J. Am. Chem. Soc. 1989, 111, 6027-6035

Mechanism of Porphyrin Reduction and Decomposition in a High-Pressure Chemical Ionization Plasma

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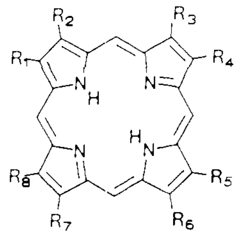
Abstract: Mechanisms for the in situ reduction and decomposition of porphyrins that occur in a high-pressure ammonia and hydrogen chemical ionization (CI) plasma are proposed based on studies of selected model porphyrins and porphyrinogens, both in a high-pressure CI ion source and under low-pressure CI conditions in a three-dimensional quadrupole ion trap. Stepwise reduction of the porphyrin by radical hydrogen, aided by interaction of the porphyrin with the interior surfaces of the ion source, followed by ionization, forms molecular species, $(M + nH)^+$, where $n = 0, 2, 4$, and 6 when using hydrogen, and where $n = 1, 3, 5$, and 7 when using ammonia. The major fragment ions in the CI mass spectra, although arising from the reduced molecular species, are not products of simple unimolecular dissociation of the $(M + nH)^+$ species. Mass spectrometry/mass spectrometry (MS/MS) studies show that the $(M + nH)^+$ species, where $n \geq 2$, fragment to form tri-, di-, and monopyrrolic units with masses two or more mass units less than the most abundant pyrrolic fragment ions observed in the CI mass spectra. Therefore, gas-phase unimolecular decomposition of the reduced ionic species can contribute somewhat to the overall CI mass spectrum. However, the major pyrrolic fragment ions observed in these spectra (as well as the majority of the other fragments) more likely result from unimolecular and thermal decomposition of the reduced species (both ionic and neutral) in the gas-phase and on the source surfaces, forming neutrals that possibly undergo additional reactions in the CI plasma prior to ionization.

High-pressure chemical ionization–mass spectrometry (CI–MS) of porphyrins (e.g., **1** and **2**), with ammonia[1,2] or hydrogen[3-6] as the reagent gas, has been shown to promote formation of structurally significant tri-, di-, and monopyrrolic fragment ions from the porphyrin macrocycle. This discovery was a major breakthrough in porphyrin structure determination using mass spectrometry, which previously had been severely limited by the inability to fragment the porphyrin macrocycle. Macrocycle fragmentation is a crucial step necessary to determine the pyrrole sequence and location of substituent groups on the macrocycle. Using electron ionization–mass spectrometry (EI–MS), for example, the structural information obtainable is limited to identification of the substituent groups on the macrocycle.[7-9] From the masses and pattern of the pyrrolic fragment ions in such a CI mass spectrum, the pyrrole sequence of the porphyrin and limited information on the location of the substituent groups can be determined.



1: $R_{1,3,5,8} = CH_3$

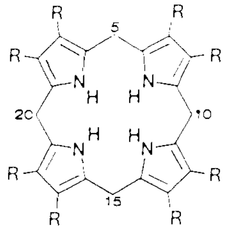
$R_{2,4,6,7} = C_2H_5$

(MW = 478 u)

2: $R_{1,2,3,4,5,6,7,8} = C_2H_5$

(MW = 534 u)

Although both ammonia and hydrogen CI–MS have been used to obtain structural data on porphyrins,[1-6] the precise phenomena responsible for the fragmentation patterns observed remain to be determined. The high-pressure CI fragmentation pattern of a porphyrin is similar to that observed in the EI mass spectrum of the corresponding porphyrinogen. Porphyrinogens (*meso*-hexahydroporphyrins (**3**) are much less stable than porphyrins, fragmenting by cleavage at the meso positions (i.e., bridging carbons C-5, C-10, C-15, and C-20 in **3**) to form pyrrolic ions when using electron ionization.[10,11] This similarity in fragmentation patterns, along with the observation of ions corresponding to the mass of the porphyrinogen in the CI mass spectra, led Djerassi and coworkers[1,2] to hypothesize that, in high-pressure ammonia CI, the porphyrin is reduced sequentially in the gas phase, by multiple interactions with the reagent gas, forming the porphyrinogen. Additional, nonspecific, attack of the reagent gas at the meso positions of the protonated porphyrinogen, $(M + 7H)^+$, was proposed to fragment the macrocycle producing the pyrrolic ions. Shaw et al.[3] interpreted their hydrogen CI data as indicating continual addition of neutral H_2 molecules to the macrocycle forming the porphyrinogen, $(M + 6H)^{\bullet+}$, which subsequently



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decomposed to yield the pyrrolic fragment ions.

As is demonstrated in this work, reduction of the gas-phase neutral or ionized porphyrin via multiple interactions with the reagent gas cannot account for formation of the $(M + nH)^+$ ions observed in the molecular ion region of the ammonia and hydrogen CI mass spectra. The reduction of porphyrins, like other reductions that have been observed to occur in high-pressure CI plasmas,[12-17] more likely results from interactions other than gas-phase ion–molecule reactions. For example, gas-phase addition of hydrogen radicals[15] or surface-assisted hydrogenation[16] are two mechanisms that have been put forward to explain the reduction of other compound classes. Furthermore, unimolecular decomposition of the ionized reduced porphyrin species (i.e., $(M + 6H)^{\bullet+}$ or $(M + 7H)^+$) to form the major pyrrolic ions observed in high-pressure CI mass spectra has not been confirmed.

In this paper, mechanisms are presented for the reduction and decomposition of the porphyrin macrocycle in a high-pressure CI plasma based on studies of selected model porphyrins and porphyrinogens in both a high-pressure CI ion source and under low-pressure CI conditions in a three-dimensional quadrupole ion trap. Spectral changes that occur with variation of high-pressure CI ion source conditions and the results of mass spectrometry/mass spectrometry (MS/MS) studies (metastable ion dissociation and collision-induced dissociation (CID)), of the in situ reduced molecular species, provide the foundation for the proposed mechanisms. The three-dimensional quadrupole ion trap, which affords greater control for the study of gas-phase ion-molecule reactions than is possible with a conventional high-pressure CI ion source, was used to obtain confirmatory data.

Experimental Section

Samples. Porphyrins were obtained from a commercial supplier (Strem Chemicals, Newburyport, MA) and used without further purification. Porphyrinogens were prepared from their respective porphyrins via a Raney nickel (Aldrich Chemical, Milwaukee, WI) hydrogenation. Raney nickel was activated immediately prior to use by bubbling hydrogen into an aqueous slurry of the catalyst. Following activation the water was decanted and the catalyst transferred into toluene by a series of washes with distilled water, methanol, and finally toluene. Porphyrin was dissolved in toluene/methylene chloride (10:1 v/v) and stirred under a nitrogen atmosphere for 5 min to flush oxygen from the system, after which the activated catalyst was added (20 mg of catalyst/mg of porphyrin). The reaction mixture was stirred under nitrogen, in the dark, until the solution was colorless (approximately 5–15 min), signaling complete conversion to the porphyrinogen. The reaction solution was decanted from the catalyst and the porphyrinogen isolated by removal of the solvent with a stream of nitrogen. Care was taken to limit exposure of the product to the atmosphere so as to minimize oxidation back to the porphyrin.

Mass Spectrometry. A modified VG ZAB of BE geometry (B = magnetic sector, E = electric sector) at Oak Ridge National Laboratory and a VG ZAB-EQ of BEQQ geometry (Q = quadrupole) at the University of Tennessee, both equipped with an EI/CI ion source, were used to perform the bulk of experiments presented in this study.

Porphyrin and porphyrinogen samples were introduced into the ion source of the BE instrument via a direct insertion probe or were dissolved in methylene chloride and deposited directly on the ion source repeller plate. Ion source temperature, as measured by a platinum resistance thermometer, ranged from approximately 413 to 523 K. Reagent gases (Linde, Danbury, CT) were maintained at a pressure of approximately 0.15 Torr and ionized using 70–100-eV electrons with an emission current of 0.3–1.5 mA. To obtain metastable ion spectra the ion source voltage (5 keV) and magnetic field were fixed to allow ions of the desired momentum-to-charge ratio to pass into the second field-free region. The ionic products formed from dissociations in this region were then analyzed by scanning the electric sector of the instrument. For CID spectra, nitrogen or helium was admitted into the second field-free region via a syringe needle collision cell[18] to a pressure sufficient to attenuate the main beam by ≤80%.

