A Study on the Solution and Gas-Phase Chemistry of Mn(III) and Fe(III) Tetraarylporphyrin Complexes by Fast-Atom Bombardment Mass Spectrometry.

2: Synthesis and Characterization of Molecular Complexes

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The chemical behavior of Fe(III) and Mn(III) tetraarylporphyrin (TAP) complexes with *N*-alkylimidazoles and other suitable ligands was studied by direct reaction in the fast-atom bombardment matrix and in the gas phase. The coordination reaction occurs at the metal center and yields molecular adducts of porphyrin/ligand (PL) and PL₂ stoichiometry. Coordinative competition between free and covalently linked ligands can be used to probe the conformation of "tailed" Mn(III)-TAP. (*J Am Soc Mass Spectrom* 1993, 4, 255-258)

A number of biological systems, ranging from cytochrome P-450 to catalase, chloroperoxidase, and ligninase, base their action on redox chemistry of a metal porphyrin active site. A crucial role in controlling the chemistry of the metal center, and therefore in dictating the action of a particular enzyme, is played by coordination to the metal center from a ligand, which is usually an electron-donating group in the side chain of an amino acid of the apoenzyme. Thus, the Fe(III) center of cytochrome P-450 is coordinated by a thiolate group from cysteine [1], whereas in horseradish and cytochrome *c* peroxidase, this role is played by a histidine imidazole [2], and a phenoxo group from tyrosine is the axial ligand in catalase [3]. A similar trend in selectivity has also been observed for some tetraarylporphyrin (TAP)-based biomimetic systems [4] that can be modulated to catalase activity when the "basket handle" contains a phenoxo or alkoxo ligand, whereas they show oxygenase activity when the ligand is a covalently linked imidazole [5].

The characterization of five- and six-coordinated

metal porphyrin species is therefore important owing to their involvement in the catalytic cycle of both cytochrome P-450 and its biomimetic analogs, latter of which are receiving ever-increasing interest as mild, selective, and ecologically compatible oxidation catalysts; however, the paramagnetic character of Mn(III)-TAP precludes the use of simple nuclear magnetic resonance techniques for the measurement of binding constants with suitable ligands. Mass spectrometric techniques have been recently applied to the generation and characterization of several supramolecular species, among which are host-guest complexes of calixarenes in the gas phase by direct chemical ionization [6] and receptor-ligand [7] and enzyme-substrate-inhibitor complexes [8] in solution by electrospray ionization. In the porphyrin field, studies have encompassed gas-phase metallation of porphyrins [9], measurement of the electron affinity of Ni(II)-TPP by equilibrium technique [10], generation of ion-molecule complexes between fluoride ions and M(II)-TPP [11], and the formation of gas-phase complexes of Fe(III) porphyrins with *N*-methylimidazole [12] and with NO₂ [13] in a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer.

Furthermore, desorption ionization mass spectrometry has been used for the qualitative study of alkali metal complexation by crown ethers and other related ligands [14], and an example of the quantitative evalu-

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ation of the binding constant between alkali cations and cyclodextrins has also been reported [15].

In this report, we apply mass spectrometric techniques to investigate (1) the formation of intermolecular coordinative complexes in solution and in the gas phase with suitable molecules, such as *N*-alkylimidazoles and 4-*t*-butylpyridine; and (2) the conformation of "tailed" porphyrins carrying a number of side-chain functions, such as pyridine, imidazole, and carboxylate.

Experimental

The structure and enumeration of the compounds used in this study are shown in Table 1. *N*-Butyl and *N*-hexyl imidazole were prepared as described previously [17]. Fast-atom bombardment (FAB) matrices [3-nitrobenzyl alcohol (mNBA) and thioglycerol], *N*-methylimidazole, 4-*t*-butylpyridine, thiophenol, and *m*-cresol are commercially available and were used as received. Porphyrin solutions at known concentrations were prepared in ethyl acetate and mixed with the matrix shortly before analysis.

