The ortho, meta, and para isomers of manganese(III) 5,10,15,20-tetakis(4-carboxyphenyl)porphyrin, MnTM-2-PyP4+, MnTM-3-PyP4+, and MnTM-4-PyP4+, respectively, were analyzed in terms of their superoxide dismutase (SOD) activity in vitro and in vivo. The impact of their interaction with DNA and RNA on the SOD activity in vitro and in vivo has also been analyzed. Differences in their behavior are due to the combined steric and electrostatic factors. In vitro catalytic activities are closely related to their redox potentials. The half-wave potentials (E1/2) are +0.220 mV, +0.052 mV, and +0.060 V versus normal hydrogen electrode, whereas the rates of dismutation (k1/2) are 6.0 × 107, 4.1 × 106, and 3.8 × 106 M−1 s−1 for the ortho, meta, and para isomers, respectively.

However, the in vitro activity is not a sufficient predictor of in vivo efficacy. The ortho and meta isomers, although of significantly different in vitro SOD activities, have fairly close in vivo SOD efficacy due to their similarly weak interactions with DNA. In contrast, due to a higher degree of interaction with DNA, the para isomer inhibited growth of SOD-deficient Escherichia coli.

Due to their high stability and chemical versatility, manganese porphyrins are a promising group of compounds for development as SOD mimics. Introducing β-electron-withdrawing substituents and modifying meso substituents of water soluble porphyrins can have a major impact on the redox and electrostatic properties of manganese porphyrins (1–7). Such substitution allows the redox potential to approach the potential of SOD itself, E1/2 ≈ +0.26 V (8). This is approximately midway between the redox potentials of the two half-reactions of the dismutation process (9, 10). While maintaining the redox potential at a value close to that of SOD, it is important to also ensure electrostatic facilitation. We have already shown that β-bromination (11, 12) of the manganese(III) 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin increases their SOD-like activities (13). However, the large positive redox potential of the former, E1/2 = +0.480 mV versus NHE, (13) stabilizes manganese in its 2+ state and diminishes stability, and the latter compound had only modest activity, probably due to lack of electrostatic facilitation (13).

It has been shown that ortho substitution on the meso phenyl ring exhibits an ortho effect that is as strong as if the same substituent were placed directly on the meso position (14). Thus, meso-tetrakis(2-methylphenyl)porphyrin behaves similarly to the meso tetramethylporphyrin (14). The same was found for other porphyrins in which meso phenyl groups have different ortho substituents, as well as when meso groups are ortho pyridyls, such as 5,10,15,20-tetrakis-(N-methylpyridinium-2-yl) (H2TM-2-PyP4+) and its zinc complex, ZnTMP-2-PyP4+ (15–19). Such a profound impact on the properties of the ortho isomers, known as the ortho effect, is due to the combined effect of inductive, resonance, and steric factors (20, 21). For this reason, we explored the ortho isomer of manganese(III) 5,10,15,20-tetrakis(N-methylpyridyl)porphyrin (MnTMPyP5+) and expected more favorable electrostatic facilitation due to the positively charged pocket formed in both the aaaa and aaaaβ atropoisomers (22, 23). Herein, we describe and compare the in vitro and in vivo SOD-like properties of the ortho, meta, and para isomers of MnTMPyP5+. The relevant structures are shown in Fig. 1, together with force field molecular mechanics (MM2) model of the major aaaaβ atropoisomer.

