broadened band, would be expected. This is not observed, suggesting that most of the porphyrin units in the film have one saturated and one intact vinyl group and that the degree of cross-linking is low. Whether the apparent deactivation of the second vinyl group should be attributed to steric or electronic effects is uncertain.

We do not know how the polymer is attached to the electrode surface, nor what the distribution of chain lengths is. The fact that a fraction of the electroactive material is lost when an electrode with preformed film is placed in fresh electrolyte and its voltammogram successively cycled suggests that at least some of the polymer segments are relatively small and dissociate readily from the rest of the film.

In this work we have shown that stable adherent metalloporphyrin films, showing good electroactivity over substantial thicknesses (>1000 monolayer equivalents), can be prepared by oxidative electropolymerization of metalloprotoporphyrin complexes. The reaction depends on ring oxidation of the porphyrin, as expected if the mechanism is cationic vinyl polymerization, and the considerable variations encountered among different complexes can largely be understood on the basis of differing degrees of ring vs. metal oxidation at the relevant potentials. The metalloporphyrin sites are accessible at least to small ions, as evidenced by facile chloride binding to the ZnPP film, and should be capable of carrying out redox chemistry on coordinated ligands. This chemistry is currently under investigation.

Acknowledgment. We thank Joseph R. Perno for work with chromium protoporphyrins and Lisa A. Miller for experiments on replacement of Zn^{2+} in zinc protoporphyrin films. This work was supported by Grant DOE-AC02-81ER10861 from the U.S. Department of Energy.

Registry No. ZnPP, 15304-09-3; CoPP, 14932-10-6; NiPP, 15304-70-8; (CrPP)O, 86238-33-7; (FePP)Cl, 15741-03-4; (MnPP)Cl, 86238-34-8; $(CrPP)_2O$, 86259-39-4; $(FePP)_2O$, 36655-90-0; NiMp, 15892-09-8; $(FeMP)_2O$, 58280-33-4; (FeMP)Cl, 14126-91-1; FePP, 18922-89-9; SnO_2, 18282-10-5; Mn, 7439-96-5; Zn, 7440-66-6; Pt, 7440-06-4; TBAP, 1923-70-2; TBAH, 3109-63-5; TBAC, 1112-67-0.

Bioorganic Applications of Mass Spectrometry. 3.[1] Fast-Atom-Bombardment-Induced Zwitterionic Oligonucleotide Quasimolecular Ions Sequenced by MS/MS

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Abstract: Both the structure and sequence of DNA segments are determined by mass spectrometry (MS). Unprotected deoxyoligonucleotide triethylammonium salts TACC (**1**) and GGTA (**2**) have been directly analyzed from their aqueous solution by fast-atom-bombardment mass spectrometry (FABMS), with the aid of the MS/MS analytical approach. The desorbed gaseous oligomers are negative monocharged ions and possess a zwitterionic structure with three phosphodiester anionic moieties and two protonated bases. Sequence information can be derived both from the FAB-induced mass spectra of primary and metastable ions (MI) and the collisional activation (CA) secondary ions.

Introduction

DNA segments from both synthetic and natural sources can be sequenced after chemical or enzymatic degradation by conventional chromatographic techniques.[3] Fast-atom-bombardment mass spectrometry (FABMS), originally introduced for otherwise intractable molecules,[4] has been recently applied to determine the structure and chemistry of deoxyoligonucleotide salts, sampled from their water solution into a glycerol matrix and analyzed in the negative mode, after sputtering caused by a 6-keV argon atom beam.[5] The introduction of ionization procedures alternative to the classic electron impart (EI)[6] appears to have overcome an original drawback of mass spectrometry, i.e., the volatilization of polar[5,7] and extremely large molecules.[8] However, the lower abundances of information-bearing fragment ions often produced represents a severe limitation to the employment of those soft methods for other than molecular weight determination.

