Copper resistance in Cupriavidus metallidurans CH34: functional study of CopB protein

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Introduction

Cupriavidus metallidurans CH34 is a bacterium able to colonize and adapt at industrial biotopes, such as those containing heavy metals. This bacterium has established resistance mechanisms allowing it to survive at high concentrations of multiple heavy metals (~1mM). This is a choice model for studies of heavy metal resistance (Cu, Zn, Pb, Au, Ni, Cr, ...). In this study, we are focused on copper resistance. Copper draws our attention owing to its presence in many applications. It is an essential trace element for biological systems fulfilling many functions as a catalyst (respiration, protection against oxidative stress, iron transport, etc.). However even if it’s essential, copper can become toxic when its concentration is too high. Therefore copper regulation is crucial.

Up to now, studies show that copper resistance in C. metallidurans CH34 would essentially involve cop cluster, present on pMOL30 plasmid of the bacterium (fig. 1). Even if function of some Cop proteins is known, the others are always unknown such as CopB protein. CopB from C. metallidurans CH34 arises interest due to presence of a repetitive motif, rich in methionine residues QGS[MG][Q][MG][MG]Q[M][Q][M][G] (fig. 2). This sequential motif repeated 11 times in the protein is susceptible to interact with copper by methionine residues of the motif. The motif could also interact with silver. In this work, we are focused exclusively on the methionine rich sequence named CopB(Met) and on synthesized peptides containing one or two methionine rich motifs.

Results

\[ \text{Cu(II) peptide interaction} \]

Studies of Cu(II)/peptide interactions were realized in presence of different molar equivalents of copper(I) and copper(II). Only doubly charged state is represented on these spectra.

\[ \text{Interaction specificity} \]

In this work, we also focused on the interaction with other metals such as silver, nickel and zinc. Peptide n°1 is able to bind 1 Ag(II) ion with high affinity. In contrast with silver, it’s not able to bind Ni(II) and Zn(II) ions (fig. 7).

\[ \text{CopB(Met) recombinant protein} \]

The CopB(Met) protein has been induced by isopropyl-1-thio-D-galactopyranoside, IPTG (fig. 8B), extracted and purified by affinity chromatographies (HIMAC and anion-exchange chromatography) (fig. 8C). Then, it has been characterized by mass spectrometry.

Only the copper(I) affinity was investigated. In presence of Cu(I) excess, protein structural changes appear in the gas phase. Indeed, charge state changes resulting from copper binding are observed (fig. 9).

CopB(Met) protein could bind at least 5 Cu(I) ions. Technical limitations prevent to see more.

Conclusion & perspectives

Studies of copper/peptide interaction by mass spectrometry showed that peptide n°1 is able to bind one Cu(II) ion with high affinity (K_a = 4.2x10^6M^-1) but has a Cu(II) less affinity. In contrast to Cu(II), the holodine residue seems to be involved to Cu(I) interaction. This peptide n°1 can also bind silver ions with high affinity but not Ni(II) and Zn(II) ions.

The CopB(Met) protein, including 11 sequential motifs QGS[MG][Q][MG][MG]Q[M][G][M][M][G], seems to have a certain structure in the gas phase and is able to bind at least 5 copper(I) ions.

As previously discussed the function of most Cop proteins is always unknown and particularly CopB. Our objective will be to improve knowledge about the CopB localization (intramembrane or periplasmic protein), we will focus on copper/interaction with entire CopB protein and identify the potential protein partners.

References:

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Figure 1: Cop cluster organization in C. metallidurans CH34

Figure 2: Nano-ESI-QToF mass spectra corresponding to peptide n°1 with some different molar equivalents of Cu(I). Only doubly charged state is represented on these spectra.

Figure 3: Mass spectrometry approach allowed to analyze the stoichiometry of copper/peptide interaction and also the interaction affinity. As shown in figure 3, peptide n°1 (1-124aM) is able to bind one Cu(I) ion with high affinity. The same results have been observed at 8µM and a dissociation constant K_a has been evaluated to 6.4µM (fig. 4).

The peptide n°3 also binds 1 Cu(I) ion with affinity similar to peptide n°1. As for peptide n°2, it interacts with 2 Cu(I) ions (data not shown).

Figure 4: Nanoelectrospray ionization mass spectrometry analysis of a recombinant CopB protein.

Figure 5: Evolution of Cu(I)/peptide n°3 interaction in presence of increased molar equivalents of Cu(I). Only doubly charged state has been considered.

Figure 6: Evolution of Cu(I)/peptide n°3 interaction in presence of increased molar equivalents of Cu(I). Only doubly charged state has been considered.

Figure 7: Interaction specificity.

Figure 8: Nano-ESI-QToF mass spectra corresponding to peptide n°1 with ~ 5 molar equivalents in Ag(II), Ni(II) and Zn(II) respectively. Only doubly charged state is represented on these spectra.

Figure 9: Nanoelectrospray ionization mass spectrometry analysis of a recombinant CopB protein.