A desorption chemical ionization (DCI) probe with a platinum DCI coil was used as the means of sample introduction on the BEQQ instrument. Ion source temperature was 423 K, ammonia (National/Bower, Philadelphia, PA) pressure 8×10^{-5} Torr (measured in the source vacuum housing), electron energy 150 eV, emission current 1.0 mA, and accelerating potential 8 keV. Porphyrin samples were dissolved in chloroform and 1–5 μL of the solution was loaded onto the DCI coil (2–10 μg of porphyrin). Porphyrin was desorbed from the DCI probe by ramping the heating current from 0 to 1.5 A at 3.75 mA s^{-1}.

A three-dimensional quadrupole ion trap at Oak Ridge National Laboratory, the Finnigan-MAT Ion Trap Mass Spectrometer™ (ITMS), was used for supporting or confirmatory experiments. General operation of the ITMS[19,20] and CI-MS using the ITMS[21] have been discussed in detail elsewhere. Helium bath gas and CI reagent gases (Linde) were used at uncorrected gauge pressures of 1×10^{-4} and 2–4×10^{-5} Torr, respectively. An ion trap temperature of 448 K was used for the analysis of porphyrins and a temperature of 398–423 K for the analysis of porphyrinogens. A direct insertion probe was used to introduce both sample types into the ion trap.

Results and Discussion

High-Pressure Chemical Ionization. High-pressure CI mass spectra of 2,7,12,18-tetramethyl-3,8,13,17-tetraethyl-21*H*,23*H*-porphine (etioporphyrin III, **1**) and 2,3,7,8,12,13,17,18-octaethyl-21*H*,23*H*-porphine (octaethylporphyrin, **2**) were obtained on the BE and BEQQ instruments. Typical CI mass spectra acquired with the BE instrument are shown in Figure 1a–c. To obtain these mass spectra, a sample of **1** (MW = 478 u) was coated onto the repeller plate of the ion source prior to the analysis. The spectra were recorded as the temperature of the source slowly increased due to radiative heating from the electron ionization filament. Similar CI mass spectra, at the respective temperatures, were obtained on either the BE or BEQQ instruments if the direct insertion probe or DCI probe was used as the means of sample introduction instead of the direct deposition method. The DCI probe and direct deposition of the sample facilitate introduction of the relatively nonvolatile porphyrins into the ion source at source temperatures below 473 K.

The CI mass spectra shown in Figure 1a–c are representative of spectra obtained at increasing source temperatures for the direct deposition method, but the relative abundances of the ions at any one temperature vary from analysis to analysis. The major ions in each spectrum are the pseudomolecular species, $(M + nH)^+$, and tri-, di-, and monopyrrolic fragments. The relative abundances of these ions, especially those in the molecular ion region, are highly dependent on the ion source temperature. The base peak in the mass spectrum at 424 K (Figure 1a) is $(M + 7H)^+$, presumably the protonated porphyrinogen, formed via reduction of **1** in the ion source. The major pyrrolic fragment ions in each spectrum occur at masses corresponding to the protonated form of the respective pyrrolic compounds as shown in Figure 2. Each individual mono-, di-, or tripyrrole is observed as a triad of peaks corresponding to the respective pyrrolic unit containing 0, 1, or 2 terminal meso carbons. Each member of the respective triads is surrounded in the mass spectrum by a cluster of lesser abundant ions.

As the ion source temperature increases, a major change in the nature of the molecular species is seen. At 450 K (Figure 1b) the fragmentation pattern remains similar to that of the 424 K spectrum (Figure 1a), but the relative abundance of the $(M + H)^+$ ion increases markedly. Correcting for isotopic contributions, the major species in the molecular ion region of the 450 K spectrum are $(M + H)^+$, $(M + 3H)^+$, $(M + 5H)^+$, and $(M + 7H)^+$, corresponding to the protonated molecule and presumably the protonated forms of the dihydro-, tetrahydro-, and hexahydroporphyrin (i.e., porphyrinogen), respectively. At 498 K

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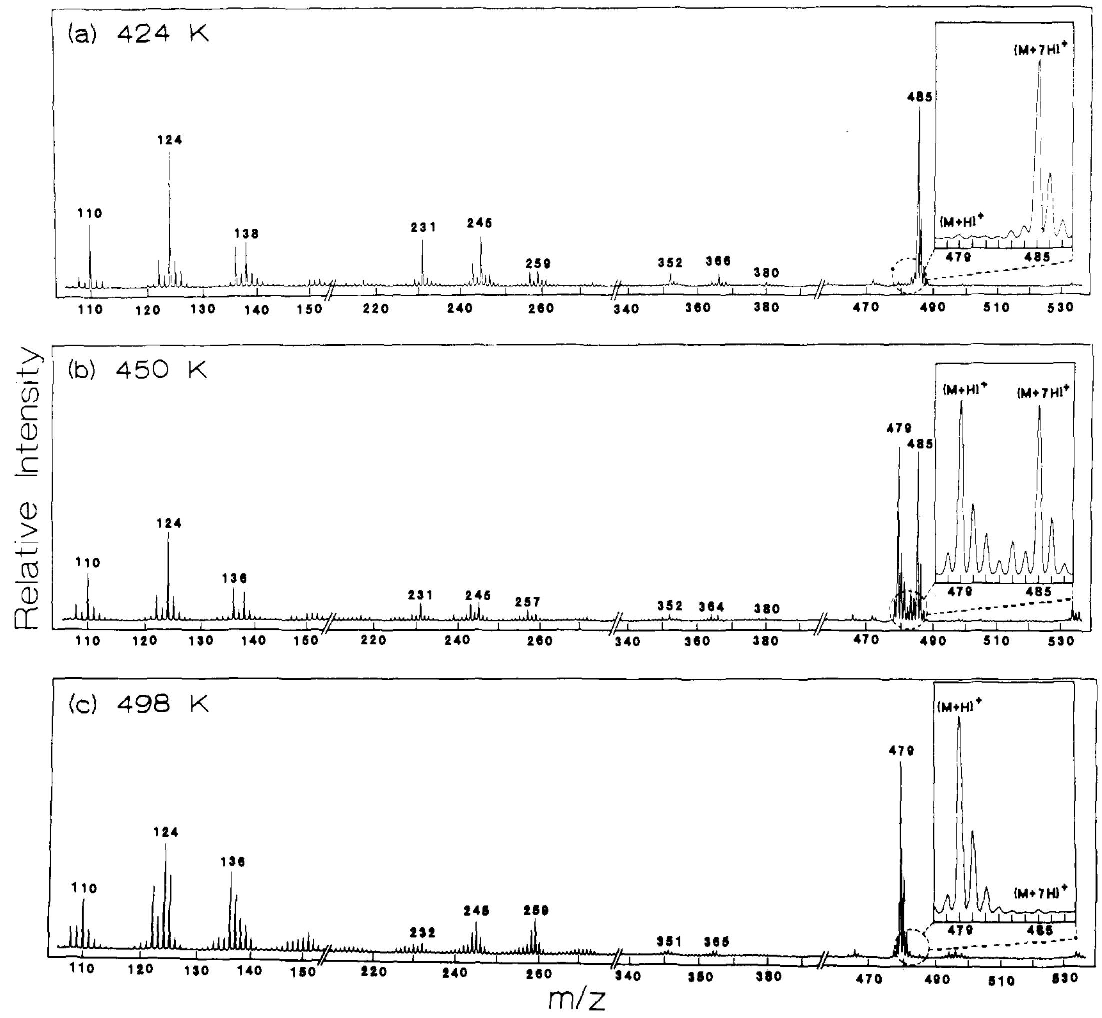
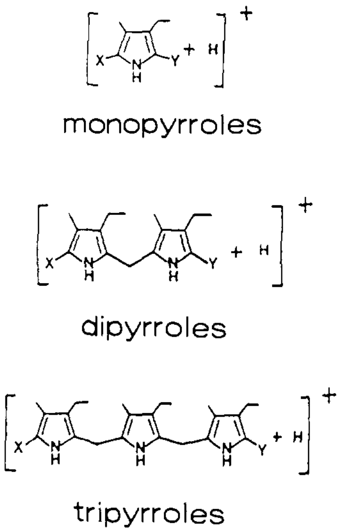


Figure 1. High-pressure ammonia CI mass spectra of etioporphyrin III (**1**, MW = 478 u) obtained at ion source temperatures of (a) 424 K, (b) 450 K, and (c) 498 K. Porphyrin was deposited on the ion source repeller plate of the BE instrument prior to analysis, and the spectra were recorded at the respective temperatures as the source temperature increased owing to heating from the electron ionization filament.