Fast atom bombardment of organic molecules dissolved in viscous matrixes usually provides sufficient structural information by the appearance of diagnostic fragments in the spectra of the primary ions thus produced. The observed electron-impact-like behavior, the simplicity of sample preparation and manipulation, and, last but not least, the availability of commercial FAB guns have promoted an extensive application of the technique in the structure elucidation of complex biomolecules. However, no definite theoretical models have yet been proposed to explain the ionization mechanism of very large molecules by particle-induced desorption, while some "hidden variables" seem to affect the obtainment of the spectra.[6b] Therefore, if this approach is applied to the analysis of unblocked DNAs, much attention has to be paid to the rationalization of the eventually observed fragmentation pattern. Furthermore, the biological important pieces of information stored in a oligodeoxyribonucleotide are a function of the sequence of only four chemical variables, i.e., the cytidine, thy-

(1) Work supported by Progetto Finalizzato del CNR—Chimica Fine e Secondaria. For part 2, see ref 10.
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midine, guanidine, and adenosine nucleotide monomers. Therefore, there is a very high probability that fragments originating from the degradation of impurities can overlap with isobaric species obtained from the main molecule. On the other hand, particularly in the case of long DNA stretches, it is not possible to exclude, a priori, the occurrence of some surface fragmentation processes as a consequence of the impinging-particle energy transfer, before the desorption process takes place.[6b] Interference of fragment peaks due to impurities of surface decompositions can be eliminated by the use of the MS/MS analytical approach.[9]

Secondary mass spectra can be obtained from primary ions of the required half-life (10^{-5} s) to decompose between the sectors of a reversed geometry mass spectrometer. The metastable ion (MI) spectrum thus obtained displays only gas-phase decomposition of low internal energy reacting precursors. It has been recently shown that the isomeric dithymidylate quasimolecular anions, FAB-desorbed from the glycerol mulls of their triethylammonium salts, gave diagnostic MI spectra,[10] complementing the information obtained from the primary mass spectrum.[5] Higher energy decompositions than those found in the MI spectra can be obtained from the internal energy content of the primary ions, selected by the magnet, by collision with an inert gas.[9,11] It has been pointed out that in the case of radical ions, the resulting collisional activation (CA) decompositions are similar to those found in the spectra of the EI-induced primary ions.

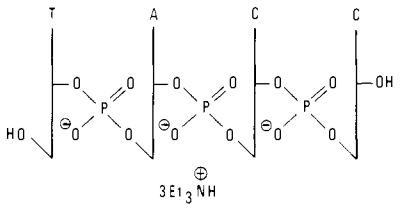
Oligonucleotides cannot be vaporized intact to undergo conventional EI ionization.[12] The use of collisions, therefore, in connection with such a useful device as FAB, enables "hard" experimental conditions to be simulated in the second drift region of the instrument for ionic species which can only be sampled in the gas phase by applying a "soft" ionization procedure.[10] The gas-phase chemistry of isomeric dideoxyoligonucleotide quasimolecular anions has been fully characterized from the mass spectra of their FAB induced primary and MI and CA secondary ions.[5,10] The possible extension to structure determination of long DNA stretches by MS requires the definition of the chemical behavior of unblocked oligomers containing more than two residues, and therefore, more than one phosphodiester moiety.

Results and Discussions

FAB-induced desorption of oligonucleotides from glycerol matrix causes the formation of positive and negative quasimolecular ions. The chemistry of both species can be suitably investigated in a noninteracting environment by a conventional double-focusing mass spectrometer. Since protonated species undergo extensive degradation resembling the lability of nucleotide oligomers in acid media,[5] it is far more straightforward to determine their structure from the spectrum of the negative rather than positive ions.

The tetradeoxyoligonucleotide triethylammonium salts d-TACC (**1**) and d-GGTA (**2**), sampled in the usual way,[5] and exposed to a 6-keV xenon atom beam, in a glycerol mull, gave negative ion spectra characterized by the presence of abundant quasimolecular and fragments anions.

