INVESTIGATING THE POSSIBLE ISOMERIC FORMS OF METHANOBACTIN-SB2

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INTRODUCTION & OBSERVATIONS

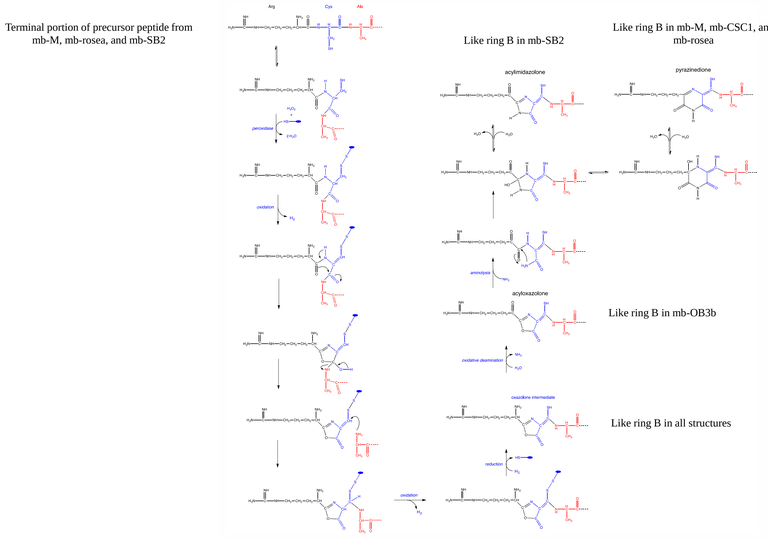
Methanobactins (mb) are peptide-derived molecules produced by bacteria called methanotrophs.

- Mbs are very strong binders of copper ions and show promise as a possible therapeutic agent for curing Wilson's disease, a copper storage disease in humans.
- To date, mbs from five different methanotrophs have been chemically characterized in their copper-bound states (Methanobactin Structures).[1]
- The copper binding sites for all five involve two heterocyclic rings, which are each derived from two of the amino acids that make up the precursor peptide. For all five mbs, the second ring (ring B) is an oxazolone ring, while the first ring (ring A), is either an acyloxazolone or a pyrazinedione ring. The ring A for the mb from *Methylocystis* strain SMB (mb-SMB) was originally characterized as an acylimidazolone[3] (Methanobactin Structures), however, evidence that we present here suggests it too may be a pyrazinedione.
- Based on observations made by others of side reactions that can take place during peptide synthesis,[4] as well as those of a group of researchers who chemically synthesized a model for the yellow fluorescent protein chromophore, which contains an acylimidazolone ring,[2] we have proposed a scheme for the synthesis of all three ring structures from the amino acids arginine (Arg) and cysteine (Cys),[1] which are known to exist in the precursor peptide for mb-SB2,[3] mb-M,[1] and mb-rosea[1] (Biosynthesis Scheme).
- Yampolsky *et al.*[2] observed that if they exposed their acylimidzolone containing structure for the to acidic conditions, that it would spontaneously rearrange to form a pyrazinedione ring.
- The chemical structure for the mb from *Methylocystis* strain M is very similar to that for mb-SB2, and its X-ray crystal structure has definitively shown that it contains a pyrazinedione ring and not an acylimidazolone ring.[5]

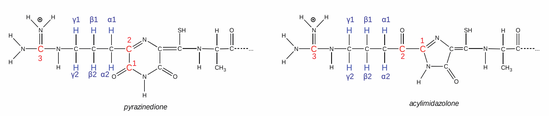
These observations have caused us to revisit our assignment of an acylimidazolone for the ring A in mb-SB2.

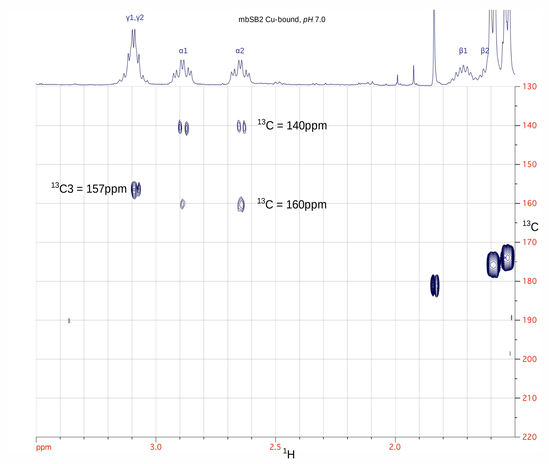
- The two rings have the same mass, so they cannot be distinguished by mass spectrometry. We instead chose to use nuclear magnetic resonance spectroscopy.
- Collecting NMR data on the rings is difficult because the carbons in the rings do not have any hydrogen atoms bonded directly to them. We needed to turn to a sophisticated, indirect 2D NMR experiment called HMBC, which is able to find the NMR resonances for carbon atoms that are three or four bonds away from a hydrogen atom. The NMR results for experiments carried out on mb-SB2, copper-bound at pH 7.0 and copper-free at both pH 7.0 and pH 9.6, suggest that mb-SB2 may indeed contain a pyrazinedione instead of acylimidazolone ring under these conditions (NMR Results).
- When we lower the pH of the Copper-free form of mb-SB2, we observe a distinct color change below pH 4 (Inset in Acid Degradation). We know that the observed color is due to light absorption by ring A. This color change suggests it might be associated with a rearrangement of ring A.
- Our attempts to collect NMR data on mb-SB2 under acidic conditions have been hampered by the fact that mb-SB2 is acid labile and undergoes degradation with extended exposure to acidic conditions (Acid Degradation). After 12 hours at pH 4, the ring A oxazolone undergoes hydrolysis and decarboxylation to form a thioglycine residue. After two weeks at pH 4, we observe the peptide backbone fragmenting between the alanine (Ala) and serine (Ser) residues, leaving the ring A intact.
- Our next step is to isolate the ring A containing fragment using HPLC and characterize it by NMR.
- Interestingly, we have some evidence to suggest that the small ring A-containing fragment may still be able to bind copper ions.

