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Spectral and thermodynamic properties of Ag(I), Au(III), Cd(II), Co(II), Fe(III), Hg(II), Mn(II), Ni(II), Pb(II), U(IV), and Zn(II) binding by methanobactin from *Methylosinus trichosporium* OB3b

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Abstract

Methanobactin (mb) is a novel chromopeptide that appears to function as the extracellular component of a copper acquisition system in methanotrophic bacteria. To examine this potential physiological role, and to distinguish it from iron binding siderophores, the spectral (UV–visible absorption, circular dichroism, fluorescence, and X-ray photoelectron) and thermodynamic properties of metal binding by mb were examined. In the absence of Cu(II) or Cu(I), mb will bind Ag(I), Au(III), Co(II), Cd(II), Fe(III), Hg(II), Mn(II), Ni(II), Pb(II), U(VI), or Zn(II), but not Ba(II), Ca(II), La(II), Mg(II), and Sr(II). The results suggest metals such as Ag(I), Au(III), Hg(II), Pb(II) and possibly U(VI) are bound by a mechanism similar to Cu, whereas the coordination of Co(II), Cd(II), Fe(III), Mn(II), Ni(II) and Zn(II) by mb differs from Cu(II). Consistent with its role as a copper-binding compound or chalkophore, the binding constants of all the metals examined were less than those observed with Cu(II) and copper displaced other metals except Ag(I) and Au(III) bound to mb. However, the binding of different metals by mb suggests that methanotrophic activity also may play a role in either the solubilization or immobilization of many metals *in situ*.

Keywords: Chalkophore; Copper-binding compound; Methanobactin; Membrane-associated methane monooxygenase; Methanotroph; *Methylosinus trichosporium* OB3b; Siderophore

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1. Introduction

Methanobactin (mb) is a low molecular mass (1154 Da) chromopeptide observed in both the extracellular and membrane fraction in many if not all aerobic methanotrophs [1–5]. When isolated from the membrane fraction, mb contains one copper atom and is predominately associated with the membrane-associated or particulate methane monooxygenase [5–7]. In the extracellular fraction, the majority of mb is metal free [2,5], and appears to be the extracellular component of a copper acquisition system similar to bacterial siderophore-based iron acquisition systems [2–6,8–12]. This proposed copper-siderophore, or chalkophore role [3], is based on copper uptake and localization studies [2,4–7,11], chelation of copper in soil systems [11], characterization of constitutive soluble methane monooxygenase mutants in *Ms. trichosporium* OB3b [2,4,9,12], and copper-binding studies [2,5,6,8,10].

The structure of copper containing mb (Cu–mb) following exposure to high copper concentrations showed the molecule bound one copper atom in a novel S, and N coordination by the 4-thiocarbonyl-5-hydroxy imidazolate (THI) and 4-hydroxy-5-thiocarbonyl imidazolate (HTI) moieties [3]. However, spectral, kinetic and thermodynamic studies indicate that initial coordination of Cu(II) and Cu(I) differs from the coordination observed in the crystal structure [8]. Mb appears to initially coordinate Cu(II) as tetramer or oligomer by THI and possibly Tyr (Fig. 1). This initial coordination is followed by a reduction of Cu(II) to Cu(I), and then followed by a change in metal ligation resulting in coordination by both THI and the HTI. At Cu(II) to mb ratios above 0.25 the Cu(II) is coordinated as a dimer, followed by coordination as a monomer at Cu(II) to mb ratios above 0.5 Cu per mb (Fig. 1).

The structural similarities of mb to siderophores in the pyoverdin class [13–16] suggested that mb may prove to be a siderophore with a capacity to bind Cu(II) as well as Fe(III). Several other observations suggest mb may be involved in the mobilization of non-cuprous metals. The coupled increase in iron uptake with increased copper uptake, or copper-induced iron uptake, suggest that mb may be involved in iron uptake [5,17]. Given that mb is the major if not sole extracellular metal binding compound produced by *Ms. trichosporium* OB3b [2,6,8,10], the observation by Jenkins et al. [18] that this bacterium mobilizes Cd(II) in soil columns suggest mb may bind Cd(II). To determine if mb can function as a siderophore and/or to mobilize metals other than copper, the metal binding properties of mb were examined. In this report the initial spectral and thermodynamic properties of Ag(I), Au(III), Co(II), Cd(II), Fe(III), Hg(II), Mn(II), Ni(II), Pb(II), U(VI) and Zn(II) binding were examined with special attention given to metals which are coordinated and reduced via a mechanism similar to copper, i.e., Ag(I), Au(III), Hg(II), and Pb(II). The results show that mb is primarily involved copper mobilization, but the binding

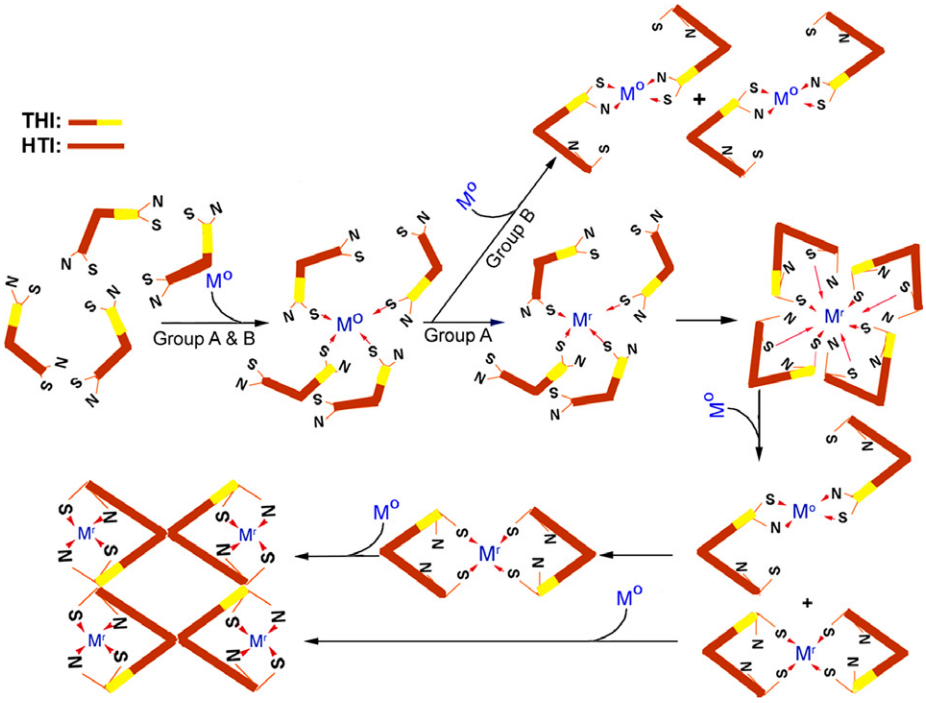

Fig. 1. Model for the binding of group A (Ag(I), Au(III), Cu(II), Hg(II), Pb(II) and U(VI)) and group B (Cd(II), Co(II), Fe(III), Ni(II), and Zn(II)) metals by mb. Mb is represented as two bars ending in the N^{ε} atom of each imidazolate and the S atom from the thiocarbonyl group on 4-thiocarbonyl-5-hydroxy imidazolate (THI; yellow and orange bar) and on 4-hydroxy-5-thiocarbonyl imidazolate (HTI; orange bar). Abbreviations: M^{o}, metal in the oxidation state added to mb solutions, and M^{r}, metal reduced by mb. (For interpretation of the references in colour in this figure caption, the reader is referred to the web version of this article.)