**MATERIALS AND METHODS**

MnCl2·4H2O, and Baker-flex silica gel IB were purchased from J. T. Baker. 2-Propanol (99.5+%), N,N-dimethylformamide (99.8+ % anhydrous), NH4PF6 (99.99%), sodium L-ascorbate (99%), NaCl, tetraethylammonium chloride, and potassium sulfate were from Sigma and Aldrich. RNA (type III from baker’s yeast, DNA (type III from salmon sperm), xanthine, and cytochrome c (oxidized form) were purchased from Sigma. Methanol (anhydrous and absolute), ethanol (absolute), acetone, ethyl ether (anhydrous), KNO3, hydrochloric and sulfuric acids, phosphate salts, EDTA, glucose, inorganic salts, and KOH were from Mallinkrodt; acetonitrile was from Fisher; casamino acids were from Difco; and bovine Cu,Zn-SOD was from Diagnostic Data, Inc. Xanthine oxidase was prepared by R. Wiley and was supplied by K. V. Rajagopalan (24). Catalase was from Boehringer Mannheim. Ultrapure argon was supplied from National Welders Supply Co. Molecular weight 3000 cut-off ultrafiltration (Centricon) concentrators were purchased from Amicon.

**Metal-free Porphyrins**

The metal free porphyrins were obtained from Aldrich, Fluka, and Porphyrin Products (tosylate salts of the meta isomer, H2TM-4-PyP4+).

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and from Mid-Century Chemicals (Posen, IL) (chloride salts of the ortho isomer, H$_2$TM-2-PyP$_4^+$, and of the meta isomer, H$_3$TM-3-PyP$_5^+$). The compounds were analyzed in terms of elemental analysis, UV-visible spectral characteristics of their di- and tetraprotonated forms (in the range of 0.5–50 μM), as well as in terms of thin-layer chromatography. Ortho isomer, H$_2$TM-2-PyP$_{Cl_5}$ (C$_{22}$H$_{16}$N$_8$O$_5$Cl$_5$), calculated = C, 58.61; H, 5.25; N, 12.43; Cl, 15.73. Found = C, 58.62; H, 5.19; N, 12.31; Cl, 15.60. UV-visible for H$_2$TM-2-PyP$_{Cl_5}$, $\lambda_{max}$ (log ε) = 413.2(5.32), 510.4(4.13), 544.3(4.49), 581.3(4.72), 634.6(6.23). For H$_3$TM-3-PyP$_6$: $\lambda_{max}$ (log ε) = 441.0(5.41), 578.4(4.17), 632.0(4.41). TLC (Baker-flex silica gel IB, KNO$_3$sat:H$_2$O:acetonitrile 1:1:8), $R_f$ = 0.08. Meta isomer, H$_2$TM-3-PyP$_{Cl_5}$ (C$_{22}$H$_{16}$N$_8$O$_5$Cl$_5$), calculated = C, 61.69; H, 4.94; N, 13.08; Cl, 16.55. Found = C, 61.88; H, 5.01; N, 13.05; Cl, 16.33. UV-visible for H$_2$TM-3-PyP$_{Cl_5}$, $\lambda_{max}$ (log ε) = 416.5(6.50), 514.1(6.50), 550(shoulder), 581.2(3.77), 635.6(2.96). UV-visible for H$_3$TM-3-PyP$_6$: $\lambda_{max}$ (log ε) = 445.8(5.55), 593.7(4.03), 645.6(4.41). TLC (Baker-flex silica gel IB, KNO$_3$sat:H$_2$O:acetonitrile 1:1:8), $R_f$ = 0.14. Para isomer, H$_2$TM-4-PyP$_{tos}$ (C$_{72}$H$_{66}$N$_8$O$_{12}$S$_4$): calculated = C, 62.11; H, 5.04; N, 8.09; S, 9.14. UV-visible for H$_2$TM-4-PyP$_{tos}$, $\lambda_{max}$ (log ε) = 422.0(5.37), 518.4(4.19), 553.8(3.75), 584.2(3.18). For H$_3$TM-4-PyP$_6$: $\lambda_{max}$ (log ε) = 459.4(5.45), 603.8(3.94), 656.0(4.34). TLC (Baker-flex silica gel IB, KNO$_3$sat:H$_2$O:acetonitrile 1:1:8), $R_f$ = 0.11.

