Characterization of a manganese-complex drug candidate using hydrophilic interaction liquid chromatography LC-MS in an inflammatory bowel disease context

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Introduction

Inflammatory bowel diseases (IBDs *e.g.* Crohn's disease), are a major public health issue, and are known to be multifactorial. Among their causes, oxidative stress seems to have a key role. A deficit in detoxifying mitochondrial Mn-superoxide dismutase (Mn-SOD) being regularly observed, a peptidomimetic ligand (L1) coordinating with manganese II (Mn1, see below) was selected for its SOD-like activity.

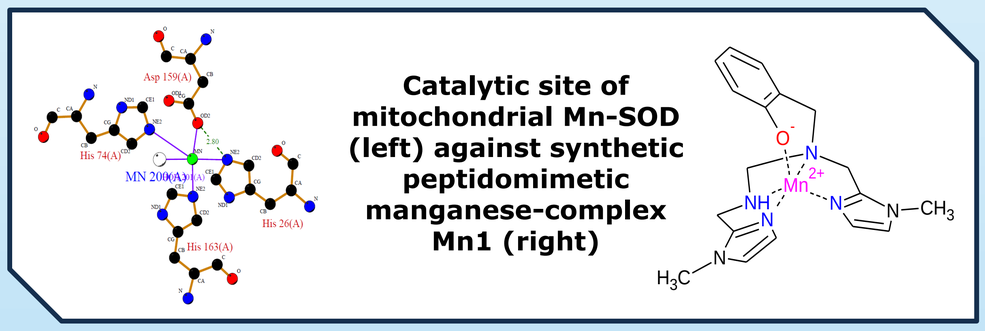
Fragmentation methods needed to be optimized in order to identify Mn1 and some of its variants, namely L1 associated with Ni^{2+} (Ni1) or Zn^{2+} (Zn1), using MS/MS. Furthermore, another variant of Mn1 with a mass loss of 1 Da was analyzed using ultra-high-resolution FTICR-MS to clarify its origin. Hydrophilic interaction liquid chromatography (HILIC) was used to isolate Mn1 from peptides for its compatibility with polar complex analysis.

Methods

The complex Mn1 was formed by mixing synthesized ligand and Mn^{2+} in HEPES solution.

Ultra-high resolution analysis was performed in direct-infusion ESI-FTMS on a LTQ FT Ultra (Thermo Fisher Scientific) with 10^6 input resolution. MS4 spectra were obtained in ESI-ITMS, with CID fragmentation.

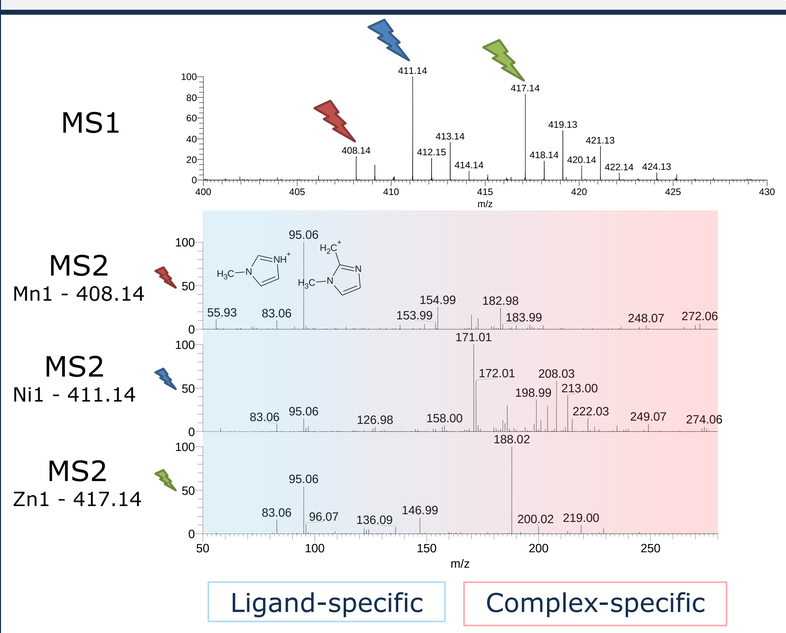
MS and MS2 spectra were obtained from a QExactive (Thermo Fisher Scientific). Acquisition method consists in FT full scan MS and systematic HCD fragmentation of selected m/z ratios.



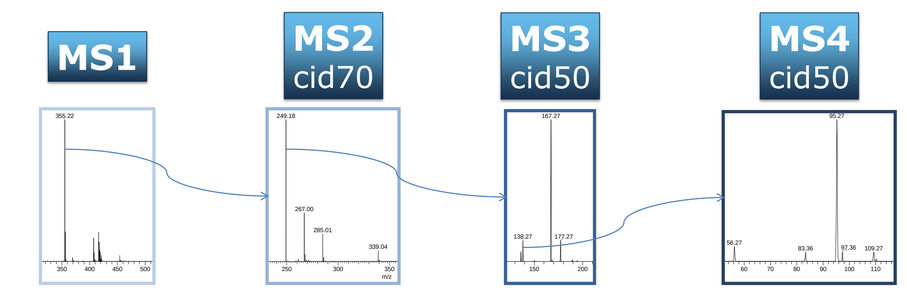
Catalytic site of mitochondrial Mn-SOD (left) against synthetic peptidomimetic manganese-complex Mn1 (right)

