The Environmental and Genetic Rhythm Governing the Production of Methanobactin

Benjamin D. Krentz[1] *, J. Colin Murrell[2], Alan A. DiSpirito[3], and Warren H. Gallagher[1]

[1] University of Wisconsin – Eau Claire: Department of Chemistry
[2] University of Warwick: Department of Biological Sciences
[3] Iowa State University: Department of Biochemisty, Biophysics, and Molecular Biology

Introduction

Methanobactin is peptide-derived, copper-binding molecule that is produced by methanotrophic bacteria.[1-8]

❖Methanobactins scavenge and reduce copper (II) ions to provide copper as an enzymatic co-factor for *particulate methane mono-oxygenase* (pMMO), which converts methane to methanol

❖pMMO requires copper ions. Methanotrophs synthesize methanobactin for the purpose of scavenging copper ions from the environment to meet this needs for this enzyme

❖mb-O3b3 binds Cu^{2+} ions, reduces them to Cu^{+} ions, and stabilizes the Cu^{+} ions using a mechanism that is thus far not well understood

❖Recently , the mapping of the genome of *Methylosinus trichosporium OB3b* has revealed that the methanobactin produced by this methanotroph (mb-OB3b) is derived from a ribsomally-produced peptide

❖Two structurally characterized methanobactins: from the methanotroph *Methylosinus trichosporium O3b3* (mb-O3b3) and *Methylocystis* strain SB2 (mb-SB2)

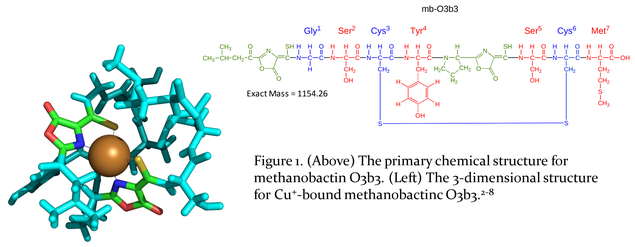


Figure 1. (Above) The primary chemical structure for methanobactin O3b3. (Left) The 3-dimensional structure for Cu^{+}-bound methanobactinc O3b3.[2-8]

Here, we report an ongoing experiment designed to elucidate the role of methanobactin the physiological pathways of *M. trichosporium OB3b*. The experiment is combination of efforts between researchers at the University of Warwick, Iowa State University, and the University of Wisconsin – Eau Claire. Specifically, our team has designed a *M. trichosporium OB3b* methanobactin knockout and observed the excreted and spent media from the bacteria. This study delineates our findings and inferences about a genetic regulator in the production of methanobactin and in methanotroph metabolism.

Figure 2. (Left) Geothermal vents are a unique high pressure and high temperature environment where some species of methanotrophs thrive [10]. (Right) Hot Springs like the Devil's Gate in New Zealand are another example of environments where some methanotrophic species can survive [11].

Methanotrophs and the Biosphere

Methanotrophic bacteria are highly ubiquitous within the biosphere [12].

❖ Not only do they occupy a significant quantity of all life on earth, but bacteria that eat methane are able to live in some of the most harsh and formidable areas on the planet.

Ex: Some methanotrophs have been known to thrive inside of hot aquifers at the bottom of the Atlantic Ocean [13].

The role that these bacteria play in balancing the amount of atmospheric methane produced is absolutely remarkable.

❖ Following the Deepwater Horizon Spill of 2010, it was demonstrated that nearly 99.7% of the methane gas produced by this pipe leak never reached the surface of the ocean [12].

❖ Rather, based on the absence of soluble oxygen and increased colonies of methanotrophic bacteria on sampled water pipes near the Gulf floor suggest that methanotrophic bacteria consumed the vast majority of methane gas produced.

These findings suggest that methanotrophic bacteria play a previously undervalued role as environmental methane buffers.

Acknowledgements

We wish to acknowledge the following contributions to this project:

UW-Eau Claire Office of Research and Supported Programs

Dr. Jeremy Semrau (University of Michigan) for supplying us with the methanobactin samples

Dr. Scott Hartsel for sharing mb-SB2 copper binding results

Ashley Van Galen and Sarah Bretle for their contributions to the Gallagher group and helpful discussions

