Characterization of a Manganese Superoxide Dismutase Mimic, potential metallodrug against Inflammatory Bowel Diseases by Mass Spectrometry

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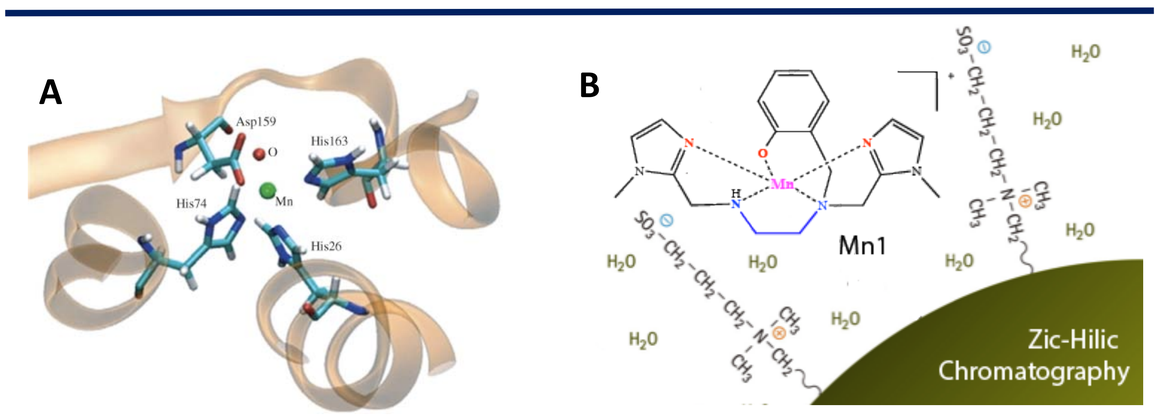
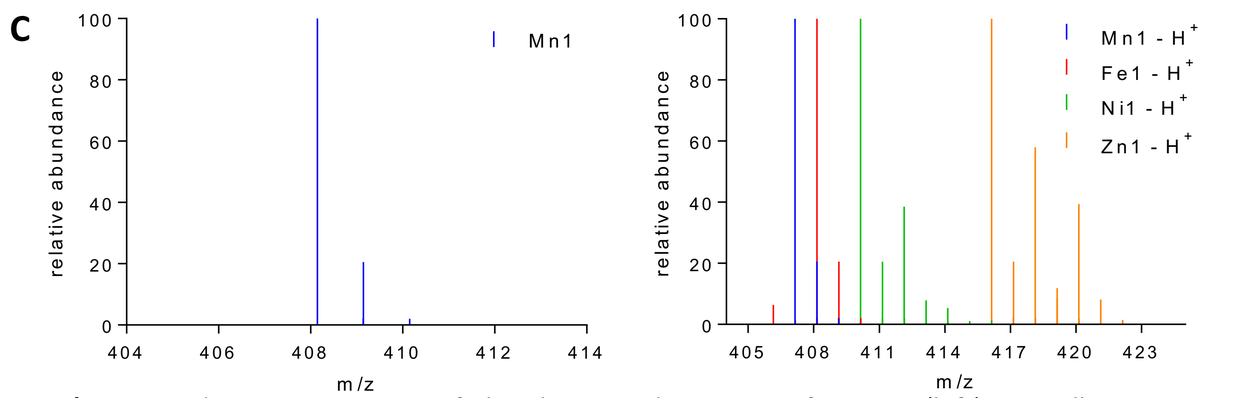
Abstract

Superoxide Dismutases (SOD) are metalloenzymes involved in the cellular antioxidant protection pathway controlling reactive oxygen species (ROS). SOD activity is weakened in epithelial cells from inflammatory bowel diseases (IBDs), leading to an increase in ROS. The manganese complex Mn1, mimicking SOD, is a promising metallodrug against IBDs, with an intracellular anti-superoxide and anti-inflammatory activity. In order to detect Mn1 (small hydrophilic molecule) inside cells and to understand its metabolization and speciation intracellularly we characterized the spectra of the Mn1 complex and its ligand in a non cell environment, using hydrophilic interaction chromatography and MS/MS fragmentation. In the obtained spectra we observed the coordination of the ligand to different metal ions, interfering with the Mn1 MS signature. The goal is to develop a method of separation and detection of Mn1 in a metal-free environment in order to detect SOD mimic metal complexes in cell lysates.

