Unraveling the Mysteries of How Methanobactin Reduces and Stabilizes Copper Ions

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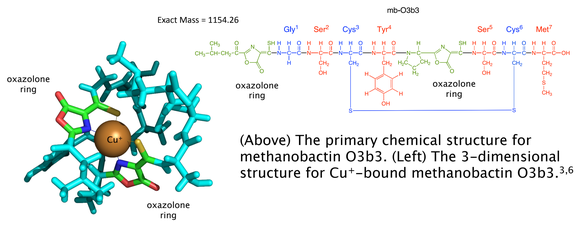
Abstract:

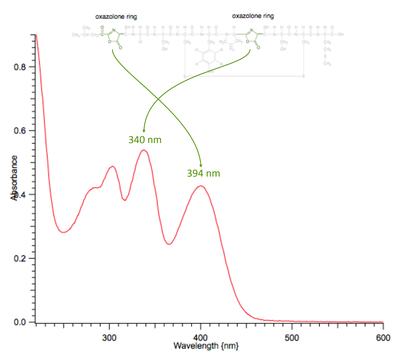
Methanobactins (mb) are peptide-derived, copper-binding molecules produced by methanotrophic bacteria, which use methane as their primary source of carbon and energy.(1-8) They synthesize mb to scavenge copper ions from the environment and use this copper to meet the needs of the enzyme that catalyzes the conversion of methane to methanol. The best characterized methanobactin is isolated from the bacterium Methylosinus trichosporium O3b3 (mb-O3b3). mb-O3b3 displays the remarkable property of binding and reducing copper(II) ions to copper(I) ions and stabilizing them in an aqueous environment. mb-O3b3 can also bind and reduce Mercury(II), Silver(I), and Gold(III) ions. Currently we do not know how mb-O3b3 is able to carry out these reactions. We do know from the structure of copper-bound mb-O3b3 that there are two oxazolone rings that are intimately involved in the binding of Copper(I) ions.(6) Here we will report on a method we have developed for selectively opening these rings and isolating the products. Our future work will focus on using these chemically modified mb molecules to further investigate the metal binding properties of mb, by assessing the role that the oxazolone rings play in reducing and stabilizing metal ions.



(Above) The primary chemical structure for methanobactin O3b3. (Left) The 3-dimensional structure for Cu^{+}-bound methanobactin O3b3.[3,6]

Introduction:

In an earlier study, we concluded that mb-O3b3 contains two oxazolone rings.(6) Some of the evidence for this included the fact that these rings underwent methanolysis when exposed to a saturated solution of HCl in methanol. This finding may help to explain our observation that uncomplexed mb-O3b3 is unstable and difficult to separate by High Performance Liquid Chromatography (HPLC). Oxazolone rings are known to be unstable. Here we report on a project that grew out of our efforts to find conditions that might stabilize uncomplexed mb-O3b3 and allow us to better purify it. While we have not yet succeeded in these efforts, we have been able to isolate and characterize various degradation products of mb-O3b3, which may, in the end, prove useful for developing a better understanding of how this remarkable molecule is able to bind, reduce and stabilize Copper(II) ions.

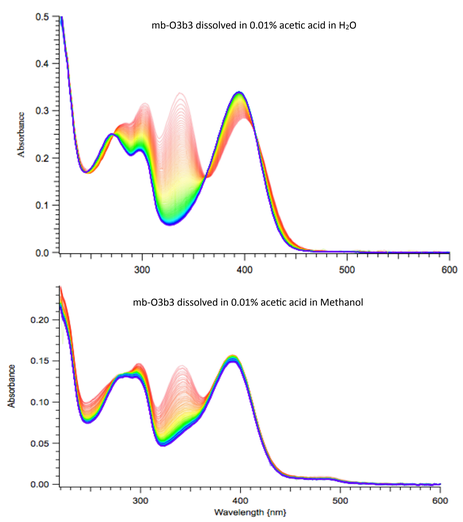


Ultraviolet/Visible (UV/Vis) spectrophotometry of mb-O3b3.