Mass spectrometric measurements were performed as described in the first part of this report [16].

Table 1. Metallotetraaryl porphyrins studied

9a n = 1; 9b n = 3; 9c n = 5	10	11

Ion-molecule reactions between FAB-desorbed metalloporphyrins and neutral molecules in the gas phase were accomplished by leaking the selected reagent from the reference inlet (kept at 180–200 °C) into the ion source until the desired pressure was reached (operational pressure limit of the instrument 8×10^{-3} mPa).

Results and Discussion

Coordinated species with suitable neutral ligands can be easily prepared from Mn(III)- and Fe(III)-TAP in the gas as well as in the condensed phase. The spectra shown in Figure 1 were obtained in the positive ion mode by FAB-desorbing Mn(III)-T(Cl₂P)P(Cl) (3b) and Fe(III)-T(Cl₂P)P(Cl) (11) from an mNBA solution while pressurizing the source to approximately 3×10^{-3} mPa.

Figure 1. Positive ion spectra of Mn(III)-T(Cl₂P)P (3b) in the presence of gas-phase *N*-methylimidazole (Melm) and 4-*t*-butylpyridine (BuPy) and Fe(III)-T(Cl₂P)P (11) and gas-phase *N*-methylimidazole (matrix is mNBA).

with *N*-methylimidazole and 4-*t*-butylpyridine. It is worth noting that with these two ligands, only the monoligated species at m/z 1024 and 1078 (most intense peak of isotope cluster), respectively, could be detected, even when the pressure was raised near the operational threshold for the instrument ($6-8 \times 10^{-3}$ mPa). The higher coordinating ability of *N*-methylimidazole versus 4-*t*-butylpyridine is reflected in the lower free versus monoligated ratio observed (approximately 5:1 and 7:1, respectively). Other possible ligands, such as methanol, *m*-cresol, thiophenol, and methyl phenyl sulfide, failed to give rise to any adduct signal. Collisional stabilization of the desorbed species in the high-pressure gas-phase environment is also likely to play a role because the absolute intensity of FAB signals increases with increasing the pressure of the gaseous reagent into the source, irrespective of the formation of any supramolecular species. It is also possible that formation of the coordinated species occurs at least partially in solution because at the pressures used, a considerable amount of the gas may dissolve in the FAB matrix. (We acknowledge a referee for this suggestion).*

Ion-molecule reactions may also give rise to some signals in the spectrum obtained by gas-phase complexation of the poorly coordinating Mn(III)-TPP (**1**) with *N*-methylimidazole (e.g., the weak ion at m/z 683, which can be attributed to the usually elusive Mn-oxo species [18] formed by ion-molecule reaction with mNBA; the signal at m/z 749 is due to the complex with *N*-methylimidazole and that at m/z 820 is the adduct with mNBA. Sometimes a weak signal at m/z 702/704 is also detected, possibly an adduct with a Cl⁻ species. It is worth noting that the corresponding signals do not occur when the experiment is performed with the strongly coordinating Fe(III) and Mn(III) complexes of T(Cl₂P)P. This somewhat frustrating failure of observation of the bis-ligated adduct of Mn(III)-TAP in the gas-phase complexation experiments was, however, used to provide evidence of intramolecular complexation of some "tailed" porphyrins [9]. The same experiment as described above was executed on compounds bearing covalently anchored functions, such as imidazole (**6**), pyridine (**7**), ester (**8**), and carboxyl (**9a-c**). In this series, the adduct with *N*-methylimidazole was only observed for compound **8** carrying the ester group. A control experiment with a porphyrin carrying an alkyl chain without coordinating group (**10**) yielded the expected adduct, thus ruling out steric hindrance as a possible cause for lack of complex formation.

