Effect of Zn(II) on the Folding of Metallo-β-lactamase L1

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Metallo-β-lactamase L1, secreted by pathogenic *Stenotrophomonas maltophilia*, is a dinuclear Zn(II)-containing enzyme that hydrolyzes almost all known penicillins, cephalosporins, and carbapenems. The presence of Zn(II) ions in both metal binding sites is essential for full enzymatic activity; however, the number of Zn(II) bound under physiological conditions is unknown. To probe *in vivo* metal incorporation, L1 was over-expressed in minimal media with (mmL1+Zn) and without (mmL1-Zn) Zn(II) added to the media, and the resulting proteins were purified and characterized. The mmL1+Zn sample was bound by a Q-Sepharose column, exhibited steady-state kinetic properties, bound Zn(II), existed as a tetramer, and yielded fluorescence emission and CD spectra similar to L1 over-expressed in rich media. On the other hand, the mmL1-Zn sample did not bind to a Q-Sepharose column, and gel filtration studies demonstrated that this protein was monomeric. The mmL1-Zn sample exhibited a lower k_{cat} value, bound less Zn(II), and yielded fluorescence emission and CD spectra consistent with this enzyme being folded improperly. We have also probed the effect of Zn(II) on *in vitro* folding of L1. Taken together, these data demonstrate that the proper folding of L1 requires the presence of Zn(II) and suggest that *in vitro*, thermodynamic metal binding studies do not accurately reflect physiological metal incorporation into L1.

mmL1-Zn **mmL1+Zn**