(Figure 1c) the protonated molecule, $(M + H)^+$, is the dominant molecular species, with barely detectable amounts of the reduced species present. Even though the reduced species are greatly decreased in intensity at 498 K, the relative abundance of the pyrrolic fragment ions has changed little compared with their relative abundance in the spectra obtained at lower temperatures. Overall, however, more fragment ions appear in this spectrum than in the spectra obtained at the lower temperatures. The ammonia CI mass spectra obtained for **2** (MW = 534 u) under the same conditions are basically identical with those of **1** (Figure 1a–c), allowing for the 14, 28, 42, and 56 mass unit increase of the mono-, di-, and tripyrrole, and molecular ion regions, respectively, in the spectra of **2**.

High-pressure hydrogen CI mass spectra are comparable to those obtained when using ammonia as the reagent gas. However, the odd-electron molecular species $M^{\bullet+}$, $(M + 2H)^{\bullet+}$, $(M + 4H)^{\bullet+}$, and $(M + 6H)^{\bullet+}$, and fragment ions dominate the spectra instead of the even-electron ions observed when using ammonia. Also, the monopyrrolic ions dominate the hydrogen CI mass spectra, and the overall amount of fragmentation is increased at all ion source temperatures relative to the ammonia CI mass spectra. Spectra similar to those obtained using high-pressure ammonia CI were acquired using methane or isobutane as the reagent gas. For the latter two gases, however, the relative abundance of the reduced molecular species at all source temperatures is low (<10% of the base peak) and the abundance of the di- and tripyrroles was significantly decreased compared with the ammonia CI mass spectra.

Porphyrin reduction was observed to decrease dramatically, and pyrrolic fragment ions were of low abundance or absent from the ammonia CI mass spectra, when porphyrin was introduced via the direct insertion probe or DCI probe at high ion source temperatures (>543 K). This observation, along with the data from



X	Y	m/z
H	H	110
H	CH_3	124
CH_3	CH_3	138
H	H	231
H	CH_3	245
CH_3	CH_3	259
H	H	352
H	CH_3	366
CH_3	CH_3	380

Figure 2. Proposed structures for the major pyrrolic fragment ions in the high-pressure ammonia CI mass spectra of etioporphyrin III (**1**, MW = 478 u). The possible isomeric pyrrole sequences for the tri- and dipyrroles cannot be distinguished in the mass spectra and are, therefore, not shown.

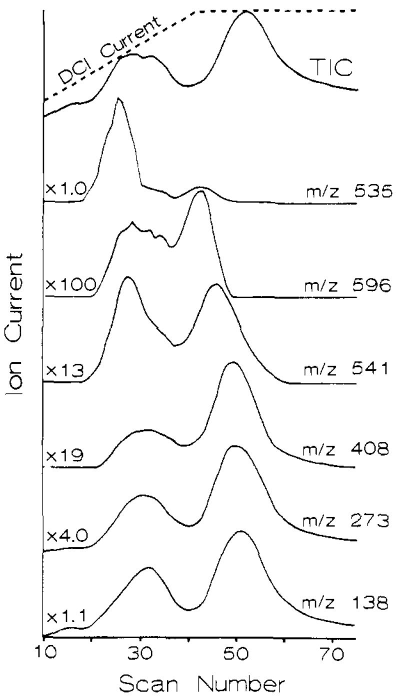


Figure 3. Total and single ion current desorption profiles from the high-pressure ammonia CI mass spectra of octaethylporphyrin (**2**, MW = 534 u). A 10-μg sample of **2** was desorbed from a DCI probe into the ion source of the BEQQ instrument, maintained at 423 K, by ramping the probe heating current from 0 to 1.5 A at 3.75 mA s–1, and then holding the current at 1.5 mA for the duration of the experiment.

the direct deposition method presented above, indicated that porphyrin–surface interactions could be critical in the reduction/decomposition process. The total and single ion current desorption profiles presented in Figure 3 provide further evidence for the importance of porphyrin–surface interactions in this process. These desorption profiles were obtained from an experiment on the BEQQ instrument in which the ion source temperature was maintained at 423 K and ammonia CI mass spectra were recorded as porphyrin (**2**, MW = 534 u) was desorbed from the DCI probe by ramping the probe heating current (3.75 mA s^{-1}). The molecular species is desorbed early in the current ramp (first hump in the desorption profile) and is protonated producing the ion at *m/z* 535 (i.e., $(M + H)^+$). The second hump present in the *m/z* 535 desorption profile (as well as the second hump in all the desorption profiles) was confirmed, by additional DCI experiments, to result from that fraction of porphyrin liberated from the probe early in the current ramp (first hump) that is deposited on the source surfaces and subsequently desorbed from those surfaces owing to radiative heating from the DCI probe.

The two-hump nature of the desorption profiles in Figure 3 and the observation of ions at *m/z* 589 (not shown) and *m/z* 596, corresponding the protonated iron–octaethylporphyrin complex and multiply reduced metalloporphyrin, respectively, indicate that, at 423 K, a substantial fraction of the gas-phase porphyrin condenses on surfaces within the ion source. The iron–porphyrin complexes are formed via contact of the free-base porphyrin with the stainless steel surfaces within the ion source. Metalation of porphyrins in this manner is well documented.[7,8] A portion of the porphyrin that contacts the source surfaces is metalated/reduced and liberated from the surface very quickly (first hump), but a more substantial fraction desorbs only after the surfaces are warmed by radiative heat transfer from the DCI probe (second hump).

Simultaneous with appearance of iron–octaethylporphyrin, and lagging behind the appearance of the protonated porphyrin, is the appearance of $(M + 7H)^+$ and the pyrrolic fragment ions. Desorption profiles for $(M + 7H)^+$ (*m/z* 541) and the major tripyrrole (*m/z* 408), dipyrrole (*m/z* 273), and monopyrrole (*m/z* 138) fragment ions are shown in Figure 3 for illustration. The maximum intensity of $(M + 7H)^+$ and each pyrrolic fragment ion is reached well after the porphyrin has been completely desorbed from the DCI probe, indicating that their formation is associated with reactions involving porphyrin deposited on the source surfaces. The di- and monopyrrole (*m/z* 273 and 138, respectively) are observed, however, in low abundance, even before the porphyrin is observed. This indicates that some decomposition is occurring while the porphyrin is on the DCI probe. Clearly, the ion source surfaces (including the DCI probe) are involved in the reduction/decomposition process, and the appearance of the pyrrolic fragment ions in the high-pressure CI mass spectra is associated with the appearance of the reduced porphyrin species.

Low-Pressure Chemical Ionization. The ion trap was used to study the low-pressure CI behavior of porphyrins and porphyrinogens prepared in vitro. The ion trap affords greater control for the study of gas-phase ion-molecule reactions than is possible in a high-pressure CI ion source. Porphyrin reduction did not occur, however, under any CI conditions normally used with the ion trap.[21] With ammonia as the reagent gas, for example, the protonated porphyrin was produced with no apparent reduction and *without* fragmentation. Similar spectra were noted using methane and isobutane as the reagent gases. Unconventional means of CI on the ion trap were also used in an attempt to promote the reduction/decomposition process. For example, the helium bath gas normally present in the ion trap at a pressure of 1 mTorr was replaced with hydrogen. Electron ionization and ammonia chemical ionization experiments were then performed at temperatures from 398 to 448 K. Reduction of MH^+ or $M^{\bullet+}$, even with extended reaction times (up to 2 s) with NH_3 or H_2 did not occur. Additionally, the neutral porphyrin could not be reduced by prolonged interaction with the neutral or charged reagent gas species.