A striking feature of the spectra of stable and fast-decomposing anions is that they enable well-defined regions to be recognized from which it is then possible to obtain selected information concerning the oligomer examined. The mass range which is up to 350 amu (Figure 1) displays the expected[5] behavior of a mixture of deoxynucleotide monophosphate anions. The three monomeric units present in the structure of d-GGTA (**2**), for instance, can be clearly identified from the peaks at m/z 321, 330, and 346 which correspond to the anions $(TMP - H)^-$, $(AMP - H)^-$ and $(GMP - H)^-$, respectively. The products of their further degradation, shown downwards, include fragment ions at m/z 195, 150, 135, and 127 which give additional information concerning the structure of the sugar, purine, and pyrimidine moieties, respectively. The first one, in fact, corresponds to an isobaric mixture of the 2- and 3-monophosphate anions of 2-hydroxymethyl-3-hydroxydihydrofuran, the others to $(Gua - H)^-$, $(Ade - H)^-$ and $(Thy + H)^-$, respectively; this is similar to the behavior patterns found for isomeric dinucleotides.[5,10] The anionic fragments appearing at very low masses correspond to an extensive degradation of the sugar and phosphate moieties. Similar results have been obtained by EI pyrolysis of DNAs.[12] While in FAB experiments such data are used only to gather additional structural evidence, in the EI analysis they represent the sole information source.



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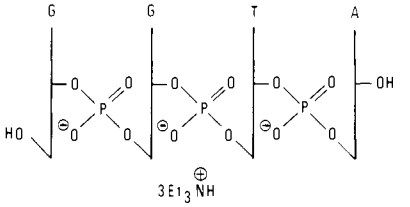

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The upper portion of the FAB spectra reported in Figure 2 provides data concerning the composition of the monocharged quasimolecular anions $(M - H)^-$; this is clearly shown by the presence of peaks at 1212, 1234, and 1250, and 1132, 1154, and 1170, corresponding to $(GGTA - H)^-$, $[(GGTA + Na) - 2H]^-$, and $[(GGTA + K) - 2H]^-$, and $(TACC - H)^-$, $[(TACC + Na) - 2H]^-$, and $[(TACC + K) - 2H]^-$, respectively. However the most important aspect of FAB-induced gaseous oligonucleotide anion reactivity, as seen from their fast decompositions processes, is the preference for a stepwise degradation at the phosphodiester bond level. In fact, all the fragment ions reported in Figure 2 correspond to trimeric and dimeric sequence peaks which enable the oligomer to be easily "rebuilt" starting from the dinucleotide species.

The structure of the parent $(M - H)^-$ is then unambiguously defined by linking the dimers according to the information stored in the trimeric fragments. However, an analysis of the stable ion spectra does not allow a clear identification of the first nucleotide of the sequence which bears a 3′-phosphate linkage; this can be extremely significant in the case of natural segments.[13] Nevertheless, an explanation for the chemical reactions displayed requires both that the structure of the precursor monocharged anions **1** and **2** be accurately defined and that those processes, if any, occurring in other than the gas phase be identified. Therefore, specific MS/MS analysis of the parent quasimolecular anions **1** and **2** has been undertaken in order to determine the chemistry of those species in a noninteracting environment. The spectrum of the daughter ions formed by unimolecular decays of $(TACC - H)^-$ (m/z 1132, Figure 3a) shows, in the upper part,