**Manganese Porphyrins**

The manganese complexes were prepared by metatallation of the porphyrin ligands in either water or in methanol at porphyrin:manganese ratios of 1.5, 1.13, and 1.10. In both solvents and at all metal to porphyrin ratios, the same compound, as evidenced by the Soret band, was obtained. Metallation in methanol was accomplished under reflux conditions, whereas in water it was achieved at room temperature. When the pH of the water was raised to 12.3 (20, 25)°C, metatallation was accomplished in ~15 min. Routinely, a 1:20 ratio of porphyrin to metal was used for prolonged periods. Thus, in 48 h, the molar absorptivity of a metal-free porphyrin falls to 50% of its initial value. The same happened when metatallation was attempted at room temperature (28, 29), should be avoided whenever possible. We have found that both metatallation (MnTMPyP$_5^+$) and its parent ligand (H$_2$TMPyP$_4^+$) suffer significant changes when exposed to either anhydrous DMF or DMSO/H$_2$O (9:1). The modification of the porphyrin ring, hydrogen bonding interaction of the pyrrole nitrogen hydrogen with DMF (30), dimerization, and metal-centered reduction are among possible routes that can lead to the complete destruction of the porphyrin ring.

**Stability of the Metalloporphyrins**

The stability of manganese porphyrins was studied under strongly acidic and alkaline conditions and in the presence of up to 1000-fold excess EDTA. All three porphyrins were resistant to protonation as well as to ligand exchange. Even after ~1 h in 36% hydrochloric acid, no

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3. I. Batinić-Haberle, unpublished results; pK$_a$ (ortho) = 10.97, pK$_a$ (meta) = 13.1, pK$_a$ (para) = 12.9. (H$_2$TMPyP$_4^+$)[H(TMPyP$_5^+$/[H$^+$]).

4. I. Batinić-Haberle, unpublished results.
Xanthine oxidase was the source of O$_2^-$ and ferricytochrome c was its indicating scavenger (33). Reduction of cytochrome c was followed at 550 nm. Assays were conducted in the presence and absence of 0.1 mM EDTA in 0.05 M phosphate buffer, pH 7.8, ± 15 μg/ml of catalase. Rate constants for the reaction of metalloporphyrins with O$_2^-$ were based upon the competition with 10 μM cytochrome c, $k_{red} = 2.6 \times 10^{11} \text{M}^{-1} \text{s}^{-1}$ (34). O$_2^-$ was produced at the rate of 1.2 μM/min. Possible interference through inhibition of the xanthine/xanthine oxidase reaction by test compounds was examined by following the rate of urate accumulation at 295 nm in the absence of cytochrome c. All measurements were done at 25°C.

The Interaction of Metalloporphyrins with Nucleic Acids

These interactions were followed by UV-visible spectroscopy, by cyclic voltammetry, through inhibition of their SOD-like activity, and by ultrafiltration.

UV-visible Spectroscopy—The interaction of metalloporphyrins with nucleic acids was performed both in the presence (27 mM) and absence of ascorbic acid in 0.05 M phosphate buffer, pH 7.8, at 6 μM porphyrin, 4.7 mM DNA and RNA. The RNA stock solutions, ranging between 18 and 76 mM, were prepared in water, whereas DNA stock solutions ranging between 18 and 38 mM were prepared in buffer due to the lower DNA solubility. The concentration of nucleic acids was calculated on the basis of mononucleotide. All the experiments with ascorbate were performed anaerobically in a specially designed cuvette (35) purged with argon.

Ultrafiltration—The retention of the porphyrins by the molecular weight 3000 cut-off filter ± the nucleic acids (11 and 22 mM) was determined filtering the 0.5 mM solutions of porphyrin in 0.05 mM phosphate buffer, pH 7.8, 0.1 mM NaCl. SOD Assay—The assay was performed at the porphyrin concentration that caused 50% inhibition of the cytochrome c reduction (IC$_{50}$). At the IC$_{50}$ porphyrin concentration, its SOD activity was titrated with nucleic acid in the concentration range of 0.4–21 μM, depending upon the isomer investigated.