The ionization and fragmentation behavior of in vitro prepared porphyrinogens was studied on the ion trap using selected reagent ion chemical ionization.[22] Using this method one particular ion from the suite of reagent ions normally present in the CI plasma can be selected to react with the analyte. In this way, the mechanism and energetics of the ionization process involving the reaction of a particular reagent ion and the gas-phase porphyrin could be studied. (It was not possible to study the reactions of $H_2^{\bullet+}$ or H_3^+ with the porphyrins since these low mass reagent ions cannot be stored effectively in the ion trap and still trap the ionized analyte.)

Using ammonia and selecting NH_4^+, protonated etioporphyrinogen III was produced *without* fragmentation (Figure 4a). However, when using methane and selecting CH_5^+, the protonated prophyrinogen was produced along with a tri- and dipyrrolic fragment ion (Figure 4b). This demonstrates, as expected, that the exothermicity of the ionization process, which increases as the proton affinity (PA) of the conjugate base of the respective reagent ion decreases[23] ($PA_{methane} = 130.5$ kcal mol^{-1} $< PA_{ammonia} = 205$ kcal mol^{-1}), determines the extent of porphyrinogen fragmentation. Based on relative proton affinities, protonation by H_3^+ ($PA_{hydrogen} = 100.7$ kcal mol^{-1}) would be expected to result in more porphyrinogen fragmentation than protonation by CH_5^+. It should be noted that the fragment ions observed from protonated etioporphyrinogen III (Figure 4b) are two mass units less than the mass of the most abundant fragment ions in the ammonia CI mass spectrum of **1** (Figure 1a–c and Figure 2). These ionization produced pyrrolic fragments are the same, however, as those fragments produced by the unimolecular dissociation of $(M + 7H)^+$ produced in situ from **1** (vide infra).

Porphyrinogen fragmentation was also studied using the charge exchange (i.e., electron transfer) reagent ions NO^+ and $N_2O^{\bullet+}$,

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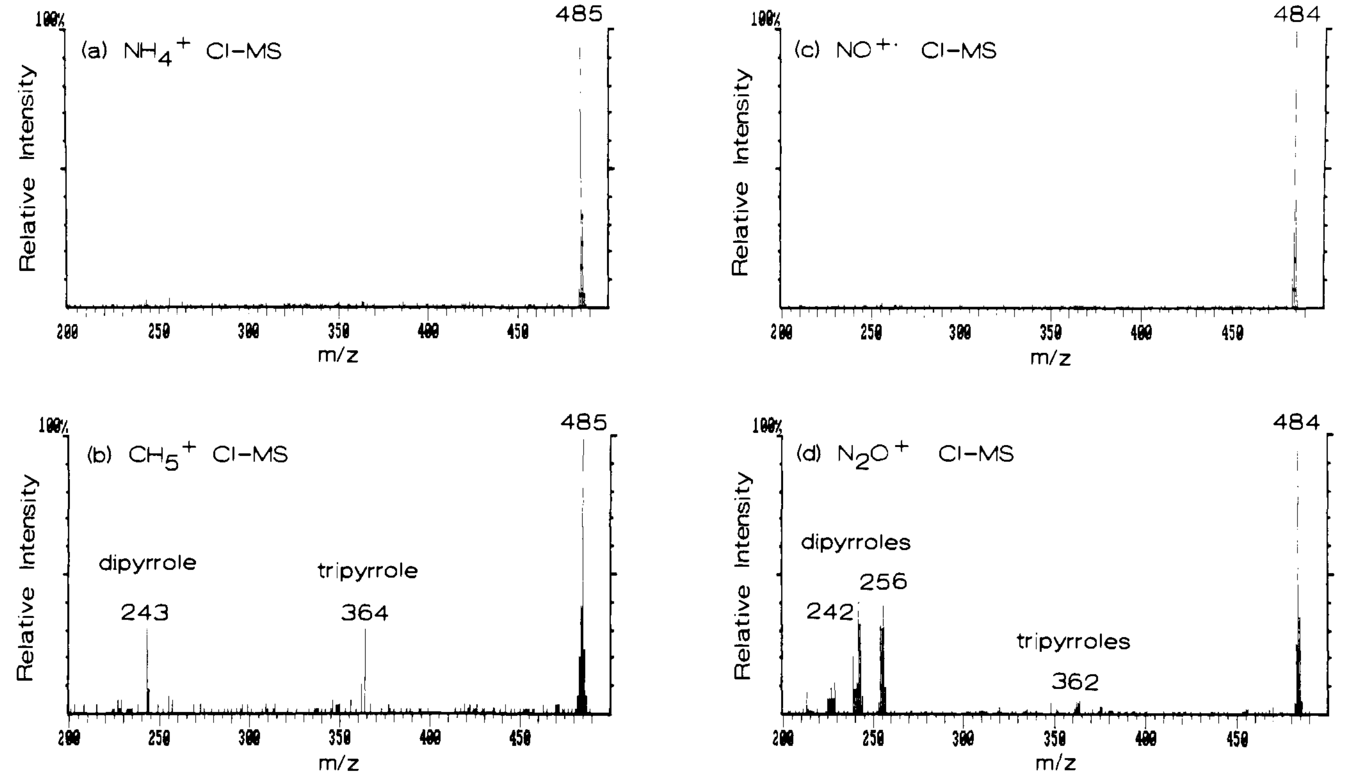
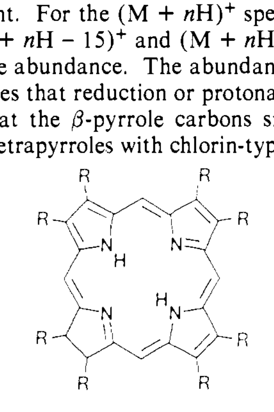


Figure 4. Ion trap CI mass spectra of in vitro prepared etioporphyrinogen III (MW = 484 u) using selected proton transfer reagents: (a) NH_4^+, PA = 205 kcal mol^{-1} and (b) CH_5^+, PA = 130.5 kcal mol^{-1} and selected charge exchange reagents, (c) NO^+, RE = 9.25 eV, and (d) $N_2O^{\bullet+}$, RE = 12.9 eV.

produced from nitrous oxide. (As is the case for proton transfer, the amount of fragmentation observed in charge exchange is dependent on the exothermicity of the reaction. For charge exchange with a given analyte species, the exothermicity increases as the recombination energy (RE) of the reagent ion increases.[23]) In this case, the molecular ion of etioporphyrinogen III was produced without fragmentation when using NO^+ as the reagent ion (RE_{NO^+} = 9.25 eV, Figure 4c), but when using $N_2O^{\bullet+}$ ($RE_{N_2O^{\bullet+}}$ = 12.9 eV, Figure 4d) the molecular ion was produced along with tri- and dipyrrolic fragment ions. These ionization-produced pyrrolic fragment ions from the molecular ion of etioporphyrinogen III are two mass units less than the mass of the most abundant fragment ions in the hydrogen CI mass spectrum of **1**. The fragment ions are the same, however, as those produced by the unimolecular dissociation of $(M + 6H)^{\bullet+}$ produced in situ from **1** (vide infra).

More extensive fragmentation of the porphyrinogen would be expected if $H_2^{\bullet+}$ ($RE_{H_2^{\bullet+}}$ = 15.4 eV) was the charge exchange reagent ion. However, $H_2^{\bullet+}$ is not an abundant species in the high-pressure CI plasma. As $H_2^{\bullet+}$ is formed in the high-pressure CI source, it rapidly reacts with H_2 to form H_3^+ and $H^\bullet$. It is possible that H_3^+ reacts with the porphyrin species by both proton transfer and dissociative charge exchange (i.e., $H_3^+ + (M + nH) \rightarrow H_2 + H^\bullet + (M + nH)^{\bullet+}$). The recombination energy for H_3^+ to form H_2 and $H^\bullet$ is roughly 9.3 eV, indicating that the energetics of dissociative charge exchange with H_3^+ is approximately equivalent to charge exchange with NO^+. The combination of a highly exothermic protonation, which results in significant fragmentation of the reduced species, and a milder dissociative charge exchange process probably accounts for the abundance of odd-electron molecular species in hydrogen CI mass spectra rather than the even-electron species present when using ammonia.

