of (A) 0.1 mM $Mn^{III}TMPyP$ (buffer 1) and of (B) 0.1 mM $Fe^{III}TMPyP$ (buffer 2) with sonicated CT DNA. Points represent the experimental data and solid curves represent the best fit obtained assuming mobile equilibria. Error bars correspond to $\pm 2\sigma$ for five measurements.

reduced Mn^{II} form. The E_p^{av} of $Fe^{II}TMPyP$ in the presence of 4.8 mM NP shifted to more positive values by only 8 mV. This small shift, however, suggests that the reduced Fe^{II} form interacts with CT DNA slightly more strongly than the oxidized Fe^{III} form.

On the other hand, the peak current (i_p) measured for both compounds was greatly affected by the presence of DNA. For 0.1 mM $Mn^{III}TMPyP$, the i_p decreased to 8% and for 0.1 mM $Fe^{III}MPyP$ to 22% of that in the absence of DNA (Figure 3, parts B and D). The decrease of the currents at a given R value is a measure of the strength

Table III. Binding Constants (K) and Site Sizes (s) of 0.1 mM Mn^{III}TMPyP[a] and 0.1 mM Fe^{III}TMPyP[b] with Sonicated CT DNA Based on Titration Data

		experimental parameters[c]		best fit[d]			
metalloporphyrin	model	10^6D_f, cm^2/s	10^9D_b, cm^2/s	10^6D_f, cm^2/s	10^9D_b, cm^2/s	$10^{-5}K$, M^{-1}	s, bp
Mn^{III}TMPyP	mobile	0.9 (0.3)	0.8 (0.4)	0.9	0.4	50 (20)	3
	static	0.9 (0.3)	0.8 (0.4)	0.9	0.4	36 (3)	4
Fe^{III}TMPyP	mobile	0.30[e] (0.03)	5 (3)	0.3	2	0.44 (0.02)	5
	static	0.30[e] (0.03)	5 (3)	0.3	4	0.19 (0.01)	6

[a] Supporting electrolyte, buffer 1. [b] Supporting electrolyte, buffer 2. [c] Numbers in parentheses represent $\pm 2\sigma$ for five measurements. [d] Numbers in parentheses represent 95% of interval confidence of value determined by nonlinear-regression calculations. [e] This value differs from that found by CV and is probably not the actual free diffusion coefficient (see the text).

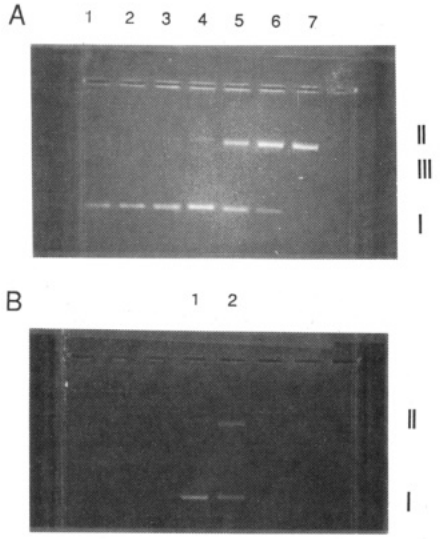

Figure 6. Electrochemically activated cleavage of pBR322 DNA with Mn^{III}TMPyP. (A) Electrolysis was carried out at −0.4 V for samples in lanes 5–7. Lane 1, pBR322 alone; lanes 2, 3, and 4, pBR322 and Mn^{III}TMPyP at concentrations of 1×10^{-8}, 1×10^{-7}, and 1×10^{-6} M, respectively; and lanes 5, 6, and 7, pBR322 and Mn^{III}TMPyP (electrochemically reduced) at concentrations of 1×10^{-8}, 1×10^{-7}, and 1×10^{-6} M, respectively. (B) Lane 1, pBR322 alone; and lane 2, pBR322 control (electrolyzed at a Pt cathode in oxygen-saturated solution at −0.4 V with no metalloporphyin).

of interaction of these molecules with DNA. The current decreases because the diffusion of the metal complex, when bound to DNA, is much slower than when it is free in solution (*83*, *84*).