Electrochemical Characterization

Measurements were performed using CH Instruments (computer supported) model 600 Voltammetric Analyzer. A three-electrode setup system in a small volume cell (0.5–3 ml) with a 3 mm-diameter button glassy carbon working electrode (Bioanalytical Systems) was used. Prior to each experiment, the electrode was cleaned with 0.3 μM alumina, sonicated in deionized water for 1 min, rinsed with stream of deionized water, dried with a paper tissue, and allowed to air-dry for 5 min.

The reference electrode was standard Ag/AgCl electrode (Bioanalytical Systems, 3 M NaCl gel filling solution), and the auxiliary electrode was a 0.5-mm platinum wire. Solutions containing 0.05 mM phosphate buffer, pH 7.8, 0.1 M NaCl and 0.5 mM metalloporphyrin were used. The effect of nucleic acids (0.05–45 mM) on the redox properties of the metalloporphyrins was studied. Ultrapure argon (less than 1 ppm oxygen), humidified, was purged through all the solutions for 30 min. Scan rates were 10–500 mV/s, typically 100 mV/s.

RESULTS

The isomers of manganese(III) meso-tetrakis[N-methyl-pyrindyl]porphyrin were prepared from their parent metal-free ligands and were characterized. The metal-free ligands were characterized as well, and their UV-visible data, given under “Materials and Methods,” agree well with literature data (15). The metalloporphyrins resist strongly acidic (36% HCl) and basic conditions (1 M NaOH). The ortho isomer appeared thrice as resistant toward oxidative degradation with H$_2$O$_2$ than the para one, which is consistent with the anodic shift of the redox

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**FIG. 2.** The optical spectra of the ortho (solid line), meta (dashed line), and para (dotted line) isomers of MnTMPyP$^{5+}$. The spectra were obtained at ~6 μM (A) and 60 μM (B) (visible region) porphyrin concentrations.

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Demetallation was observed at ~6 μM porphyrin concentration. A very slow demetallation was observed in 88% sulfuric acid in the case of meta compound, where ~50% of demetallation occurred at 6 μM porphyrin in a 24-h period at room temperature. Under the same conditions, only negligible demetallation was observed in the case of para, whereas the ortho isomer appeared to be the most resistant, showing no observable demetallation in 24 h. Their stability can be ascribed to the combination of steric hindrance and electronic effects (31, 32).

The stability toward H$_2$O$_2$ was measured at 25°C with 5 μM porphyrin, 5 mM H$_2$O$_2$ in 0.05 M phosphate buffer at pH 7.8. The half-times for the oxidative degradation of the porphyrin ring were 105, 28, and 30 s for the ortho, meta, and para isomers, respectively. This observation is consistent with their redox properties, i.e., resistance toward oxidation (anodic shift of both reduction and oxidation potentials) (1–7), due to the closer position and therefore greater inductive effect of the ortho positive charges on the porphyrin ring.
The porphyrins were prepared and characterized as chloride salts, and spectral characteristics were determined in water. The estimated errors in ε are within ±2%.

### Table I

| Soret bands | Q bands | Charge transfer |
|-------------|---------|-----------------|
| Ortho       | 362.8 (3.64), 455.4 (5.11) | 556.0 (4.03), 686.0 (2.93) | 781.0 (3.18) |
| Meta        | 372.8 (4.70), 395.2 (4.67) | 557.2 (4.11), 675.8 (3.13) | 765.6 (3.34) |
| Para        | 459.8 (5.14) | 560.0 (4.07), 678.0 (3.08) | 769.5 (3.25) |