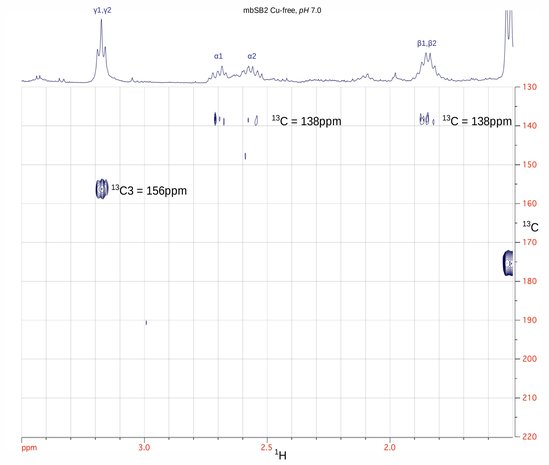
PROPOSED BIOSYNTHESIS SCHEME

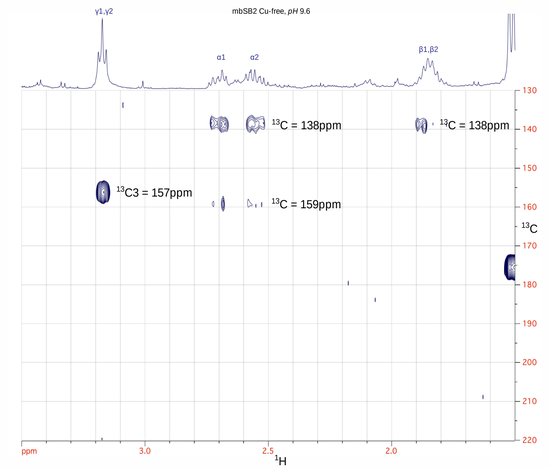


NMR RESULTS









Carbon-13 Chemical Shifts - Observed for Methanobactin Strain SB2

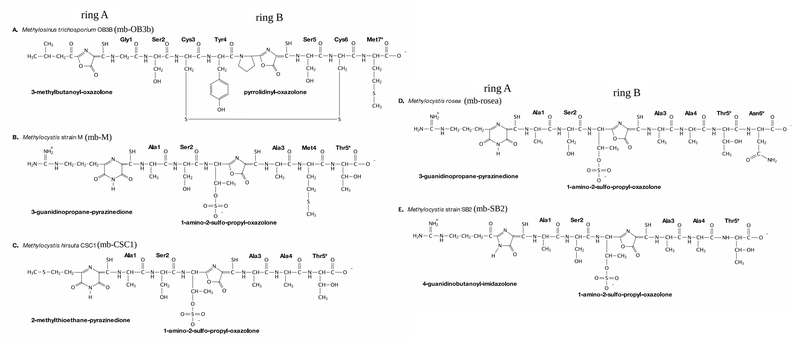
	α	β	α	β	γ
	C1 or C2		C1 or C2		C3
mbSB2 Cu-bound, *pH* 7.0	140ppm	-	160ppm	-	157ppm
mbSB2 Cu-free, *pH* 7.0	138ppm	-	-	-	156ppm
mbSB2 Cu-free, *pH* 9.6	138ppm	138ppm	159ppm	-	157ppm

Carbon-13 Chemical Shifts - Calculated and Observed by Yampolsky et al., 2009

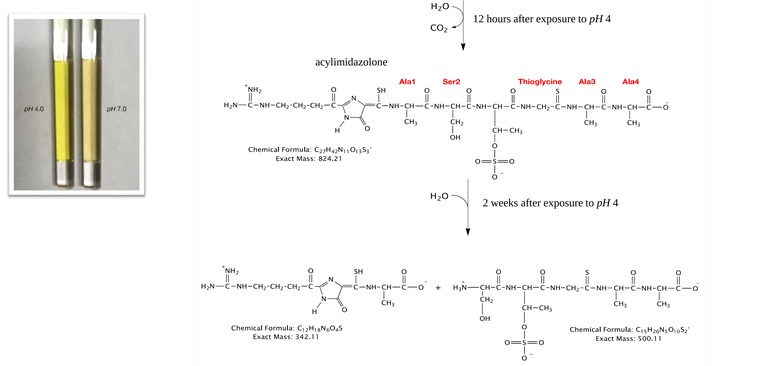
	pyrazinedione			acylimidazolone		
	C1	C2	C3	C1	C2	C3
Yampolsky *et al.*, 2009	159ppm	156ppm	-	153ppm	196ppm	-
Computational Prediction	160ppm	148ppm	160ppm	145ppm	191ppm	158ppm

We see no evidence for a ring A carbon with this high of a chemical shift value.

METHANOBACTIN STRUCTURES



ACID DEGRADATION OF mbSB2



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