Introduction

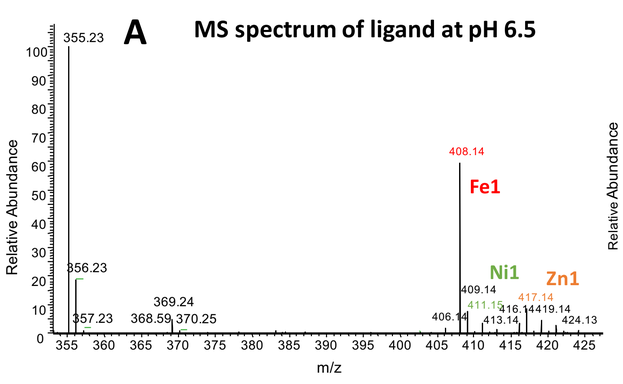


A) Manganese Superoxide Dismutase (MnSOD) active site showing Mn^{2+} coordination sphere congaing three histidines (His74, His26, His163) and an asparagine (Asp159). **B)** Inspired from MnSOD we synthesized the manganese complex Mn1, based on an ethanediamine ligand and mimicking SOD activity. Here we show the interaction of the hydrophilic Mn1 with hydrophilic (zic-hilic) chromatography stationary phase containing sulfobetaine functional groups.

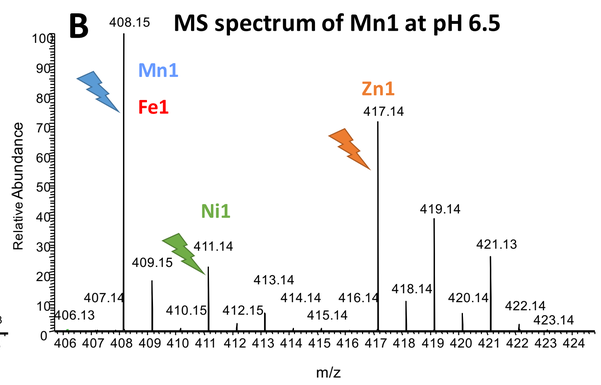


C) Expected isotopic pattern of the theoretical spectrum for Mn1 (left) as well as its dehydrogenated version among with other coordinated metal ions like Fe^{2+}, Zn^{2+} and Ni^{2+} forming the complexes Fe1, Zn1 et Ni1 with the same ligand (right).