### Table II

| MnTMPyP5+ | IC50 μM | kcat s⁻¹ | % of SOD activity | Specific activity units/mg |
|-----------|--------|----------|-------------------|---------------------------|
| Ortho     | 4.3 x 10⁻⁵ | 6.0 x 10⁷ | 3.00 | 8500 |
| Meta      | 6.5 x 10⁻⁷ | 4.1 x 10⁶ | 0.20 | 560 |
| Para      | 6.7 x 10⁻²⁷ | 3.8 x 10⁶ | 0.18 | 550 |
| Cu,Zn-SOD | 1.3 x 10⁻¹⁶ | 2.0 x 10²⁶ | 100 | 5100 |

*Conditions: 0.05 M phosphate buffer, 0.1 mM EDTA, pH 7.8, 40 μM xanthine, 2 μM xanthine oxidase. The rate of cytochrome c reduction by O2⁻, kcat = 2.6 x 10⁶ M⁻¹ s⁻¹ (34) is used for calculation of kcat. The estimated errors in IC50 are within ±10%.

### Table III

| MnTMPyP5+ | DNA | RNA | DNA | RNA | Affinity a |
|-----------|-----|-----|-----|-----|------------|
| Ortho     | 4.1 x 10⁴  | 0.8 x 10⁴  | 3.2 x 10⁹  | 3.7 x 10⁹  |
| Meta      | 2.2 x 10⁴  | 2.1 x 10⁴  | 2.4 x 10⁹  | 6.0 x 10⁹  |
| Para      | 17.0 x 10⁴ | 2.4 x 10⁴  | 7.0 x 10⁵  | 8.1 x 10⁵  |

*The association includes the interaction of DNA and RNA with metal complexes of both Mn(II) and Mn(III), the reductant being O2⁻.*

SOD Activity in Vitro—Inhibition of cytochrome c reduction by the porphyrins, when plotted as (v/v)_o – 1 versus (MnTMPyP5+) concentration (38) yielded a straight line as shown for the ortho isomer in Fig. 3. The concentration that causes 50% of the inhibition of cytochrome c reduction by O2⁻, v_o is the rate of reduction of cytochrome c inhibited by the porphyrin catalyst in the presence of 0.1 mM EDTA in 0.05 mM phosphate buffer at pH 7.8, 40 μM xanthine, 2 μM xanthine oxidase. The total volume of the assay solution was 3 ml.

The association includes the interaction of DNA and RNA with metal complexes of both Mn(II) and Mn(III), the reductant being O2⁻.

The SOD assay was determined by the means of SOD cytochrome c¹ assay and by the ultrafiltration experiment.

 ultrafiltration data.

### Diagram

**FIG. 3.** The plot of (v_v_o) - 1 versus MnTMPyP5+. v_o is the rate of reduction of 10 μM cytochrome c by O₂⁻; v_v_o is the rate of reduction of cytochrome c inhibited by the porphyrin catalyst in the presence of 0.1 μM EDTA in 0.05 μM phosphate buffer at pH 7.8, 40 μM xanthine, 2 μM xanthine oxidase. The total volume of the assay solution was 3 ml.

Data obtained from the SOD assay, under the conditions where manganese cycles between 2⁺ and 3⁺ state, correspond to porphyrins both oxidized and reduced at the metal center. Each addition of nucleic acids decreased SOD activity by an amount that is linearly related to the concentration of bound porphyrin. This is shown in Fig. 4. The association constants (K_a) for all three isomers are presented in Table III. The K_a values for the interactions of the meta isomer with DNA and RNA are essentially equal, whereas they are greater for the interaction of the ortho and para isomers with DNA than with RNA. The association constants are on average 2 orders of magnitude higher than those calculated from ultrafiltration data when manganese was in its 3⁺ state.

Spectrophotometric monitoring of the interaction of metalloporphyrin with nucleic acids was performed anaerobically in the absence and presence of ascorbic acid. (When no ascorbic acid was present, the same interaction of porphyrins with
The interaction of the ortho (squares), meta (triangles), and para (circles) isomers of MnTMPyP5+, with DNA as determined by cytochrome c assay. The SOD-like activity was titrated by the addition of DNA in 0.05 M phosphate buffer, pH 7.8, and porphyrin was at its IC50 concentration, cytochrome c at 10 μM, xanthine at 40 μM, xanthine oxidase at ~2 nM. The reaction was started by the addition of ~2 nM xanthine oxidase.