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(13) While the manuscript was being edited the same ionization technique was applied to the sequencing of some unprotected deoxyoligonucleotides, using information derived from stable ion mass spectra only.[14] The observed different reactivity of the 3′- and 5′-phosphodiester bonds of isomeric deoxyoligonucleotides[5] was suggested as a criterion for defining the sequence origin of the oligomer. This proposal appears to lack general applicability as shown by the present and other unpublished results.[15] In fact, the relative abundances of $(pACC - H)^-$, $(TACp - H)^-$, $(pCC - H)^-$, and $(TAp - H)^-$ displayed by the MIKE spectrum (Figure 3a) clearly show that the 3′-P/5′-P ratio, even within the same molecule, undergoes significant changes in the course of the experiment itself.
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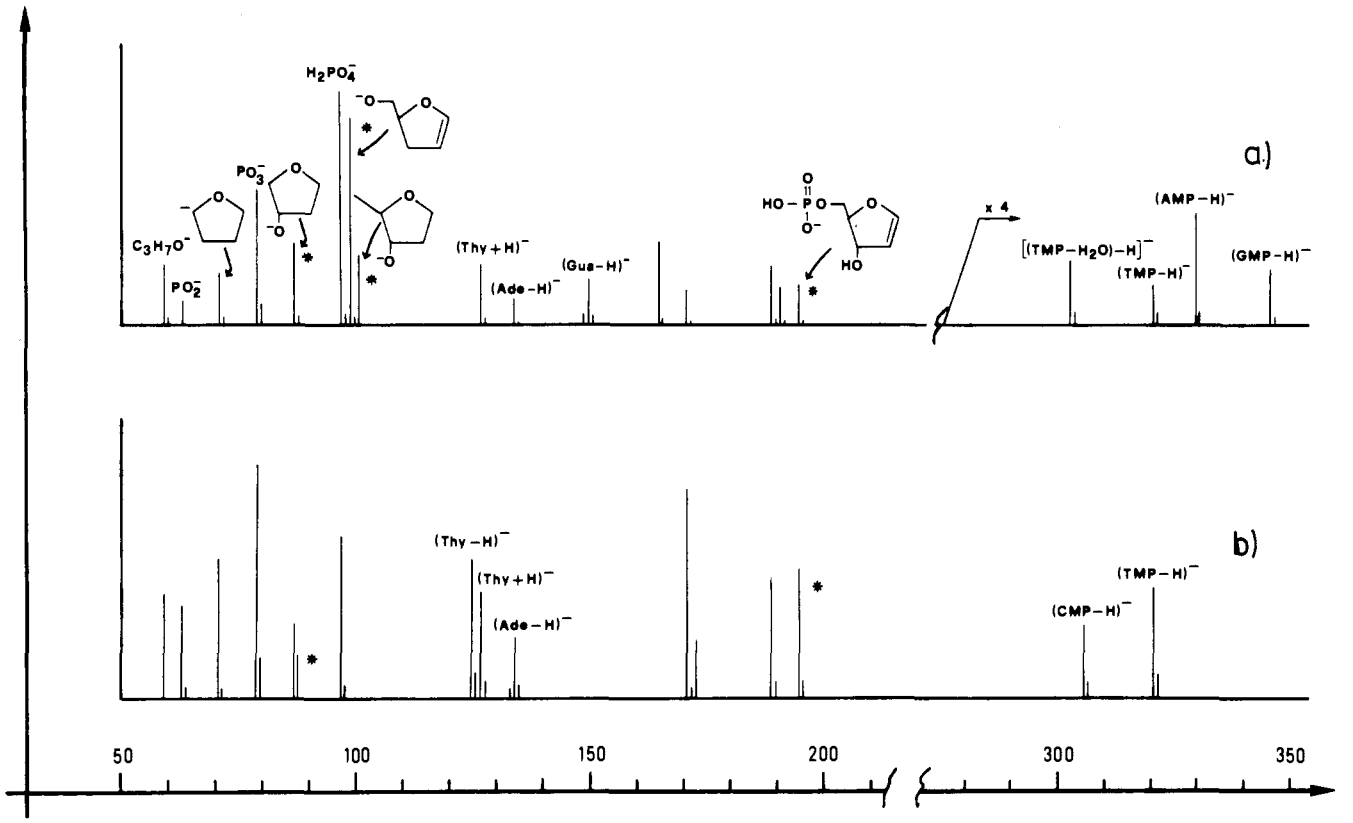

Figure 1. Low-mass-range FAB spectra of d-GGTA (a) and d-TACC (b). Asterisked peaks correspond to isomeric mixtures.