Mass Spectrometry/Mass Spectrometry (MS/MS). To further investigate which, if any, of the reduced porphyrin species might be the source of the pyrrolic fragment ions observed in the CI mass spectra, MS/MS spectra (metastable ion spectra and/or CID spectra) of the $(M + nH)^+$ ions in the ammonia and hydrogen CI mass spectrum of **1** were recorded using the BE instrument. Metastable ion spectra of the major reduced molecular species ($(M + 2H)^{\bullet+}$, $(M + 4H)^{\bullet+}$, and $(M + 6H)^{\bullet+}$) in the hydrogen CI spectrum of **1** are shown in Figure 5a–c. Each metastable ion spectrum in Figure 5 exhibits fragment ions resulting from cleavage of the substituent groups on the macrocycle and pyrrolic fragment ions resulting from macrocycle decomposition. The predominant fragment from $M^{\bullet+}$ (*m/z* 478, spectrum not shown) results from β-cleavage of an ethyl group, $(M - 15)^+$, with a much lesser amount of $(M - 29)^+$, arising from α-cleavage of an ethyl group, also evident. For the $(M + nH)^+$ species, where n = 2, 4, and 6, the $(M + nH - 15)^+$ and $(M + nH - 29)^+$ fragments are of comparable abundance. The abundant α-cleavage fragmentation indicates that reduction or protonation has occurred, to some degree, at the β-pyrrole carbons since α-cleavage is characteristic of tetrapyrroles with chlorin-type structures (**4**).[7-9]



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The relative abundance of the pyrrolic fragment ions in the metastable ion spectra increases as the degree of reduction increases. Monopyrrolic fragments are not observed, however, in any of the metastable ion spectra. The appearance of di- and tripyrrolic fragment ions indicates that reduction at the meso positions (or rearrangement to give an equivalent structure) takes place since interruption of the aromatic system at these points, and probably ring opening, is a necessary requirement for macrocycle decomposition.[7-9]

The hypothesis that the $(M + 6H)^{\bullet+}$ species produced in situ is, in fact, the porphyrinogen was supported by comparison of the metastable ion and CID spectra of $(M + 6H)^{\bullet+}$ in the hydrogen CI mass spectrum of **1** (Figure 5c,d) with the metastable ion and

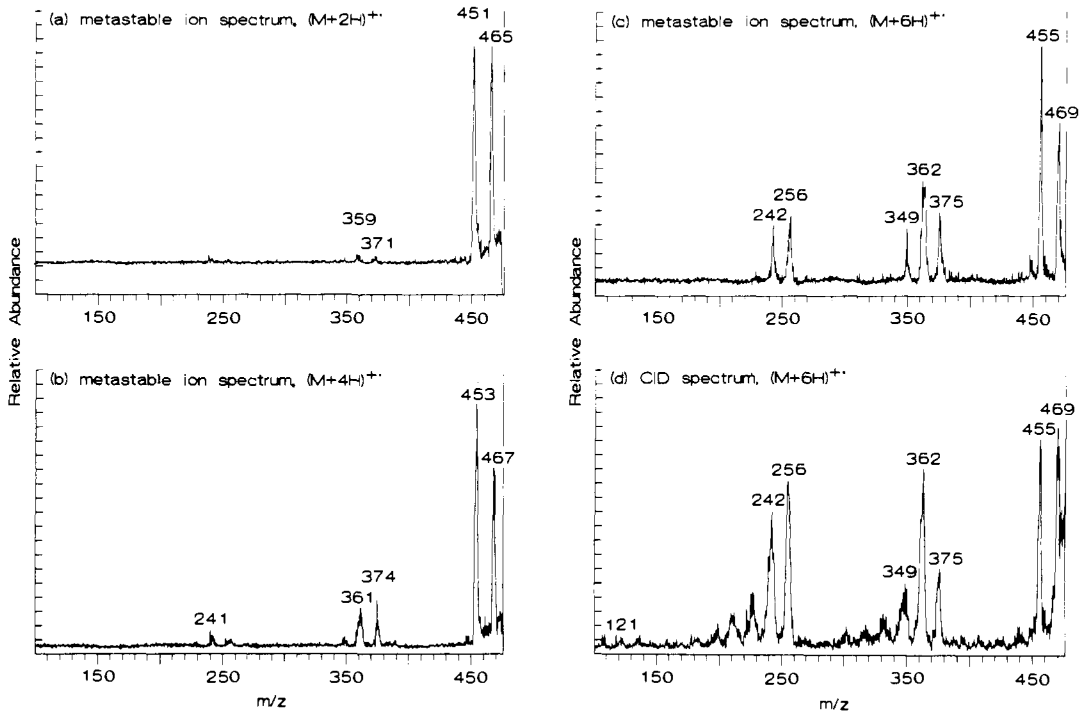

Figure 5. Metastable ion spectra of (a) $(M + 2H)^{\bullet+}$ (*m/z* 480), (b) $(M + 4H)^{\bullet+}$ (*m/z* 482), and (c) $(M + 6H)^{\bullet+}$ (*m/z* 484), and (d) the CID spectrum of $(M + 6H)^{\bullet+}$ in the hydrogen CI mass spectrum of etioporphyrin III (**1**) obtained on the BE instrument.

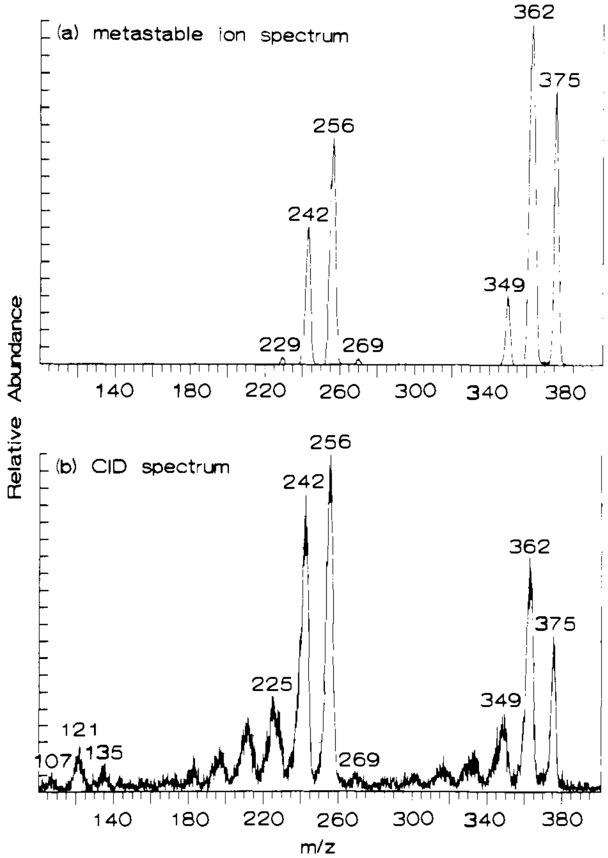

Figure 6. (a) Metastable ion spectrum and (b) CID spectrum of $M^{\bullet+}$ (*m/z* 484) in the EI mass spectrum of in vitro produced etioporphyrinogen III obtained on the BE instrument.

CID spectra of $M^{\bullet+}$ in the EI mass spectrum of in vitro produced etioporphyrinogen III (Figure 6a,b). The metastable ion spectra of each species contain the same substituent group fragments and the same tri- and dipyrrolic ions. The same tri-, di-, and monopyrrolic fragments, as well as fragment ions resulting from cleavage of the substituents from the pyrrolic units, are observed in the CID spectra of each species. (Note that the fragment ions resulting from α- and β-cleavage of the substituent groups from etioporphyrinogen III are not shown in Figure 6.) Also, the pyrrolic fragment ions in these MS/MS spectra (Figure 5c,d and Figure 6a,b) are the same as those observed in the $N_2O^{\bullet+}$ charge exchange mass spectrum of etioporphyrinogen III (Figure 4d).