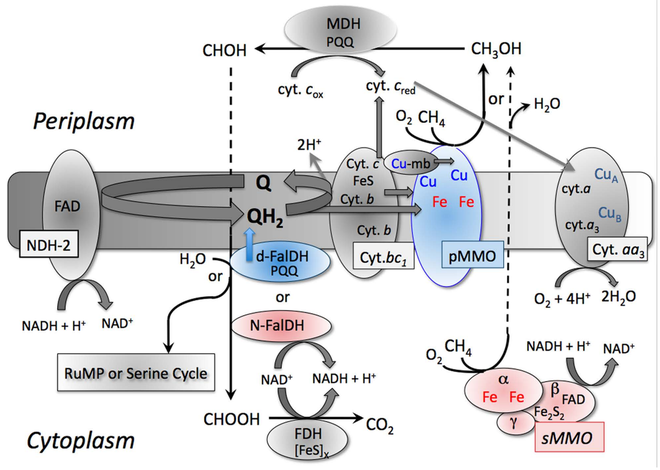


Figure 3. Fig. 2. Alan DiSpirito's working model for Cu-mb mediated electron flow from the respiratory chain to the metal centers of the pMMO from Mc. capsulatus Bath [14]. Demonstrates the known role of methanobactin-copper complexes in a methanotroph metabolism.

Methanobactin plays a Role in Methane Oxidation

Methanotrophs use methane from the atmospheric as their primary carbon and energy source; the first step in metabolizing methane is oxidizing it to methanol.

❖ This reaction is catalyzed by a family of methane monooxygenases, specifically particulate (pMMO) and soluble (sMMO)[15] .

❖ pMMO accounts for roughly 20 percent of all membrane-bound proteins in methanotrophic cells [9], and utilizes copper (I) as a cofactor in coordinating this oxidation intracellularly [2]. sMMO, a solvated analogue to pMMO, is not as well understood structurally, but has been demonstrated to perform the same reduction using iron ions [17]

❖ Methanobactin, by a mechanism not fully understood, binds and reduces Cu^{2+} → Cu^{1+}, and delivers the copper ion to pMMO [16] (Fig. 3).

❖ When methanobactin is not produced in a transctiptional knockout, sMMO is not produced. This is most likely due to positive transcription regulation by the copper-mb complex [16].

❖ From the extraneous cell material, we report an isolated iron-binding molecule that displays some of the characteristics of a siderophore which may be produced in lieu of methanobactin.

The Siderophore

❖ Originally, CAS binding tests indicated that this molecule does not bind iron. Usually, a novel siderophore is initially classified by its ability to bind and maintain binding with iron, which is demonstrated by the CAS test. This molecule did not appear to bind Fe^{3+}. This was also later confirmed by UV/Vis and MS spectra that demonstrated a failure of Fe^{3+} complexes to form.

❖ Fe^{2+} appears to bind to this particular molecule in the fray of post-translational fragments. Fe(II)SO_4 was titrated into a solution of approximately 2 mM mixture.

❖ At concentrations where the amount of Fe^{2+} is approximately equivalent to the estimated amount of molecule in the mixture.

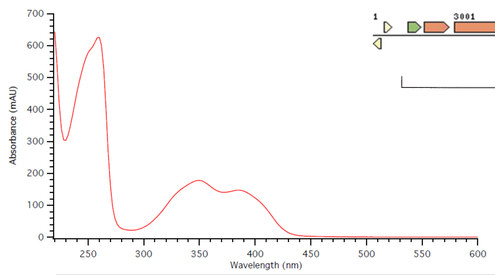
❖ This is uncharacteristic of siderophores, which usually:

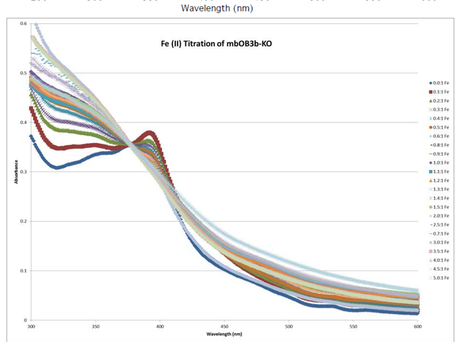
- Bind Fe^{3+}, not Fe^{2+}
- Bind iron ions at with much higher affinity or at less saturation than this molecule

❖ It is characteristic, however, that the molecule undergoes a chromophoric shift between two states, as is demonstrated by the presence of an isosbestic point in the UV titration (Fig. 4b). This titration spectra argues that the molecule must be altering conformations (which are likely to be binding conformations) due to the Fe^{2+} ligand or adduct.

❖ This molecule has been isolated via LC-MS, and found to have a mass of 680 Da (Fig. 4c). It is questionable as to what the true function of this molecule is, though it is hypothesized that this particular fragment associates with Fe^{2+} in a unique manner.