**Table IV**
The interaction of the ortho, meta, and para isomers with nucleic acids as observed by optical spectroscopy

| MnTMPyP^5+ | \( \lambda_{\text{max}} \) | A | \( \Delta \lambda \) | \( \Delta I \) |
|------------|-----------------|------|---------|---------|
| Ortho      | 453.4           | 0.785|          |        |
| Ortho + DNA | 452.0           | 1.030| -1.4    | +7     |
| Ortho + RNA | 454.2           | 0.810|          | +7     |
| Meta       | 459.8           | 0.898|          |        |
| Meta + DNA | 458.4           | 1.222| -1.4    | +22    |
| Meta + RNA | 460.2           | 0.909|          | +1     |
| Para       | 462.2           | 0.767|          |        |
| Para + DNA | 461.8           | 1.071| -0.4    | +37    |
| Para + RNA | 463.6           | 0.778| +1.4    | +3     |
| Ortho + AA | 436.2           | 0.647|          |        |
| Ortho + AA + DNA | 440.8 | 1.238| +4.6    | +91    |
| Ortho + AA + RNA | 444.1 | 0.846| +7.8    | +30    |
| Meta + AA  | 440.8           | 0.767|          |        |
| Meta + AA + DNA | 444.0 | 1.806| +3.2    | +230   |
| Meta + AA + RNA | 452.0 | 1.102| +11.2   | +43    |
| Para + AA  | 447.2           | 0.661|          |        |
| Para + AA + DNA | 453.6 | 1.125| +6.4    | +70    |
| Para + AA + RNA | 463.0 | 0.869| +15.8   | +31    |

The metal-free ligands did not express a fairly good protection, whereas the meta and para isomers and less in the case of the ortho one, was detected. These toxicities may be attributed to their metal-chelating ability as well as to their interaction with nucleic acids.

**SOD-like Activity in Vivo**—The metal-free ligands did not show any SOD-like activity in growth experiments at the concentrations at which metalloporphyrins were potent. Moreover, slight toxicity, which was equal in the case of the meta and para isomers and less in the case of the ortho one, was detected.

The effect of the metalloporphyrins on the growth of SOD-deficient and wild type strains in the casamino acids medium are shown in Fig. 8. The ortho isomer was clearly the most effective. In the minimal, five-amino acid-containing medium, the ortho isomer again appeared to be the most effective compound, meta expressed a fairly good protection, whereas the para isomer was toxic, as shown in Fig. 9. Special care must be taken to ensure that the metalloporphyrin preparations are...
devoid of free Mn(II), because supplementation of the medium with Mn(II) per se facilitates the growth of the sodAsodB strain.5

5 J. Imlay, unpublished results.

No effect on the growth of wild type was detected at a 25 \( \mu \)M concentration of each isomer, but some toxicity was introduced at higher concentrations.

The SOD activity of the porphyrin-containing cell extract was studied as well. The SOD-deficient strain of E. coli J1132 was grown aerobically in the presence of a 25 \( \mu \)M concentration of all three isomers for \( \sim 8 \) h. The crude cell extract was prepared, and the porphyrin concentration in the cell extract determined spectrally, being higher for the para isomer (10 \( \mu \)M) and lower for the ortho (3 \( \mu \)M) and meta (4 \( \mu \)M) isomers. At IC\(_{50}\) of all three isomers, 42% of the SOD-like activity was detected in the case of the ortho isomer, 20% in the case of the meta isomer, and only 6% in the case of the para isomer.

**DISCUSSION**

In the Mn(III) meso-tetrakis(N-methylpyridyl)porphyrins, moving the N-methyl group from the para to the ortho position,

**FIG. 6.** The optical spectra of the para isomer in the absence of nucleic acids (solid line) and in the presence of nucleic acids (dotted line), RNA (A), and DNA (B). The concentration of porphyrin was \( \sim 6 \mu \)M, and that of DNA and RNA was 4.7 \( \mu \)M.