The metastable ion spectra of the major reduced molecular species ($(M + 3H)^+$, $(M + 5H)^+$, and $(M + 7H)^+$) in the ammonia CI mass spectrum of **1** are similar to those shown in Figure 5. In contrast to the MS/MS spectra of $(M + 6H)^{\bullet+}$ (Figure 5c,d), however, the abundance of the pyrrolic fragment ions in the MS/MS spectra of $(M + 7H)^+$ (Figure 7a,b) is greater than the abundance of the ions resulting from cleavage of the substituent groups. It is clearly demonstrated by the MS/MS spectra in Figures 5 and 7 that the relative amount of macrocycle decomposition increases as the degree of porphyrin reduction increases. Since formation of pyrrolic fragment ions probably requires the macrocycle to ring-open forming the less stable linear tetrapyrrole, this observation is consistent with the statistical probability of forming the ring-opened species. Reduction and protonation can occur on a number of sites on the macrocycle, but only additions at certain of these sites (i.e., the meso carbons and α-pyrrole carbons) will allow the macrocycle to ring-open and fragment. Therefore, the probability that an $(M + nH)^+$ species can ring-open, and subsequently fragment to form pyrrolic ions, should increase as the degree of reduction increases.[24]

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[24] Ring-opening of the macrocycle and the fragmentation mechanism proposed for formation of the major pyrrolic fragment ions observed in the metastable ion spectra and CID spectra of $(M + 7H)^+$, and the other reduced species, are discussed in detail elsewhere: Van Berkel, G. J.; Glish, G. L.; McLuckey, S. A.; Tuinman, A. A. *Anal. Chem.*, in preparation. In that paper, the MS/MS spectrum of the $(M + 7H)^+$ species is shown to contain the same structural information as that contained in the CI mass spectrum. The fragmentation pattern in the MS/MS spectrum of $(M + 7H)^+$ is much simpler than the fragmentation pattern in the CI mass spectrum since fragment ions arise only from unimolecular decomposition of the gas-phase species. Therefore, the MS/MS spectrum of $(M + 7H)^+$ can be of considerable utility for structure determination. This is especially true if an instrument capable of obtaining unit mass daughter ion resolution, such as the BEQQ instrument, is used to acquire the spectra. MS/MS analysis of $(M + 7H)^+$ for porphyrin pyrrole sequencing is particularly advantageous in the analysis of true unknowns or asymmetrically substituted porphyrins, which typically produce extremely complex and difficult to interpret CI mass spectra. See also: Van Berkel, G. J.; Glish, G. L.; McLuckey, S. A. *Org. Geochem.*, in press.

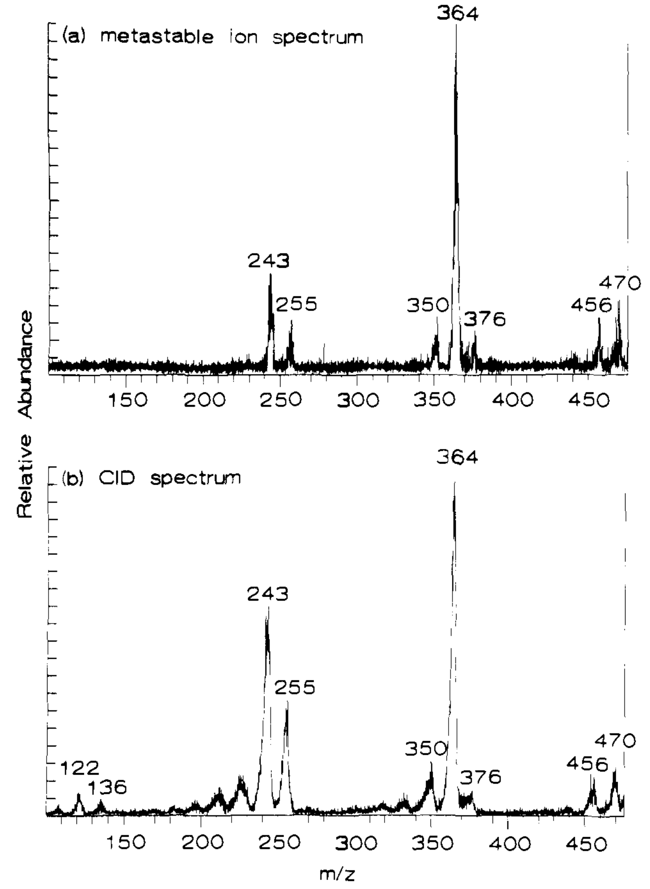


Figure 7. (a) Metastable ion spectrum and (b) CID spectrum of $(M + 7H)^+$ (*m/z* 485) in the ammonia CI mass spectrum of etioporphyrin III (**1**) obtained on the BE instrument.

The proposed structures for the major mono-, di- and tripyrrole fragment ions observed in the MS/MS spectra of $(M + 7H)^+$ from the ammonia CI mass spectra of **1** (Figure 7a,b) are shown in Figure 8. These fragment ions, resulting from the unimolecular decomposition of $(M + 7H)^+$ are the same as the fragment ions observed in the ion trap CH_5^+ CI mass spectrum of etioporphyrinogen III (Figure 4b). However, the most abundant of the fragment ions in the MS/MS spectra of $(M + 7H)^+$ formed in situ from **1** (Figure 8) are two mass units less than the mass of the most abundant fragment ions observed in the high-pressure ammonia CI mass spectra of **1** (Figure 1a–c and Figure 2).

Reduction Mechanism. The data presented above indicate that porphyrin reduction *does not* occur via gas-phase ion–molecule chemistry involving the common neutral or ionic species present in a high-pressure CI plasma (e.g., NH_3 and NH_4^+ in ammonia CI). Equally important, the data indicate that the reduction occurs via a surface-assisted mechanism. Other reactive species present in the CI plasma, which might be responsible for the reduction, include free radicals and excited neutrals. Radical species can be present in high-pressure CI plasmas at concentrations approaching 100 times that of the reagent ions,[13] and reactions, prior to ionization, between molecules and various alkyl and hydrogen radicals are known to occur.[12-17] These reactions can occur in the gas phase, but much of the data concerning such reactions suggest surfaces often play a role.[17]

Evidence for the role of hydrogen radicals in the porphyrin reduction process was obtained by plotting the intensity of the molecular species, $(M + nH)^+$, in the high-pressure ammonia CI mass spectrum of **1**, as a function of electron emission current (Figure 9). (These data were obtained using the BE instrument while introducing the porphyrin via a direct insertion probe into the ion source maintained at approximately 448 K). Previous workers have shown that as electron emission current is increased, the radical population in a high-pressure CI plasma increases at a faster rate than the reagent ion population, thereby enhancing the abundance of species produced by radical–molecule reactions.[12,13] As the emission current was increased in this experiment, the abundance of each reduced porphyrin species was enhanced relative to the protonated porphyrin, $(M + H)^+$. These data strongly suggest that hydrogen radical–porphyrin reactions produce the reduced species. (Hydrogen radicals are likely to be much lower in concentration in the ion trap than in a high-pressure CI plasma, particularly after the electron pulse used for ionization is turned off, accounting for the lack of porphyrin reduction in the ion trap.)

