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

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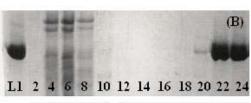
Metallo-β-lactamase L1, secreted by pathogenic Stenotrophomonas maltophilia, is a dinuclear Zn(II)-containing enzyme that hydrolyzes almost all known penicillins, cephalosporins, and carbapenems. The presence of Zn(II) ions in both metal binding sites is essential for full enzymatic activity; however, 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  $k_{\text{cat}}$ 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

(A)
L1 2 4 6 8 10 12 14 16 18 20 22 24

Before 0-150 mMgradient NaCl gradient

mmL1+Zn



Before 0 – 150 mM gradient NaCl gradient