The diffusion coefficients of the free metalloporphyrins were obtained in absence of sonicated CT DNA: Mn^{III}TMPyP, $D_f = 0.9\ (\pm 0.3) \times 10^{-6}\ cm^2/s$; Fe^{III}TMPyP, $D_f = 3.0\ (\pm 0.3) \times 10^{-7}\ cm^2/s$. The D_f of Fe^{III}TMPyP obtained by DPV is small compared to that obtained by CV ($D_f = 2.0\ (\pm 0.2) \times 10^{-6}\ cm^2/s$). This suggests that kinetic factors play a role in the observed DPV peak height. However, these factors will affect the response in the presence of DNA in the same way, assuming the kinetics are not changed, so that the titration curves of DPV response vs the amount of DNA should still yield reasonable estimates of binding constants and site sizes. The bound diffusion coefficient (D_b) was obtained at the highest R possible (considering detection limits of the instrument and solubility of CT DNA) to make sure that the metalloporphyrins were bound to the DNA macromolecule. The bound diffusion coefficient calculated at $R = 21$ for Mn^{III}TMPyP was $D_b = 8\ (\pm 4) \times 10^{-10}\ cm^2/s$ and at $R = 73$ for Fe^{III}TMPyP was $D_b = 5\ (\pm 3) \times 10^{-9}\ cm^2/s$.

The binding constant (K) and binding-site sizes (s) were calculated for both metalloporphyrins on the basis of both fast (mobile) and slow (static) equilibria with a titration curve in which the DPV peak current (i_p) was measured at several R values. The interpretation of the changes in voltammetric behavior ($E^{o\prime}$, i_p) due to the interaction of an electroactive species with DNA, as well as the approach used to fit the titration data, have been discussed in detail in previous publications (*83*, *84*).

The titration curves of Mn^{III}TMPyP and Fe^{III}TMPyP with sonicated CT DNA are shown in Figure 5, parts A and B, respectively. For the titration of Mn^{III}TMPyP, the supporting electrolyte used was 5 mM Tris–HCl, 50 mM NaCl, pH 7, while for Fe^{III}TMPyP it was 0.1 M Tris–HCl, 0.1 M NaCl, pH 7. The solid curves represent the best fit for the mobile equilibria. Results of the nonlinear regression analysis of the titration data for both complexes are summarized in Table III.

Cleavage of pBR322 DNA. Electrochemical reduction of Mn^{III}TMPyP and Fe^{III}TMPyP, in the presence of oxygen, induced DNA cleavage. This electrochemical reductive activation parallels previous studies of porphyrins with chemical reductants (e.g., ascorbate, dithiothreitol, 2-mercaptoethanol, sodium hydrosulfite) (*10–13*, *40–42*) but does not involve the addition of these reagents. Figure 6A shows the gel electrophorogram of pBR322 with different concentrations of Mn^{III}TMPyP. In lanes 2–4, Mn^{III}TMPyP was simply incubated with pBR322 DNA while in lanes 5–7 the reaction mixture was submitted to electrolysis at −0.4 V for 12 h. Production of the nicked open circular form II occurred only in lanes 5–7, where Mn^{III}TMPyP was electrochemically reduced. In lane 7, for example, there is complete conversion of the supercoiled form I to form II as well as some linear form III. To determine how much of this cleavage was really due to the reduction and therefore activation of Mn^{III}TMPyP, a control experiment was carried out in which an O_2-saturated solution of pBR322 was electrolyzed at −0.4 V. Oxygen reduction at the Pt cathode predominantly produces H_2O (*99–102*). Some DNA cleavage was observed in the control experiment (Figure 6B), however to a much smaller extent than when Mn^{III}TMPyP was present. To ascertain whether a small amount of H_2O_2 produced during the cathodic reduction of oxygen at this potential was responsible for the observed cleavage in the control experiment, a sample containing 2 μg of pBR322 was incubated for 12 h with 1.0 mM H_2O_2 and a piece of Pt metal. No cleavage was observed. This suggests that a small amount of cleavage might be promoted by some direct interaction of the DNA and oxygen at the Pt cathode. We did not consider it necessary to introduce a control experiment without oxygen, since the dependence of DNA scission on the presence of oxygen has been widely studied (*10*, *42*).