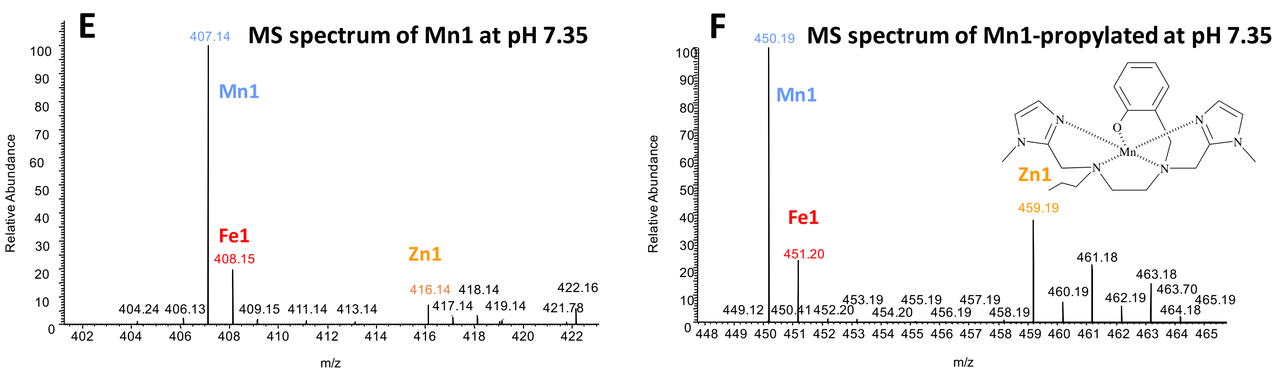
Results



Apart from its protonated majoritarian form at m/z 355.23, we observe coordination of the ligand to Fe, Ni and Zn **(A)**.

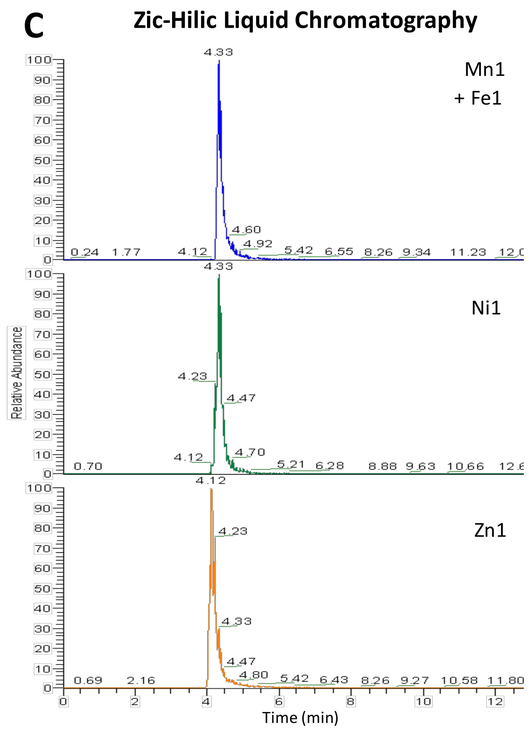


Exchanges of Mn with other metal ions are detected. The characteristic peak of Mn1 at m/z 408.14 is superposed to a dehydrogenated form of Fe1 **(B).**

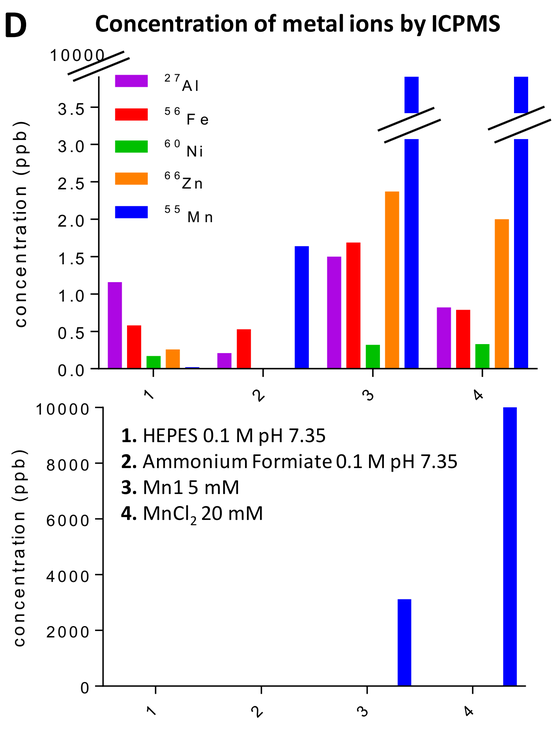


By increasing the pH we obtain the dehydrogenated form of Mn1, Fe1 and Zn1 **(E)**.

