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Characterization of a μ -oxo-bridged diiron porphyrin by ESI-LTQ-Orbitrap-MS

Dear Sir,

Metalloporphyrins are a class of important biological molecules for which an increasing number of technological applications has been described. In recent years, specific interest arose for the catalytic properties of μ -bridged metalloporphyrin dimers, which can form by bridging atoms, such as O or N, between the two metal centers.^[1–3] Although monomeric porphyrins are frequently characterized by mass spectrometry (MS), using an array of different ionization techniques,^[4] the characterization of bridged dimeric metalloporphyrins by such method is not frequent. This can be attributed to the difficulty in introducing the dimers into a mass spectrometer and their apparent low stability in the gas phase. The seminal study by Brown and Wilkins,^[5] characterizing the dimer ion of a O-bridged iron(III) porphyrin by laser desorption/ionization-Fourier transform ion cyclotron resonance (LDI-FT-ICR) MS, has remained an isolated exception until the early 21st century. Motivated by the utilization of porphyrin species in anion selective membranes,^[6] the characterization of μ -bridged dimers by electrospray ionization mass spectrometry (ESI-MS) was achieved for oxo-bridged dimer ions of Ga(III) and Zr(IV) octaethylporphyrin and mesotetraphenylporphyrin.^[7,8] However, the detection of the dimer ions and their speciation was shown to be highly dependent on the mobile phase in which they were solubilized for analysis, requiring the use of a stabilizing lipophilic agent such as sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate. Recently, ESI-MS has been successfully used for the characterization of a μ -N-bridged diiron meso-tetraphenylporphyrin ($[(\mu-N)(FeTPP)_2]^+$) with interesting catalytic properties.^[3] Despite the studies with gallium, zirconium, and μ -N-bridged iron(III) porphyrin dimers, information regarding the identification of μ -O-bridged diiron porphyrins is scarce. The study by Gozet et al. describing the gas phase generation of O-bridged and Cl-bridged iron (III) and manganese(III) porphyrin dimers^[9] is the single work reporting on the MS and MS/MS characterization of such species. However, porphyrins were prepared as monomers, and dimer formation was reported to occur in the gas phase. Being so, the work by Brown and Wilkins remains the single exception on the MS characterization of a μ -O-bridged diiron porphyrin material prepared by metallo-organic synthesis. Herein, we report on the MS characterization of a μ -oxo diiron (III) meso-tetrakis(pentafluorophenyl)porphyrin ($[(\mu-O)(FeTPFP)_2]$), (Fig. 1), which has been shown to be a promising catalyst for fuel desulfurization.^[1] ESI-MS analysis was performed in an LTQ-Orbitrap-XL instrument (Thermo Scientific), and tandem mass spectra were obtained using collision-induced dissociation (CID) and high-energy collision dissociation (HCD), demonstrating advantages in the application of the latter method for structural characterization.

Mass spectrometry experiments were performed in the positive ionization mode with the following ESI source parameters: electrospray needle voltage +3.1 kV, sheath gas nitrogen 5 (arb), capillary temperature 275 °C, capillary voltage 45 V, and tube lens voltage 250 V. Samples were infused into the electrospray source using the mass spectrometer inbuilt syringe infusion pump at a flow rate of $10 \,\mu$ l min⁻¹. Full scan positive mass spectra were acquired between m/z 400 and 3500, using the instrument high-mass set up with resolution at m/z 400 set as 30 000 or 60 000. Tandem mass spectra were acquired using a precursor ion selection window of $\pm 0.5 m/z$ both in CID and HCD mode. MS and MS/MS were recorded with Xcalibur (version 2.2).

The compound $[(\mu-O)(FeTPFPP)_2]$ was prepared using a slightly modified protocol described in the literature.^[10] A mixture of meso-tetrakis(pentafluorophenyl)porphyrin and iron(II) chloride dissolved in N,N-dimethylformamide (DMF) and pyridine (96%: 4%) was refluxed for 2 h under an argon atmosphere and afterwards exposed to air at room temperature for a period of 18 h. The solid that formed (purple crystals) was isolated and characterized by UV/Vis spectroscopy, MS, and single-crystal X-ray diffraction.

Solutions for MS measurements were prepared by dissolving $[(\mu-O)(FeTPFPP)_2]$ in methanol, acetonitrile, or dichloromethane to a concentration of 1 mg ml⁻¹. Right before MS analysis, the solution was diluted 200 times in pure solvent, setting the final concentration at 5 µg ml⁻¹. UV–visible spectra were acquired in a Cary 50 Bio UV–visible spectrophotometer.

Figure 2 shows the MS spectra of $[(\mu-O)(FeTPFPP)_2]$ prepared in methanol (A), dichloromethane (B), and acetonitrile (C), together with the respective UV-visible spectra (D). In methanol [Fig. 2(A)], the $[M + H]^+$ ion of the porphyrin dimer $[(\mu - OH)(FeTPFPP)_2]^+$ is clearly visible at m/z 2072.95768 ($\Delta m = -0.15$ ppm), presenting the expected isotopic pattern. However, this peak has an intensity of approximately 30% of the base peak at m/z 1027.97791, which corresponds to the M⁺ ion of the iron porphydrine monomer $[FeTPFPP]^+(\Delta m = 0.5 \text{ ppm})$. Despite not being quantitative, this monomer/dimer ratio roughly corresponds to the FeTPFPP solution speciation observed by UV-visible spectrophotometry [Fig. 2(D)]. Metaloporphyrins have a typical Soret band at around $\lambda = 400$ nm, and the formation of oxo-bridged dimers has been described to originate a new band, shifted toward a shorter wavelength. As described in the literature,^[11] acidification (0.1% formic acid) of the methanol solution led to dimer disruption and precluded the MS detection of the $[(\mu-OH)(FeTPFPP)_2]^+$ species.