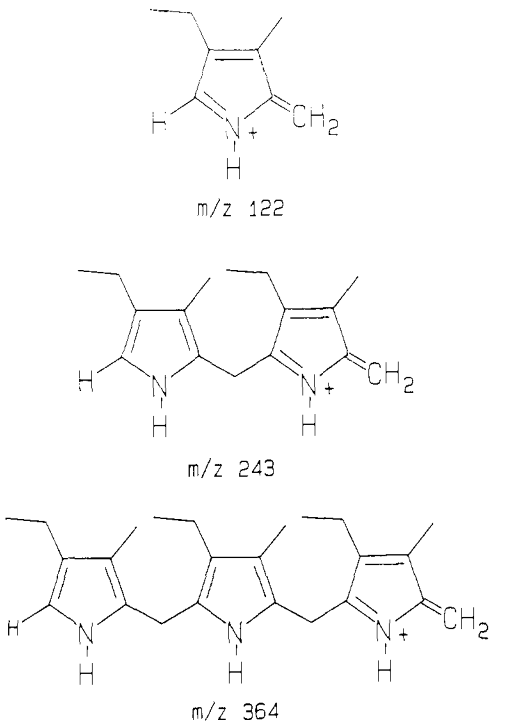


Figure 8. Proposed structures for the major monopyrrole (*m/z* 122), dipyrrole (*m/z* 243), and tripyrrole (*m/z* 364) observed in the MS/MS spectra of $(M + 7H)^+$ (*m/z* 485) in the ammonia CI mass spectrum of etioporphyrin III (**1**). The possible isomeric pyrrole sequences for the tri- and dipyrrole cannot be distinguished in the MS/MS spectra and are, therefore, not shown.

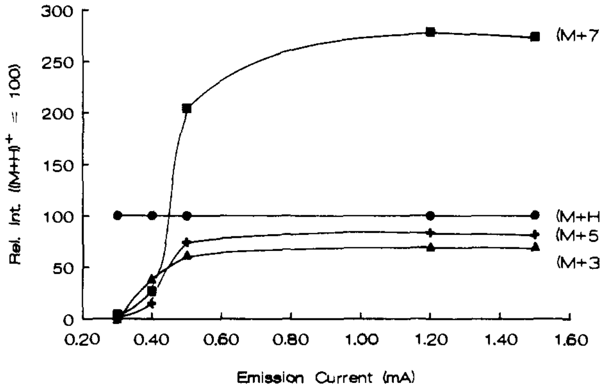
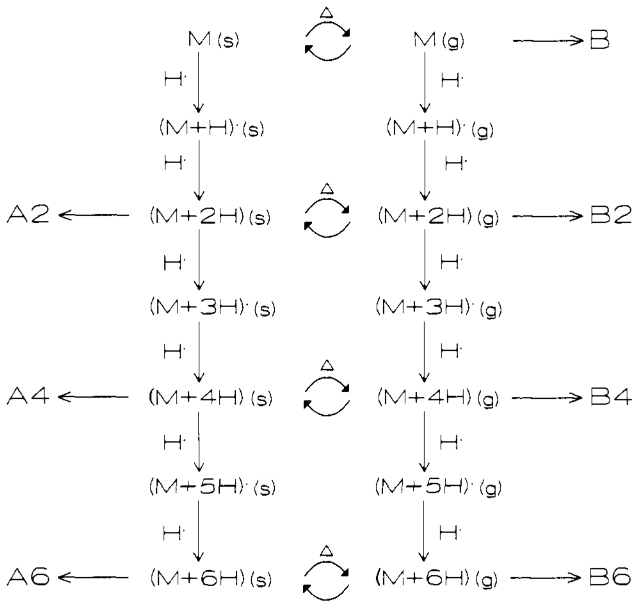


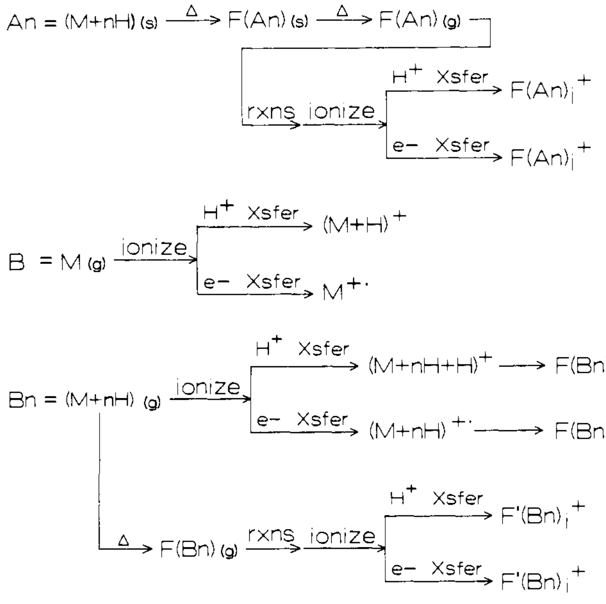
Figure 9. The effect of electron emission current on the relative intensity of the molecular species in the high-pressure ammonia CI mass spectra of etioporphyrin III (**1**).

The porphyrin reduction mechanism proposed is shown in Scheme I. In this scheme, porphyrin is sequentially reduced by hydrogen radicals from the CI plasma, aided by interaction of the porphyrin with the interior surfaces of the ion source. The reduction appears to be largely independent of the reagent gas, provided that the gas can act as a source of hydrogen radicals. Ammonia and hydrogen, however, are more effective in producing the reduced porphyrin species than either methane or isobutane. Surface assistance of porphyrin reduction is probably required, because of the combination of hydrogen radical–porphyrin reaction

Scheme I



Scheme II



kinetics and typical residence times for gas-phase neutrals in a high-pressure CI ion source.[16] Reactions of the porphyrin radicals, $(M + H)^{\bullet}$, $(M + 3H)^{\bullet}$, and $(M + 5H)^{\bullet}$, with radical hydrogen and/or neutral species in the plasma (e.g., NH_3) to form the more stable neutrals, (M + 2H), (M + 4H), and (M + 6H), are likely to be the most rapid steps in the sequential reduction because of the rapid reactions rates for radical–radical reactions[14] and the high number density of the neutral reagent gas, respectively. However, unless the rate constants for each and every step of the reduction reaction in the gas phase are as fast as the fastest observed for radical reduction (1×10^{-10} cm^3 s^{-1}),[13,16] the surface is required to hold the porphyrin in the ion source while it is reduced. At the ion source temperatures over which porphyrin reduction was observed in this study (i.e., 423–543 K), both the porphyrin and the reduction products, (M + *n*H), can probably transfer between the gas phase and source surfaces several times before ionization. Therefore, some steps of the reduction may occur in the gas phase, but the data indicate that the majority of the reduction takes place on the source surfaces. At ion source temperatures greater than 543 K, where the porphyrin remains in the gas phase during the majority of its residence time in the ion source, thereby minimizing porphyrin–surface interactions, little or no reduction is observed. Eglington et al.[25] saw similar behavior using methane as the CI reagent gas. They reported reduced abundance of $(M + H)^+$, the appearance of reduced molecular species, and formation of pyrrolic fragments from porphyrins at an ion source temperature of 473 K. At higher source temperatures no fragments were observed and $(M + H)^+$ was the major ion in the spectrum.

In the case where the porphyrin is deposited directly on the ion source repelller plate prior to the analysis, both the porphyrin and the reduced products are probably present on the source walls at low temperature (after the filament has been on for a period of minutes), but only the porphyrinogen is volatile enough to vaporize and be ionized. For example, etioporphyrinogen III will desorb from a direct insertion probe without probe heating at an ion trap temperature of 448 K, whereas the corresponding porphyrin, **1**, must be heated by the probe to greater than 448 K before it will desorb. As the temperature of the ion source increases, the partially reduced species and the porphyrin can desorb from the surfaces. Given the relatively low volatility of porphyrins, if the sample is introduced using a direct insertion probe or DCI probe at ion source temperatures less than 543 K, a substantial fraction of the sample will condense on the source surfaces and be reduced. At temperatures higher than 543 K, much less porphyrin–surface interaction occurs, and therefore, little reduction/decomposition is observed.

Decomposition Mechanism. The data already presented in this paper demonstrate that direct unimolecular decomposition of the in situ produced $(M + nH)^+$ porphyrin species *does not* produce the most abundant tri-, di-, and monopyrrolic ions observed in porphyrin high-pressure CI mass spectra. Porphyrin decomposition in a high-pressure CI plasma, rather than resulting from direct gas-phase decomposition of the ionic reduced species alone, more likely reflects products from a wide variety of reactions as illustrated in Scheme II. (This scheme describes the fate of the species (M + *n*H) (s) (i.e., An), M (g) (i.e., B), and (M + *n*H) (g) (i.e., Bn) shown in Scheme I.)