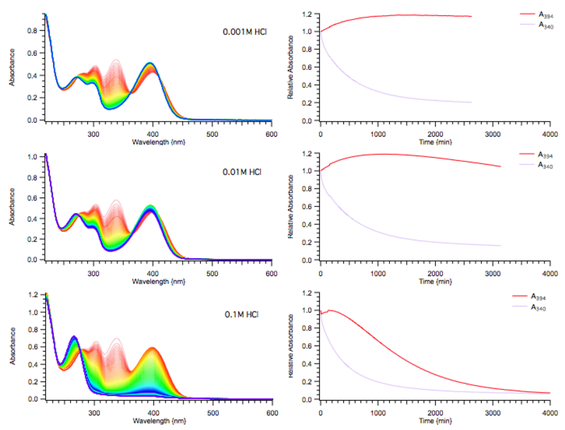
The two oxazolone rings produce peaks at 340 nm and 394 nm. These peaks are lost when the rings open.

Experimental:

Instability has been a problem in all of our attempts to isolate uncomplexed mb-O3b3 using HPLC. We first decided to examined the stability of the uncomplexed mb-O3b3 in the solvents that we were using when doing HPLC. These contain a much more dilute concentration of acid than was used in the methanolysis experiments: 0.01% acetic acid in H_2O and 0.01% acetic acid in methanol. Dilute acetic acid is commonly used when carrying out HPLC to improve the separation of peptides, such mb-O3b3. We monitored the UV/Vis absorbance of mb-O3b3 after dissolving it in each of our HPLC solvents. Below are the results we obtained by monitoring the UV/Vis absorbance for the first 24 hours after dissolving the sample. We conclude from these results that the presences of dilute acetic acid in the solvents is causing the opening of one of the oxazolone rings in mb-O3b3. However, we found that the end products obtained depend on the solvent used.



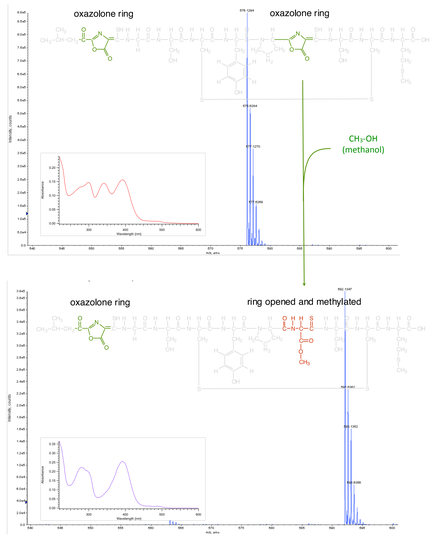
We next looked at various concentrations of dilute hydrochloric acid (HCl) in water to study the effect of acid concentration on the opening of the rings.



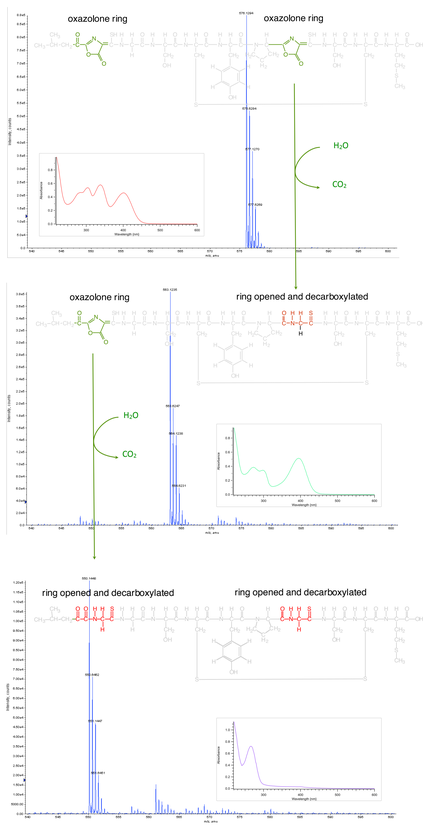
A comparison of the UV spectra of mb after reacting with different concentrations of HCl.

These three graphs compare the absorbance of the products at 340 nm and 394 nm. These spectra show that one oxazolone ring is more susceptible than the other to lower concentrations of acid and that both rings will open at higher concentrations. This led to the development of a procedure that can be used to selectively open one ring while leaving the other intact, or alternatively, opening both rings.