Quantitative data of Figure 6 is expressed in terms of the percentages of forms I, II, and III in Table IV. Table IV also shows the number of single-strand breaks per DNA molecule (S). This value was calculated with the following equation (*1*):

$$S = -\ln \chi_I \qquad (2)$$

Table IV. Results of pBR322 DNA Cleavage by Electrochemically Activated $Mn^{III}TMPyP$

lane	conditions[a]	electrolysis	% form I	% form II	% form III	S^b
			Figure 6A			
1	pBR322 alone	no	91	9	0	
2	pBR322, 0.01 μM Mn–P	no	90	10	0	
3	pBR322, 0.1 μM Mn–P	no	91	9	0	
4	pBR322, 1 μM Mn–P	no	91	9	0	
5	pBR322, 0.01 μM Mn–P	yes	50	50	0	0.60 (0.13)
6	pBR322, 0.1 μM Mn–P	yes	18	82	0	1.62 (1.15)
7	pBR322, 1 μM Mn–P	yes	0	96	4	4.54 (4.07)
			Figure 6B			
1	pBR322 alone	no	90	10	0	
2	pBR322 alone	yes	57	43	0	0.46

[a] Mn–P = $Mn^{III}TMPyP$. [b] Values in parentheses are the number of single strands produced by activated $Mn^{III}TMPyP$ (S of control experiment was subtracted).

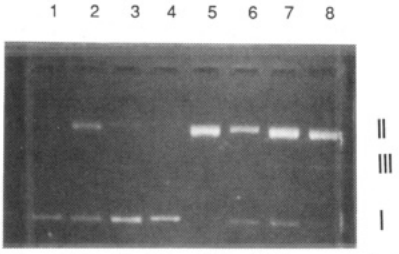

Figure 7. Electrochemically activated cleavage of pBR322 DNA with $Fe^{III}TMPyP$. Electrolysis was carried out at –0.3 V for samples in lanes 2, 6, 7, and 8. Lane 1, pBR322 alone; lane 2, pBR322 control (no $Fe^{III}TMPyP$); lanes 3, 4, and 5, pBR322 and $Fe^{III}TMPyP$ at concentrations of 1×10^{-8}, 1×10^{-7}, and 1×10^{-6} M, respectively; and 5, 6, and 7, pBR322 and $Mn^{III}TMPyP$ (electrochemically reduced) at concentrations of 1×10^{-8}, 1×10^{-7}, and 1×10^{-6} M, respectively.

where χ_I is the fraction of form I. If form III was present, then S was obtained from eq 3 where χ_{II} is the fraction

$$\chi_I + \chi_{II} = [1 - S(2h + 1)/2L]^{S/2} \quad (3)$$

of form II, h is the maximum space in base pairs between two nicks, required to produce form III (h = 16 base pairs) (*103*), and L is the total number of base pairs of pBR322 plasmid DNA (L = 4362 base pairs) (*104*). The S value reported was subtracted from that obtained for pBR322 DNA alone, in lane 1 of Figure 6, parts A and B. Numbers in parentheses are S values that account only for the catalytic effect of reduced $Mn^{III}TMPyP$ (S of the control experiment was subtracted).

The gel electrophorogram of pBR322 with $Fe^{III}TMPyP$ is shown in Figure 7. In this gel, the control experiment was included in lane 2 and the electrolysis was done at –0.3 V for samples in lanes 6–8. The percent of forms I–III and the S values obtained from Figure 7 are given in Table V. At concentrations of complex of 1×10^{-8} and 1×10^{-7} M, formation of form II occurred only when $Fe^{III}TMPyP$ was electrochemically reduced. However, 1×10^{-6} M $Fe^{III}TMPyP$ was found to produce the same amount of cleavage in the absence of electrochemical reduction. In lane 5, 98% of form I was transformed to form II, and in lane 8, form I was totally converted to 97% form II and 3% form III.