* The mechanism through which molecular complexes with *N*-methylimidazole are formed was qualitatively tested as follows: a "gas-phase" experiment in which the ligand was leaked through the reference inlet was interrupted several times by closing the metering valve to the ion source. Reproducibly, the signal due to the molecular complex disappeared within one or two scans. A separate experiment in which *N*-methylimidazole was mixed (1:1 v/v) to mNBA yielded a stable, although weaker, ion current that resisted until the whole matrix dried out (3-4 min).

When analyzed in the negative ion mode (thioglycerol or mNBA matrix), tailed porphyrins with a carboxyl group (**9a-c**) yielded only the signal corresponding to the zwitterion undergoing electron capture but no adduct signal with the matrix, thus accounting for intramolecular complexation of the metal center by the carboxyl group. In the positive ion mode, these porphyrins are desorbed as protonated species, and the COOH group is probably intramolecularly ligated because no adduct with *N*-methylimidazole is formed [19]. We previously reported the values of the constants for the mono and bis coordination of some Mn(III)-TAP by UV-visible (vis) titration with *N*-hexylimidazole in dichloromethane solution [20]. Positive ion FAB analysis of mNBA solutions of Mn(III)-T(Cl₂P)P(Cl) (**3b**) containing variable molar amounts of *N*-butylimidazole yielded the corresponding mono- and bis-ligated species at m/z 1068 and 1192, respectively (most intense peak of signal cluster), as shown in Figure 2. *N*-Butylimidazole (molecular weight 124) was used instead of the hexyl homolog, the mass of which (152 u) could possibly interfere with that of mNBA (153 u), which is the best matrix found for these measurements.

Spectra were recorded at ligand:porphyrin (L/P) ratios ranging from 1 to 2000 and an equal response factor was assumed for the free and the ligated species. Figure 3 shows the relative intensity of the signals of the mono- and bis-ligated species (m/z 1068 and 1192, respectively) plotted versus the L/P ratio. The curves are very different from those obtained by UV-vis spectroscopy and are useless for calculating the binding constants. Also at high L/P ratios, at which the porphyrin is almost completely bis-ligated (as indicated by UV-vis measurement), the relative intensity of the PL₂ signal is approximately 5%, and this is probably due to gas-phase source decomposition of the ligated species, according to the pathway PL₂ → PL₁ → P; however, decomposition of metastable mono- and bis-ligated complexes of Mn(III)-TAP with *N*-butylimidazole may be used to estimate the interaction energy between the metalloporphyrin and the coordinating ligand through evaluation of the onset potential in a

Figure 2. Positive ion FAB spectrum of a mixture of Mn(III)-T(Cl₂P)P and *N*-butylimidazole (Bulm) (1:2000 L/P molar ratio); matrix is mNBA.

Figure 3. Abundances of the ion current of the mono (PL, m/z 1068) and bis (PL₂, m/z 1192) complexes of Mn(III)-T(Cl₂P)P with *N*-butylimidazole [relative to the intensity of Mn(III)-T(Cl₂P)P, m/z 943] plotted against the L/P ratio.

triple-quadrupole instrument [21]. Work in this field is in progress.

Conclusions

The results reported here demonstrate the possibility of generating and analyzing supramolecular or coordinated metal porphyrin species by FAB mass spectrometry and of using them as probes of the condensed- or gas-phase conformation of simple as well as superstructured metallatetraarylporphyrins. These insights can be regarded as confirming the currently accepted mechanism proposed on the basis of solution kinetic measurements that showed a prominent cocatalytic effect of carboxylic or imidazole residues (or both) covalently linked to the porphyrin frame. The recent isolation and mass spectrometric characterization of a

high-valent Mn-oxo species [18] can therefore open the way to gas-phase investigation on the reactivity of this crucial intermediate in the oxidative reaction pathway.

Although ion storage devices, such as FT-ICR or ion trap mass spectrometry are certainly better suited to yield thermochemical data on isolated species and to follow their evolution by time-resolved spectroscopy, qualitative data obtained with more conventional instrumentation can also be valuable in highlighting the reactivity of these biochemical or biomimetic systems.

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