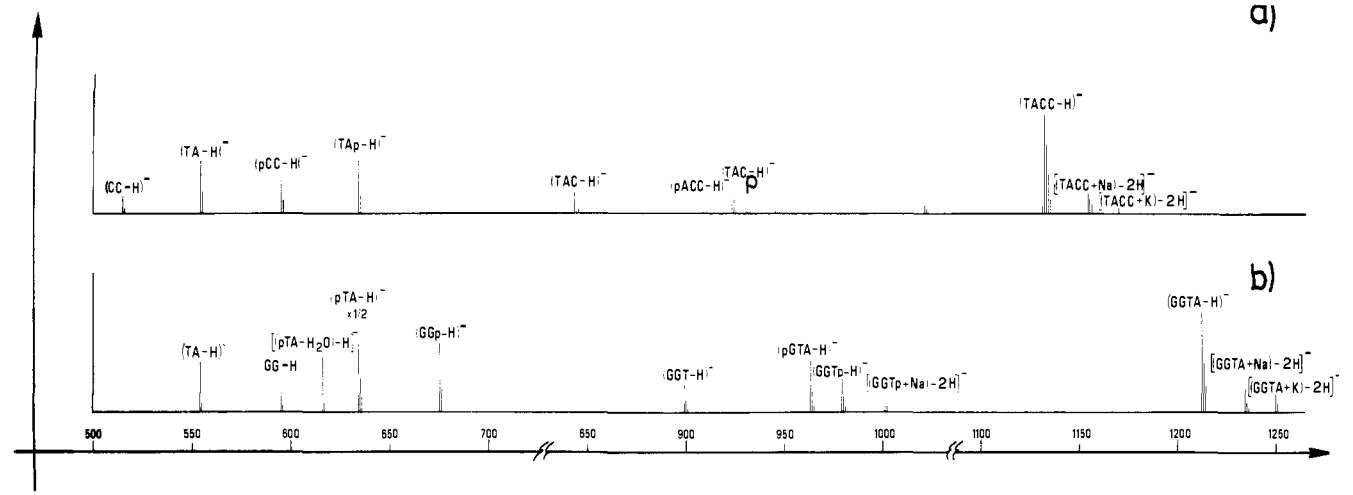

Figure 2. High-mass-range FAB spectra: (a) d-TACC (**1**) and (b) d-GGTA (**2**) tetradeoxyoligonucleotides.

i.e., in the 700–800 electrostatic sector voltage range, peaks at m/z 1039 and 1051, which may correspond to some degradative rearrangements of the purine and pyrimidine bases, and a fragment anion at m/z 1021 due to cytosine elimination; the lower portion displays a series of competitive and consecutive reaction products which provide sequence information. Those data clearly indicate that the FAB-induced desorption of oligonucleotides causes the transfer into the gas phase of quasimolecular anions containing enough internal energy to decompose after 10^{-5} s from their formation. Furthermore, the decomposition pathways evidenced by the discussed MI spectrum correspond to the population of reaction channels unaffected by interaction of the sampled molecule with the matrix or induced by the colliding Xe particles. The reactions exhibited by slow (Figure 3a) and fast (Figure 2a) decomposing $(TACC - H)^-$ anions complement each other; this agrees with the principles governing the reactivitiy of gaseous ions decomposing unimolecularly at different internal exitation energy. However, no sufficient evidence exists against the occurrence of some surface decomposition processes which may overlap with unimolecular decays displayed by the spectra of primary ions, at least in the low mass range region (Figure 1).

The abundant base elimination peak occurring at m/z 1021, which is negligible for fast reactiong ions, might be seen as a rearrangement process which predominates in the metastable window as already verified for dimeric analogues.[5,10] However, when the internal energy content of the precursor $(TACC - H)^-$ is increased by collision with air in a CA experiment, the spectrum of the product ions exhibits both a dominant cytosine elimination reaction and the appearance of thymine loss. These results indicate that rearrangement processes controlling base elimination from the parent oligomer do not occur. On the contrary, it would appear that the cytosine and thymine moieties are already "present" in the structure of the reacting species and ready to be eliminated intact as good neutral leaving groups. Furthermore, the gaseous tetranucleotide quasimolecular anions reacting under the experimental conditions described above exist as monocharged species as their m/z values indicates. These ionized tetranucleotides must be formed by proton-transfer reactions occurring in the matrix

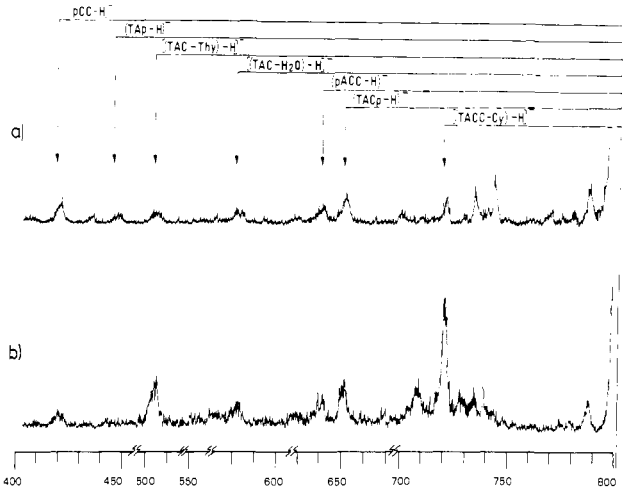

Figure 3. Metastable ion (MI) (a) and collisional activation (CA) (b) secondary mass spectra of d-TACC quasimolecular anion.

and preceding the sputtering process. A very simple view of the structure of those ions might suggest that two phosphodiester acid functions and one monophosphate anion group are present. This, however, is very unlikely since the resulting anion is an amphoteric species bearing two strong acid group ($pK_a = 1$) and hard basic centers such as heterocyclic rings.