❖ Attempts to isolate the iron-bound siderophore are still underway.





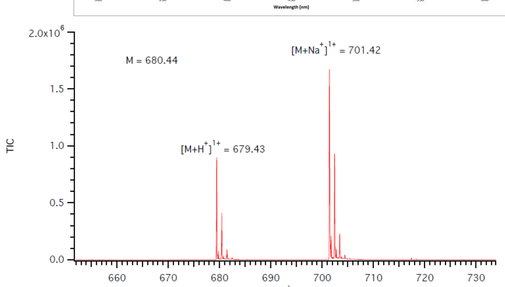


Figure 4. A) UV signature absorbance of the hypothetical siderophore. Notice the absorbance at 350 and 400 nm is indicative to resonant groups which may be responsible for binding Fe^{2+}. B) Fe^{2+} titration into siderophore. The isosbestic point at ~350 nm demonstrates a change in binding conformation as the concentration of Fe^{2+} is increased. C) Mass spectrum of the apo-form of the hypothetical siderophore. Forms shown are +1 ionized and conjugately bound to Na^{+}.

Methods Used in Our Studies

Ultraviolet/Visible (UV/Vis) Spectrophotometry

❖Ultraviolet and visible light are can be absorbed my molecule.

❖The rings mb-O3b3 and mb-Sb2 give them a characteristic absorption which can be used to characterize the presence of these rings.

High Pressure Liquid Chromatography (HPLC)

❖ Separates molecules in solutions and mixtures based on their affinity for other molecules, overall charge and polarity, molecular size, et cetera...

❖ Analogous to a highly-specific molecular filter

❖ Here, a reverse-phase column (RPC) is used on the HPLC in order to separate molecules by polarity as they are eluted through the column

Mass Spectroscopy

❖ A mass spectrometer is able to measure the mass-to-charge ratio (m/z) of charged molecules.

❖ Our Time-of-Flight is able to measure mass to charge ratios to accuracy of 2 parts per million!

Iron and the genetic production of methanobactin

The uptake of iron by non-methanobactin producing knockouts has several possible interpretations. In any case, it is clear that methanobactin is not entirely necessary for cellular survival since the knockouts are non-lethal.

1. Iron is required for the operation of both pMMO and sMMO; moreover, it is the sole metal cofactor required by sMMO.

   ❖ Hyperproduction of siderophore may either stimulate overdrive behavior of sMMO, since pMMO cannot use Fe^{2+} as a proxy for copper [15].

   ❖ This behavior may explain why the knockout mutation is non-lethal.

2. It has previously been demonstrated that methanobactin acts as a genetic regulator for the expression of both sMMO and pMMO *in vivo* [15].

   ❖ If methanobactin is not present to behave as a copper-chelating genetic regulator that a siderophore or siderophore-emulating structure may be produced to induce an alternative metabolic step

   ❖ This siderophore may then behave a genetic activator or silencer, or as a transcription riboswitch., as evidenced by the proximity of coding for a TonB-receptor to the coding for a methanobactin precursor[18] (Fig. 5).

   - TonB-receptor transports siderophores across the cell membranes

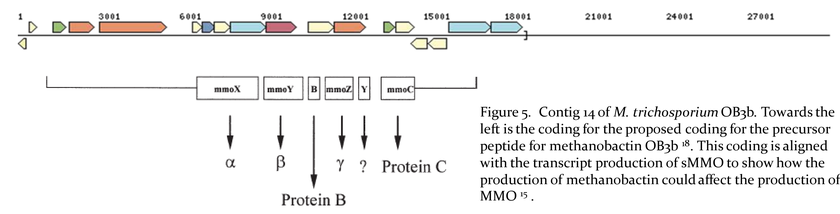


Figure 5. Contig 14 of *M. trichosporium* OB3b. Towards the left is the coding for the proposed coding for the precursor peptide for methanobactin OB3b [18]. This coding is aligned with the transcript production of sMMO to show how the production of methanobactin could affect the production of MMO [15] .

Final Thoughts

Physiological and metabolic role of methanobactin in methanotrophic bacteria is still not fully understood.

Methanobactin is thought to be both a genetic regulator and essential to the methane-oxidation metabolism, BUT methanobactin knockout bacteria are viable.

Though methanobactin is not produced, a possible siderophore is produced.

This siderophore fails the CAS characteristic test, BUT it has been spectroscopically observed to bind Fe^{2+}.

Further insights into how this siderophore operates structurally and is produced genetically will give a greater understanding of the genetic and environmental regulation surrounding methanobactin's production.