The reaction of mb-Ob3b with 0.01% acetic acid in methanol. The ring opens in the presence of methanol it creates a methyl ester as the product. This is what is known as methanolysis.



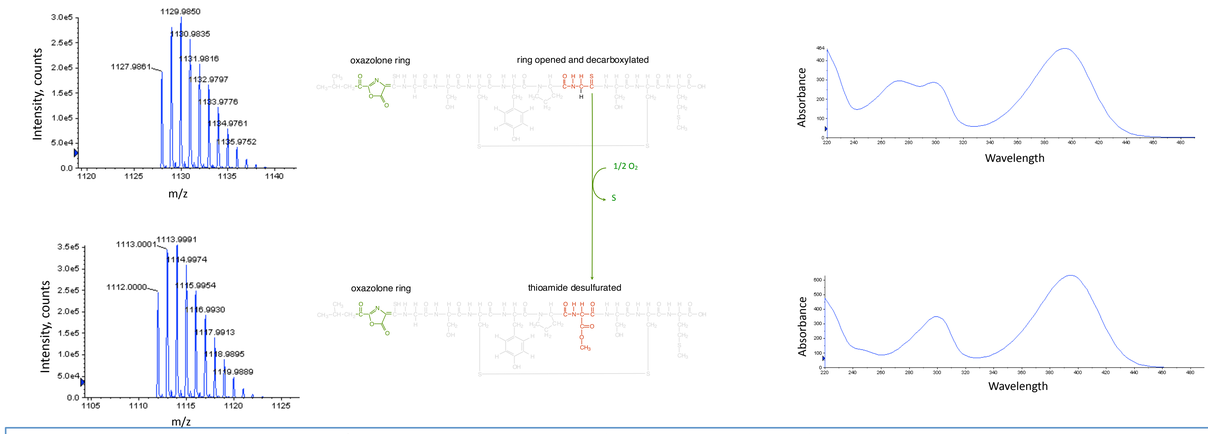
The reaction of mb-Ob3b with HCl in H_2O. First the right-hand ring opens by hydrolysis, which is followed by decarboxylation. At higher concentrations of HCl, the same reaction occurs at the left-hand ring.



The electrospray injection (ESI) time-of-flight (TOF) mass spectra of the degradation products of mb after being exposed to HCl in either the presence of methanol (CH_3-OH) or water.

ESI-TOF mass spectrometry measures mass-to-charge (m/z) ratio of a molecule. All of the mass spectra shown here are for the -2 charged species. The multiple peaks in each group are due to the distribution of isotopes for each atom in a molecule. The actual mass for each species can be obtained by multiplying the m/z by 2 and adding back the mass of two hydrogen ions. The masses we observed confirm that we obtained the reaction products shown in the reaction equations. The UV/Vis spectrum for each species is shown for comparison to the timecourse experiments.

We also found that the exposure to acid can cause desulfuration of the thioamide that forms in mb-O3b3 after hydrolysis and decarboxylation, where the sulfur is replaced with an oxygen.



The ESI-TOF mass spectra, and the UV/Vis spectra of the sulfurated (top) and desulfurated (bottom) mb after undergoing decarboxylation.

The ions shown in the ESI-TOF here have a single negative charge. The difference in m/z between the sulfurated and desulfurated is 16 amu which is consistent with the substitution of an oxygen for a sulfur. The UV/Vis spectrum of the sulfurated mb has a peak at 272 nm which is in the characteristic absorption range of thioamides. This peak is absent in the spectra of the sulfurated mb.

Where we go from here:

- With the reactions described above, we have developed procedures for producing and isolating a number of different forms of mb-O3b3.
  - If we perform the reaction in methanol, the rings open and a methyl ester is formed, and if we perform the reaction in H_2O, the rings open and decarboxylation occurs.
  - By altering the concentration of the acid, we can selectively open one or both rings.
  - Exposure to acid can also cause mb-O3b3 to undergo desulfuration.
- Since these species represent intermediates that are stable to exposure to acid, we have been able to isolate them using HPLC.
- The modifications we have observed specifically affect the regions of the mb-O3b molecule that are directly involved in the binding of copper ions. We can use these species in metal-binding studies, which may help us answer some of the questions that still remain on how mb-O3b binds and reduces metal ions.

References

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