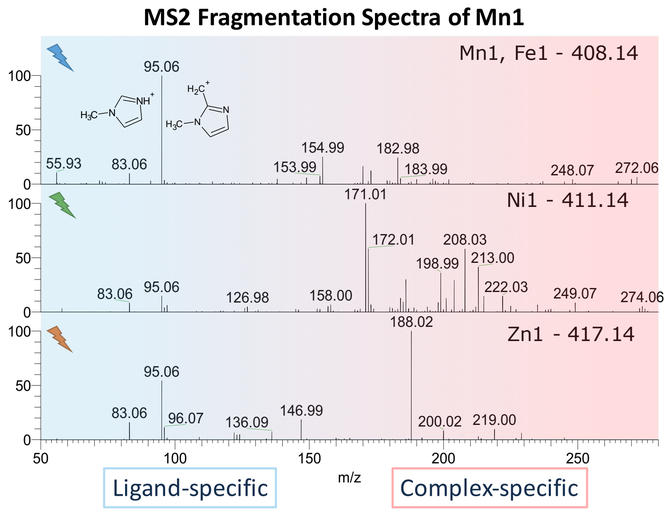
The propylated-Mn1 in a pH favoring dehydrogenation maintains the expected m/z **(F)**.

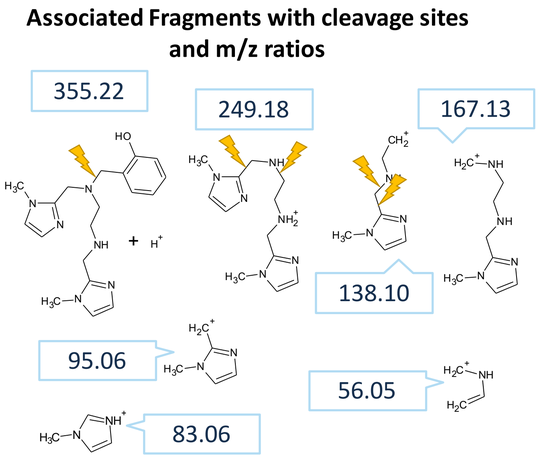


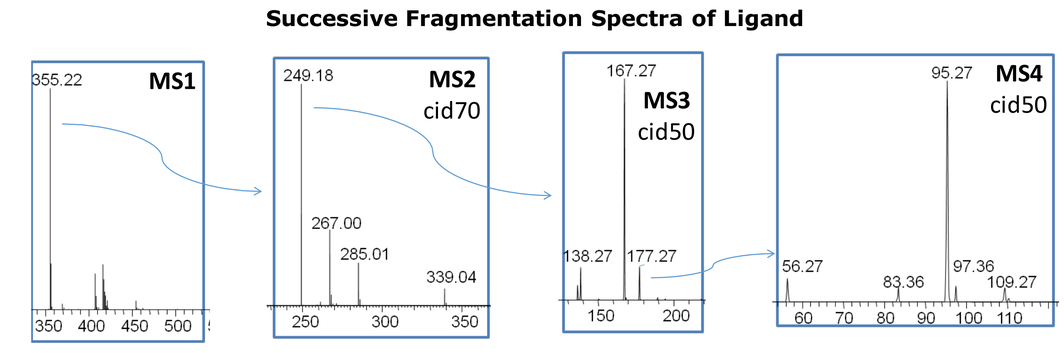
Characteristic retention times of Mn1, Fe1, Ni1, Zn1 **(C)**.



Presence of metal ions in buffers used for the Mn1 preparation, compared to Mn1 **(D)**.







Perspectives

The aim of the future studies is to validate the antioxidant and anti-inflammatory activity of Mn1 and other SOD mimic complexes in a cellular context. To do so, we will develop a method of intracellular Mn1 detection and we will determine and specify its antioxidant/anti-inflammatory activity by using bottom-up redox proteomics.

References

(1) Bernard A-S, *Dalton Trans.*, **2012**, 41, 6399 , (2) Mathieu E. et al, *Inorg. Chem*, **2017**, 56 5, 2555

Acknowledgement

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