Results

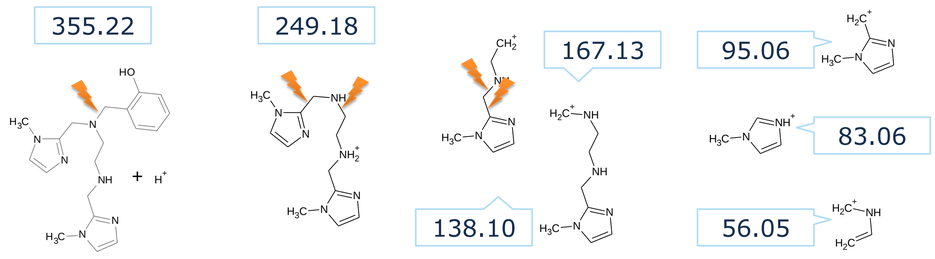
Fragmentation



MS and MS2 (NCE 70) spectra showing ligand- and complex-specific fragments



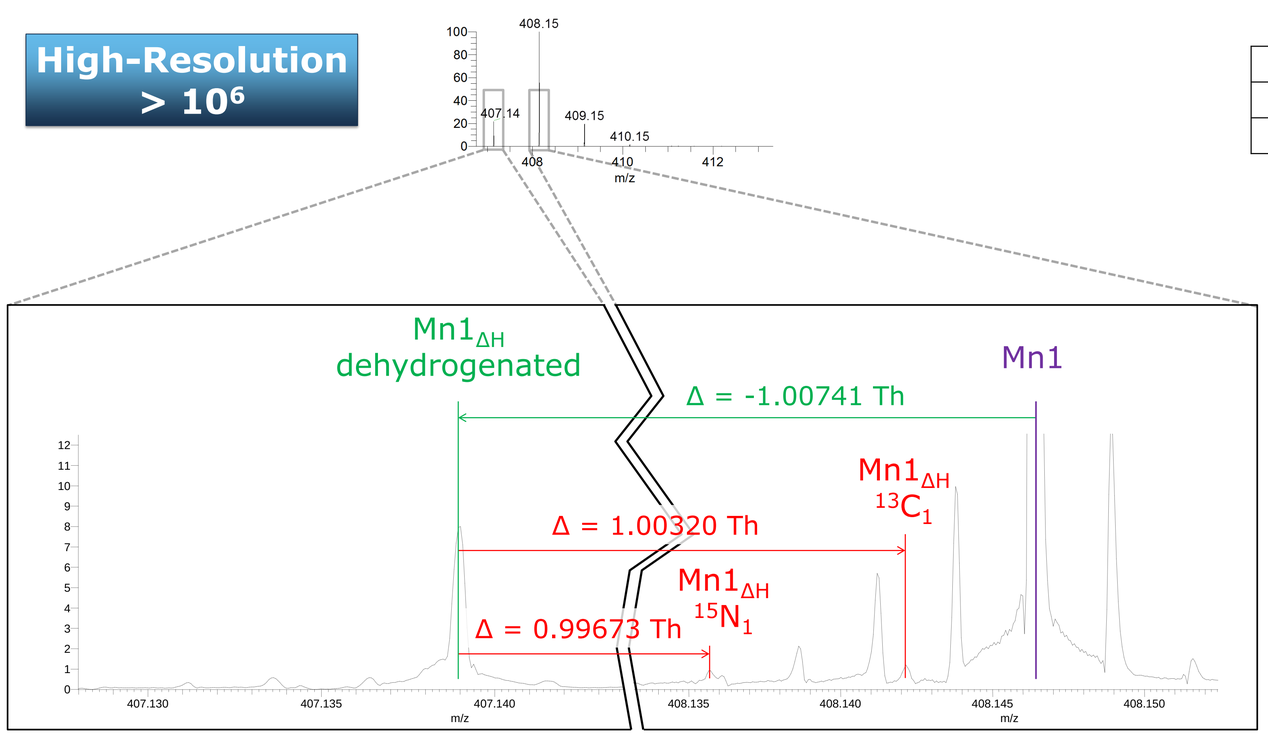
MS1 to MS4 spectra of ligand L1



Associated fragments with cleavage sites and m/z ratios

High-resolution

High-Resolution > 10^6



^1H	1.007825 Da
Δm ^{13}C-^{12}C	1.003355 Da
Δm ^{15}N-^{14}N	0.997035 Da

Table (top): Exact mass of Hydrogen and differences between Carbon and Nitrogen stable isotopes

Spectra (left): Full-scan MS magnification of ultra-high resolution Mn1 spectrum

Conclusions

Fragmentation profile was characterized for Mn1 and its variants. We refined fragments' structures using successive fragmentations up to MS4.

Ligand-specific fragments were described, allowing detection of the complex regardless of the metal involved, and eventual metabolites.

We also found metal-bound fragments, specific to the different coordination complexes.

Thanks to high resolution mass spectrometry, we proposed a structure corresponding to a dehydrogenated variant of Mn1, consistent with the reduction-oxidation catalyzed by SOD-mimetics and native SODs.