A similar behavior was observed in dichloromethane, but when acetonitrile was used, the two predominant ion species were

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Figure 1. Chemical structure of the μ -oxo diiron(III) meso-tetrakis (pentafluorophenyl) porphyrin complex [(μ -O)(FeTPFPP)₂], (Fe₂C₈₈H₁₆N₈F₄₀O).

detected at m/z 1027.97461 and m/z 1069.00159, respectively, corresponding to the [FeTPFPP]⁺ monomer ($\Delta m = -2.7$ ppm) and a solvent adduct ((CH₃CN)[FeTPFPP]⁺) ($\Delta m = -2.2$ ppm) [Fig. 2 (C)]. Despite the UV–visible data indicating the existence of the dimer species in solution [Fig. 2(D)], the ion at m/z 2072.96 could not be encountered. The contrast between UV–visible spectra and MS spectra suggests that the formation of a strong adduct with acetonitrile occurs in the gas phase, leading to the dimer dissociation. This result highlights the importance of solvent choice and indicates that solvents with a higher tendency to coordinate to the metal ion center are not suitable vehicles for the ESI characterization of μ -O-bridged iron porphyrin dimers.

Tandem mass spectra (MS/MS) of $[(\mu-OH)(FeTPFPP)_2]^{\dagger}$ were obtained, utilizing both CID [Fig. 3(A)] and HCD [Fig. 3(B)]. CID fragmentation led to a neutral loss of 5 μ ., originating a species at m/z 2067.92022. This feature corresponds to the unusual loss of five hydrogen atoms at the β -pyrrole positions of the porphyrin rings ($\Delta m = 0.65$ ppm). This species was observed when using normalized collision energy values ranging from 0% to 60%, and no additional fragmentation of the [(μ -OH)(FeTPFPP)_2]⁺ ion could be observed with CID. The observation of the ions at m/z 2067.72 when the normalized collision energy was set to 0 indicates that



Figure 3. Tandem mass spectra (MS/MS) of $[(\mu$ -OH)(FeTPFPP)₂]⁺ dissolved in methanol. (A) MS/MS spectra of the ion at m/z 2072.96 ($[(\mu$ -OH)(FeTPFPP)₂]⁺) obtained by collision-induced dissociation (CID) at a normalized collision energy of 60% (B). MS/MS spectra of the ion at m/z 2072.96 obtained by higher energy collision dissociation (HCD) at a normalized collision energy of 30%.

the parent ion probably undergoes metastable decay when

isolated in the linear ion trap. CID conditions seem to favor porphyrin



Figure 2. Mass Spectra of $[(\mu-O)(FeTPFPP)_2]$ dissolved in methanol (A), dichloromethane (B), and acetonitrile (C), together with the respective UV-visible optical spectra (D). m/z 2072.96 $[(\mu-OH)(FeTPFPP)_2]^+$, m/z 1027.98 $[FeTPFPP]^+$, and m/z 1069.00 $(CH_3CN)[FeTPFPP]^+$.



macrocycle oxidation by loss of a hydrogen atom (-H⁻). After the initial formation of a radical at the β -pyrrole position, the reaction may proceed through the formation of β - β linkages between the two porphyrin rings in the μ -O-bridged dimer. The oxidation reaction of metalloporphyrins with silver hexafluorophosphate or via an anode is one of the most widely used methods in porphyrin synthesis for connecting the porphyrin macrocycle directly through meso-meso or other positions including meso- β , β - β , and fused linkages.^[12,13] The mechanism proposed involves the reaction of a radical cation with a neutral metalloporphyrin, the regioselectivity for meso or β -pyrrolic positions being determined by differences in the HOMO orbitals of the metalloporphyrins.^[13] Cross-linking between the two rings would explain further losses of H₂, while stabilizing the porphyrin dimer and preventing its dissociation. Unfortunately, the ion at m/z 2067.72 did not prove to be sufficiently stable or to be present in an amount that could afford an additional fragmentation step (MS³), thus preventing its structural characterization.

Fragmentation of $[(\mu$ -OH)(FeTPFPP)₂]⁺ with HCD [Fig. 3(B)] produced two predominant fragment ions at *m/z* 1027.96939 and 1023.95818, showing the cleavage of the μ -O-bridged porphyrin dimer into its monomer species [FeTPFPP]⁺ (*m/z* 1027.97) and a further loss of four hydrogen atoms (*m/z* 1023.96). Further investigations will be required to understand why the preferable CID fragmentation pathways of the μ -O-bridged porphyrin dimer seem to favor loss of hydrogen atoms over dimer dissociation or other characteristic neutral losses such as those found for the monomer fragmentation. Fragmentation of the [FeTPFPP]⁺ monomer (*m/z* 1027.97) both with HCD or CID resulted in cumulative losses of hydrogen (*m/z* 1, –H⁺), hydrogen fluoride (*m/z* 20, –HF), pentafluorobenzyl radical (*m/z* 166, –C₆F₅+), and pentafluorobenzene (*m/z* 167, –C₆F₅H) as previously described in the literature.^[14]

 μ -O-bridged iron porphyrin dimers are becoming widely utilized in a number of technological applications and these experiments show that ESI instruments may become an important tool in the characterization of these chemical systems. Given the right choice of solvent, MS may report on the solution speciation of monomer – dimer equilibria. Such capability may prove useful when μ -O-bridged iron porphyrin dimers are used in chemical catalysis, allowing the study of catalyst stability, reaction mechanisms, and degradation products. Furthermore, it was shown that the gas phase chemistry of monomers and dimers differs significantly, and that a combination of CID and HCD fragmentation modes may not only provide structural information but also give insight into the chemical properties of these molecules.

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Yours,

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