References

1. DiSpirito, A. A.; Zahn, J. A.; Graham, D. W.; Kim, H. J.; Larive, C. K.; Derrick, T. S.; Cox, C. D.; Taylor, A. Journal of bacteriology 1998, 180, 3606.
2. Choi, D. W.; Antholine, W. E.; Do, Y. S.; Semrau, J. D.; Kisting, C. J.; Kunz, R. C.; Campbell, D.; Rao, V.; Hartsel, S. C.; DiSpirito, A. A. Microbiology (Reading, England) 2005, 151, 3417.
3. Kim, H. J.; Graham, D. W.; DiSpirito, A. A.; Alterman, M. A.; Galeva, N.; Larive, C. K.; Asunskis, D.; Sherwood, P. M. Science 2004, 305, 1612.
4. Martinho, M.; Choi, D. W.; Dispirito, A. A.; Antholine, W. E.; Semrau, J. D.; Munck, E. J Am Chem Soc 2007, 129, 15783.
5. Choi, D. W.; Semrau, J. D.; Antholine, W. E.; Hartsel, S. C.; Anderson, R. C.; Carey, J. N.; Dreis, A. M.; Kenseth, E. M.; Renstrom, J. M.; Scardino, L. L.; Van Gorden, G. S.; Volkert, A. A.; Wingad, A. D.; Yanzer, P. J.; McEllistrem, M. T.; de la Mora, A. M.; DiSpirito, A. A. Journal of inorganic biochemistry 2008, 102, 1571.
6. Behling, L. A.; Hartsel, S. C.; Lewis, D. E.; DiSpirito, A. A.; Choi, D. W.; Masterson, L. R.; Veglia, G.; Gallagher, W. H. J Am Chem Soc 2008, 130, 12604.
7. Krentz, B. D.; Mulheron, H. J.; Semrau, J. D.; DiSpirito, A. A.; Bandow, N. L.; Haft, D. H.; Vuilleumier, S.; Murrell, J. C.; McEllistrem, M. T.; Hartsel, S. C.; and Gallagher, W. H. (2010) Biochem. 49, 10117-10130.Balasubramanian, R.; Rosenzweig, A. C. Current Opinion in Chemical Biology 2008, 12, 1.
8. Medzihradszky, K. F.; Darula, Z.; Perlson, E.; Fainzilber, M.; Chalkley, R. J.; Ball, H.; Greenbaum, D.; Bogyo, M.; Tyson, D. R.; Bradshaw, R. A.; Burlingame, A. L. Mol Cell Proteomics 2004, 3, 429.
9. Kenney, G. E., and Rosenzweig, A. C. 2012, Acs Chem. Bio. 7, A-I.
10. Edwards, L. (Sept. 16. 2010). http://phys.org/news203835088.html
11. Dunfield, P. (2007) http://www.bio.ucalgary.ca/contact/bio.php/dunfield.html
12. J D Kessler et al. Science 2011;331:312-315
13. Hirayama, H., Suzuki, Y., Abe, M., Miyazaki, M., Makita, H., Inagaki, F., Uematsu, K., and Takai, K. 2011 Intl. J. of Syst. and Ev. Microbio. 61, 2646-2653.
14. DiSpirito, A.A. (2012) http://www.bbmb.iastate.edu/index
15. Knapp, C. W., Fowle, D. A., Kulczycki, E., Roberts, J. A., and Graham, D. W. 2007., P. of the Nat. A. of S. 104, 12040-12045.
16. Bandow, N. L., Gallagher, W. H., Behling, L., Choi, D. W., Semrau, J. D., Hartsel, S. C., Gilles, V. S., and DiSpirito, A. A. 2011. Meth. in Enz: Meth. in Meth. M., Vol 495, Pt B (Rosenzweig, A. C. R. S. W., Ed.), pp 259-269
17. Sullivan, J. P., Dickinson, D., and Chase, H. A. 1998. C. R. in Microbio. 24, 335-373.
18. Stein, L. Y., Yoon, S., Semrau, J. D., DiSpirito, A. A., Crombie, A., Murrell, J. C., Vuilleumier, S., Kalyuzhnaya, M. G., den Camp, H. J. M. O., Bringel, F., Bruce, D., Cheng, J. F., Copeland, A., Goodwin, L., Han, S., Hauser, L., Jetten, M. S. M., Lajus, A., Land, M. L., Lapidus, A., Lucas, S., Medigue, C., Pitluck, S., Woyke, T., Zeytun, A., and Klotz, M. G. 2010. J. of Bact. 192, 6497-6498.