Collisional experiments indicate that some of the heterocyclic bases present in the oligomer can be lost as neutrals by a fast single bond cleavage process; therefore, they must contain an extra proton with respect to the original structure of the sampled compound (**1**). Gas-phase data strongly suggest, therefore, a zwitterionic structure for the $(M - H)^-$ obtained after FAB of d-TACC triethylammonium salts; three negative phosphodiester groups and two protonated bases are present, which probably correspond to the cytosine and thymine pyrimidinic rings. The advantage of using a FAB sputtering source to produce gaseous DNA oligomers, rather than other classic MS approaches, relies on the fact that molecules can be released intact in the gas phase with an internal energy content allowing stepwise degradation of the molecule. Thermal procedures, in fact, bring about a complete chemical degradation to sugar, purine and pyrimidine bases before ionization. The application of MS/MS analysis, moreover, allows a specific determination of the breakdown pattern of the ionic species thus produced, in experimental conditions which do not suffer the occurrence of those "hidden variables" often affecting the obtainment of the primary ion spectra by a particle-induced desorption process. Furthermore, the existence of gaseous quasimolecular ions enable the most up-to-date weapons in the mass spectrometrist's armory to be used to fully determine the chemistry involved in a given experiment. This is, in our opinion, a necessary step toward any further applications to the analysis of unknown species.

In conclusion, unprotected deoxyoligonucleotides can be directly sequenced by FABMS from the aqueous solution of their triethylammonium salts, via an investigation of the reactivity of those monoanions possessing a zwitterionic structure. MI determination and CA experiments play a unique role in structure determination and provide a wealth of information which can be used to increase those obtained from the spectra of the primary formed ionic species.

Experimental Section

Oligonucleotides have been synthesized by the phosphotriester approach.[16] The oligomers have been sampled directly from their water solution into the glycerol layer present on the tip of FAB probe. Spectra have been recorded in a double-focusing mass spectrometer VG ZAB equipped with a commercial FAB gun. CA experiments have been performed admitting air in the collision cell of the second field-free region and reducing by a factor of 3 the intensity of the sampled quasimolecular anions.

Acknowledgment. We thank Professor Colin B. Reese for helpful discussions and Miss D. Scott for help with the MS.

Registry No. 1, 86392-57-6; **2**, 86392-58-7.

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Electrochemical and Spectral Characterization of Iron Mono- and Dinitrosyl Porphyrins

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Abstract: The electrochemistry of $(TPP)Fe^{II}(NO)$ and $(OEP)Fe^{II}(NO)$ was investigated in nine nonaqueous solvents. In weakly binding solvents, such as dichloromethane or benzonitrile, five diffusion-controlled electron-transfer reactions were observed. Three of these reactions were oxidations. The remaining two reactions involved reversible electroreductions, either at the Fe(II) center or at the porphyrin ring. In strongly binding solvents, such as Me_2SO or pyridine, similar redox reactions were observed, but a number of chemical reactions were coupled to the electron-transfer steps. Formation of dinitrosyl complexes from either the unnitrosylated or mononitrosyl Fe(II) and Fe(III) complexes was characterized, and several stability constants measured. Finally, competitive ligation between different solvents and NO as axial ligand in each solvent system was studied along the series of oxidized, neutral, and reduced complexes.

During the last 15 years, a large number of studies have been published on the electrochemistry of (TPP)FeX and (OEP)FeX.[1] These studies have included investigations of the Fe(III)/Fe(II) reaction as well as oxidation to yield $[(TPP)FeX]^+$ or reduction to yield $[(TPP)Fe]^-$. In the former case, the reaction was initially postulated to be at the Fe(III) center, producing Fe(IV),[2,3] but this assignment now appears to be incorrect, and the actual oxidation probably occurs at the porphyrin ring.[4-6] For reduction,

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