Decomposition of the ionized gas-phase reduced porphyrin species to form pyrrolic fragment ions is represented by Bn in Scheme II. When using ammonia, methane, or isobutane as the CI reagent gas, the even-electron species, $(M + H)^+$, $(M + 3H)^+$, $(M + 5H)^+$, and $(M + 7H)^+$, are the major molecular species observed, because protonation is the dominant ionization mechanism. In the case of ammonia CI, the ion trap data showed that protonation by NH_4^+ is insufficiently energetic to fragment the porphyrinogen at temperatures below 423 K (Figure 4a). When CH_5^+ was used as the protonating reagent, fragment ions were observed (Figure 4b). As discussed above, proton transfer from H_3^+ would result in significant fragmentation of the reduced porphyrin species. The odd-electron molecular species, $M^{\bullet+}$, $(M + 2H)^{\bullet+}$, $(M + 4H)^{\bullet+}$, and $(M + 6H)^{\bullet+}$, probably predominate in hydrogen CI mass spectra because protonation results in significant fragmentation of the (M + *n*H) species, whereas the less energetic dissociative charge exchange process (i.e., electron transfer) involving H_3^+ produces the ionized species with minimal fragmentation. Thus, unimolecular decomposition of the ionized reduced porphyrin species can contribute to the overall fragmentation pattern. (Ionization of the porphyrin by the most energetic charge-transfer or proton-transfer reactions (pathway B in Scheme II) will not fragment the stable aromatic macrocycle.) The relative amount of this contribution will depend largely on the energetics of the ionization process and, therefore, the reagent gas used. However, the pyrrolic fragment ions produced in this manner (Figure 7a,b, and Figure 8) are two or more mass units less than the most abundant fragment ions observed in the CI mass spectra (Figure 1a–c and Figure 2). Therefore, unimolecular decomposition of the ionized reduced porphyrin species (Bn in Scheme II) does not produce the major pyrrolic fragment ions observed in the high-pressure CI mass spectra.

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[25] Eglinton, G.; Hajalbrahim, S. K.; Maxwell, J. R.; Quirke, J. M. E.; Shaw, G. J.; Volkman, J. K.; Wardroper, A. M. K. *Phil. Trans. R. Soc. London, A* **1979**, *293*, 69–91.

Thermal decomposition of the reduced porphyrin species in the gas phase (Bn in Scheme II), but most importantly on the source surfaces (An in Scheme II), is proposed as the mechanism of formation for the major pyrrolic ions (as well as the majority of other fragments) observed in the CI mass spectra. Since, at high source temperatures (>673 K), the porphyrin macrocycle will decompose,[9] is it probable that the reduced porphyrin species, which have a less stable macrocycle than the porphyrin, are very susceptible to thermal decomposition at the temperatures employed in the present studies.

Production of the major pyrrolic fragment ions through a thermal decomposition mechanism is strongly supported by the desorption profiles shown in Figure 3. These profiles demonstrate that the majority of the reduced species and pyrrolic fragments observed in the CI mass spectra are formed from porphyrin species that condense on the source walls. These products formed from surface-bound porphyrin are observed to increase in relative abundance with time in the ion source and reach their maximum intensity after the DCI current (temperature) has reached its maximum level and continues to radiatively heat the source surfaces. As the DCI probe heats the surfaces, the less stable reduced species on the surfaces are thermally decomposed and the resulting neutral pyrrolic species can vaporize, possibly undergoing additional reactions in the CI plasma, before being ionized. (Thermal decomposition of material introduced using a DCI probe and the "double maxima" phenomena in DCI desorption profiles have been noted for other compound classes.[26]) Similar arguments can be made for the case in which the porphyrin is placed on the ion source repeller plate prior to the analysis (Figure 1a-c). The percentage of the reduced species formed on the source surfaces that decomposes, rather than vaporizing intact, increases as the ion source temperature increases. (This is also true when the direct insertion probe or DCI probe is used to introduce the sample.) Fragment ion abundance increases in these spectra as the ion source temperature increases, and pyrrolic fragment ions are observed in the spectra obtained at high source temperatures even though little or no reduced species are evident (i.e., there is total thermal decomposition of the $(M + nH)$ species).

Conclusions

Previously proposed reduction/decomposition mechanisms, invoking a gas-phase ion-molecule reduction scheme and unimolecular decomposition of the ionized reduction products, cannot fully account for the appearance of high-pressure CI mass spectra of porphyrins. The results from this study demonstrate that the phenomena responsible for the appearance of these high-pressure CI mass spectra are different than previously proposed.

The porphyrin reduction observed in a high-pressure CI plasma *does not* result from gas-phase ion-molecule chemistry. Radical hydrogen, rather than neutral or ionized reagent gas species, appears to be the most likely reducing agent, and the reduction is surface-assisted. Furthermore, the reduction process is largely independent of reagent gas, as long as a source of radical hydrogen is present.

Simple unimolecular decomposition of the reduced porphyrin species (e.g., $(M + 7H)^+$) *does not* produce the most abundant pyrrolic fragment ions observed in high-pressure CI mass spectra. This decomposition pathway can contribute, however, to the overall CI mass spectrum. The amount of this contribution is largely dependent on the energetics of the ionization process, and, therefore, the CI reagent gas. For example, less fragmentation is observed in ammonia CI mass spectra than hydrogen CI mass spectra, at the same ion source temperature, because of the lower exothermicity of ionization when using ammonia. The most abundant fragment ions, as well as the majority of the fragment ions, observed in the high-pressure CI mass spectra more likely originate via thermal decomposition of the reduced porphyrin species in the gas phase, but most importantly on the source surfaces, producing neutral pyrrolic units that possibly undergo additional reactions in the CI plasma prior to ionization. Therefore, the relative abundance of the different ions and the amount of fragmentation observed in the high-pressure CI mass spectra, when using a particular porphyrin and reagent gas, will correlate with the temperature of the ion source.

Acknowledgment. This work was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, through Contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.

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(26) Cotter, R. J. *Anal. Chem.* **1980**, *52*, 1589A-1606A.

Multiphoton Ionization of Acetone Clusters: Metastable Unimolecular Decomposition of Acetone Cluster Ions and the Influence of Solvation on Intracluster Ion-Molecule Reactions

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Contribution from the Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802. *Received February 27, 1989*

Abstract: A comprehensive investigation of the reactions of acetone ions in clusters was made to investigate and compare the similarities and differences in the ion reactions due to solvation effects. Neutral acetone clusters, prepared in a pulsed nozzle supersonic expansion, are ionized using multiphoton ionization and investigated using a time-of-flight reflectron technique. The observed major cluster ions resulting from prompt fragmentation following ionization are $[(CH_3)_2CO]_m{\cdot}H^+$ (m = 1-15), $[(CH_3)_2CO]_m{\cdot}C_2H_3O^+$ (m = 1-17), and $[(CH_3)_2CO]_m{\cdot}CH_3^+$ (m = 1-10). In a time window of a few tens of microseconds, all three classes of cluster ions unimolecularly decompose, losing only one acetone monomer. Interestingly, a reaction corresponding to the dehydration of $[(CH_3)_2CO]_m{\cdot}H^+$ and leading to the production of $[(CH_3)_2CO]_{m-2}{\cdot}C_6H_{11}O^+$ is observed for m = 2-6. The most striking finding of the present study is that the presence of water molecules in a cluster suppresses the dehydration reaction. This finding clarifies the discrepancy between earlier studies reported in the literature and, most importantly, provides further evidence for the influence of a solvent on ion reactions in clusters, and ion-molecule reactions in general.

Unraveling the influence of solvation on chemical reactions is a topic of considerable current interest. It is recognized that insight into reasons for similarities and differences in reactivity between the gaseous state and the condensed state can be gained by studying reactivities of clusters as a function of the degree of aggregation. Acetone is a widely used solvent, and studies of acetone clusters serve as a good model for unraveling reactions of hydrogen-bonded organic systems with specific reference to

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