DISCUSSION

Voltammetric Studies of $Mn^{III}TMPyP$ and $Fe^{III}TMPyP$. $Mn^{III}TMPyP$ and its reduced form undergo several acid dissociation equilibria in aqueous solution. The identification of these species and the pK for these equilibria has been determined by Harriman et al. (*94*). At pH 7, the electrochemical reduction of $Mn^{III}TMPyP$ can be described by eq 4. For $Fe^{III}TMPyP$ the equilibria of species in solution is somewhat more complicated and is not fully understood, because of simultaneous acid–base and dimerization equilibria that depend on concentration, ionic strength, and pH. The reduction of $Fe^{III}TMPyP$ can be expressed as

$$[Mn^{III}TMPyP(H_2O)_2]^{5+} + e^- \rightleftarrows [Mn^{II}TMPyP(H_2O)]^{4+} + H_2O \quad (4)$$

$$[Fe^{III}TMPyP(H_2O)(OH)]^{4+} + e^- + H^+ \rightleftarrows [Fe^{II}TMPyP(H_2O)_2]^{4+} + H_2O \quad (5)$$

according to Kurihara et al. (*89*). At this pH, the following monomer–dimer equilibrium also occurs.

$$2[Fe^{III}TMPyP(H_2O)(OH)]^{4+} \rightleftarrows [[Fe^{III}TMPyP(H_2O)]_2O]^{8+} + H_2O \quad (6)$$

Several values appear in the literature for the magnitude of the dimerization constant, K_d: 2×10^3 M^{-1} by Forshey et al. (*86*), 9×10^5 M^{-1} by Pasternack et al. (*105*), 2.1×10^3 M^{-1} by Tondreau et al. (*106*), and 3×10^5 M^{-1} by Goff et al. (*107*).

The CV behavior of both metalloporphyrins is in agreement with that reported in the literature (*85–92*). The reduction of $Fe^{III}TMPyP$ in phosphate buffer at pH 6.5–9.4 and at a glassy carbon electrode have been reported to show two cathodic peaks. The first corresponds to the reduction of the monomer form of $Fe^{III}TMPyP$ and the second, at more negative potentials, to that of the dimeric form. The peak potential of the second peak was found between –0.45 and –0.55 V vs SCE. However, our CV of this compound in 5 mM Tris–HCl, 50 mM NaCl, pH 7, and at a Pt electrode only encompassed the first cathodic peak, because we used a Pt electrode which could not be used at potentials more negative than –0.6 V vs SCE due to background limitations (i.e., evolution of hydrogen).

As we pointed out earlier, the mode of interaction of these metalloporphyrins with DNA is electrostatic in nature. These complexes possess a high positive charge (5+ or 4+) and interact very strongly with the negative phosphate groups of the DNA backbone. On the basis of this type of interaction, we should expect a more positively charged species to have a higher affinity for DNA. Thus if the oxidized form of a redox couple had a higher positive charge than the reduced form, a difference in binding strength may arise, which would be reflected as a shift in $E^{\circ\prime}$ to more negative values. In DPV, the $E^{\circ\prime}$ (or E_p^{av}) of $Mn^{III}TMPyP$ shifted 25 mV to more negative values, implying that the binding constant of the oxidized species (5+) to DNA is 2.7 times larger than that of the reduced species (4+). In the case of $Fe^{III}TMPyP$, where both forms have charge 4+, the $E^{\circ\prime}$ shifted

Table V. Results of pBR322 DNA Cleavage by Electrochemically Activated Fe^{III}TMPyP

lane	conditions[a]	electrolysis	% form I	% form II	% form III	S[b]
1	pBR322 alone	no	90	10	0	
2	pBR322 alone	yes	50	50	0	
3	pBR322, 0.01 μM Fe–P	no	89	11	0	
4	pBR322, 0.1 μM Fe–P	no	92	8	0	
5	pBR322, 1 μM Fe–P	no	2	98	0	3.81 (3.22)
6	pBR322, 0.01 μM Fe–P	yes	24	76	0	1.53 (0.94)
7	pBR322, 0.1 μM Fe–P	yes	15	85	0	1.80 (1.11)
8	pBR322, 1 μM Fe–P	yes	0	97	3	3.90 (3.21)

[a] Fe–P = Fe^{III}TMPyP. [b] Values in parentheses are the number of single strands produced by activated Fe^{III}TMPyP (S of control experiment was subtracted).

only by 8 mV to more positive potentials, indicating no large differences in binding between the two forms and DNA. These thermodynamic arguments are somewhat compromised by the quasireversibility of the CV and DPV waves; however, the averaging procedure employed should give a good estimate of the formal potential. To obtain the binding constants we assumed reversibility of the systems, although some heterogeneous electron transfer limitations occur, especially in the case of Fe^{III}TMPyP, as discussed previously. For this case, we assumed that the electron-transfer kinetics of the Fe^{III}TMPyP is not affected by the presence of DNA as demonstrated by the absence of changes on the peak width at half-height of the DPV waves.