**FIG. 7.** The interaction of the ortho, meta, and para isomers with DNA (A) and RNA (B) as observed by cyclic voltammetry. The conditions were as follows: 0.5 \( \mu \)M porphyrin, 0.1 M NaCl, 0.05 M phosphate buffer, 22 \( \mu \)M DNA, and 45 \( \mu \)M RNA. The potentials are given as obtained in V versus Ag/AgCl, and are corrected for 235 mV when given in V versus NHE in Table V.

| [Nucleic acids] | Ortho | Meta | Para |
|-----------------|-------|------|------|
|                 | \( E_{1/2} \), V vs. NHE | Current | \( E_{1/2} \), V vs. NHE | Current | \( E_{1/2} \), V vs. NHE | Current |
| 0 \( \mu \)M RNA | 0.232 1.00 | 1.60 | 0.052 1.00 | 1.60 | 0.060 1.57 |
| 0.011           | 0.222 1.00 | 1.60 | 0.084 1.57 | 0.014 1.00 | 0.060 1.57 |
| 0.045 DNA       | 0.227 0.85 | 0.05 | 0.110 0.90 | 0.56 | 0.139 0.73 |
| 0.011           | 0.229 0.67 | 0.06 | 0.072 1.00 | 0.087 0.40 | 0.060 1.57 |
| 0.022 DNA       | 0.247 0.57 | 0.094 0.68 | 0.094 0.68 | 0.087 0.40 | 0.060 1.57 |
| 0.022           | 0.247 0.57 | 0.094 0.68 | 0.094 0.68 | 0.087 0.40 | 0.060 1.57 |

\( a \) Anodic shift in the potential in the presence of nucleic acids.

\( b \) The percentage of current obtained in the presence of nucleic acids as compared to the current in the absence of nucleic acids.
minimal (five amino acids) medium as affected by the presence of casamino acids medium after a 4-h period, expressed as a percentage of wild type AB1157 growth versus concentrations of the ortho (circles), meta (triangles), and para (squares) isomers of MnTMPyP$^{5+}$. When no porphyrin was added, the SOD-deficient strain grew at 18% of the growth of wild type (diamond).

resulting in the changes known as the ortho effect (15–21), was advantageous for several reasons. When the center of positive charge is closer to the porphyrin ring, the redox potential at the center is increased (15–21), which facilitates electron transfer and specific recognition of nucleic acids. A striking feature of these compounds is their extreme stability, which makes it likely that they will persist within cells. We have also observed that the meta isomer interacts with DNA similar to the ortho (Tables III and V and Fig. 7), as a consequence of the restricted rotation of N-methylpyrrole-2-propionate (42–45). Consequently, it behaved as a good SOD mimic in vivo despite the ~16-fold less in vitro SOD-like activity (Fig. 9).

The interaction of metalloporphyrins with nucleic acids has been studied (46–54). Those capable of axial ligation, as well as those possessing other means of steric hindrance, such as the ortho and meta isomers of both H$_2$TMPyP$^{4+}$ and MnTMPyP$^{5+}$, are restricted to external association. In contrast, the absence of axial ligation and coplanarity (metal-reduced para isomer) allows intercalation. Our data support this view and indicate that in vivo utility, as a replacement for SOD, is fostered by inability to intercalate into nucleic acids.

The results reported herein for the MnTMP-2-PyP$^{5+}$ are for the mixture of atropoisomers, because we could not resolve them. However, the atropoisomers of ZnTMP-2-PyP$^{4+}$ have been resolved (55). Future work will be directed at resolving the zinc compound and then replacing Zn(II) with Mn(III) in the separated atropoisomers. We are anxious to see whether the atropoisomers will differ in catalytic activity.

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