The DPV peak currents of both metalloporphyrins decreased drastically in presence of an excess of DNA. This decrease in the current is an indication of the degree of interaction of these complexes with DNA and was used to quantify the binding of these metalloporphyrins with CT DNA. The values of the binding constants obtained for sonicated CT DNA and Mn^{III}TMPyP in 5 mM Tris, 50 mM NaCl, pH 7, and Fe^{III}TMPyP in 0.1 M Tris, 0.1 M NaCl, pH 7, are larger than those reported for other DNA electrostatic binders such as $Ru(bpy)_3^{2+}$, $Fe(bpy)_3^{2+}$, and $Co(bpy)_3^{2+}$ with native CT DNA. At 5 mM Tris, 50 mM NaCl, pH 7, $Co(bpy)_3^{2+}$ has a binding constant for native CT DNA of 1.4×10^4 M^{-1} (mobile equilibria), while the $Ru(bpy)_3^{2+}$ and $Fe(bpy)_3^{2+}$ affinity for CT DNA is negligible (*83, 84, 108*). Binding affinity studies of $Ru(bpy)_3^{2+}$ and $Fe(bpy)_3^{2+}$ with native CT DNA have been carried out at lower salt concentration (*83, 84, 109*). The binding-site sizes (*s*) found here compare quite well with those obtained for these bipyridyl complexes. The value of the binding constant for Fe^{III}-TMPyP with sonicated CT DNA determined in this study ($K = 4.4 \times 10^4$ M^{-1}; mobile equilibria) is somewhat smaller than the one determined with native CT DNA by competition binding experiments using ethidium bromide ($K = 3.5 \times 10^5$ M^{-1}) (*57*) at the same ionic strength.

Cleavage of pBR322 DNA. As is evident from Figures 6 and 7, electrochemical reduction of Mn^{III}TMPyP and Fe^{III}TMPyP (at low concentrations) was essential to activate these complexes and cause DNA breakage. In the control experiment, as illustrated in Figure 6B, the reduction of oxygen also induced DNA degradation but to a lesser extent. H_2O_2, an expected intermediate of the reduction of oxygen, did not cause cleavage, even in the presence of Pt metal that would produce some hydroxyl radicals (*110*). Fe^{III}TMPyP (1×10^{-8} M), when electrochemically reduced, was more efficient at cleaving DNA than reduced Mn^{III}TMPyP. Fe^{III}TMPyP converted 85% of the form I to form II, while Mn^{III}TMPyP cut only 50%. At this concentration Mn^{III}TMPyP produced practically the same percentages of forms I and II as the control experiment, which means that the nicking observed was not due to activated Mn^{III}TMPyP.

At 1×10^{-6} M, Fe^{III}TMPyP caused DNA breakage without being electrochemically reduced. This is consistent with the results reported by Fiel et al. (*41*). Ward et al. (*40*) attributed this phenomenon to the presence of reducing agents in the agarose. Possibly disproportionation of two Fe^{III}TMPyP molecules results in self-activation.

The long periods of time required for the activation of the metalloporphyrins via electrochemical reduction can be attributed to the slow mass transfer rates in the cell used, since the solutions were not stirred. Electrochemical activation, on the other hand, is a clean method which does not require addition of a reductant and thus makes the analysis of reaction products easier. It can also offer a wider range of potentials to activate molecules with different binding specificities which might not be activated by chemical methods and might also be useful in following formation and consumption of electroactive species to study reaction mechanisms.

CONCLUSION

We have shown that it is possible to use electrochemical methods to study the interaction of Mn^{III}TMPyP and Fe^{III}TMPyP with sonicated calf thymus DNA and to activate these complexes to produce pBR322 DNA cleavage. These results thus complement the earlier communication by Van Atta et al. (*93*) on electrochemical activation of the Fe^{III}–bleomycin complex.

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