Magnetic Circular Dichroism and Cobalt(II) Binding Equilibrium Studies of *Escherichia coli* Methionyl Aminopeptidase

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Equilibrium dialysis of methionyl aminopeptidase from *E. coli* (*Ec*MetAP) monitored by atomic absorption spectrometry (AA) and magnetic circular dichroism (MCD) shows that the enzyme binds up to 1.1 ±0.1 equivalents of Co^{2+} in the metal concentration range likely to be found *in vivo*. The dissociation constant, K_d, is estimated to be between 2.5 and 4.0 μM. Analysis of the temperature and magnetization behavior of the two major peaks in the MCD spectrum at 495 and 567 nm suggests that these transitions arise from Co^{2+} with different ground states. Ligand field calculations using AOMX are used to assign the 495 nm peak to Co^{2+} in the 6-coordinate binding site and the 567 nm peak to Co^{2+} in the 5-coordinate site. This is further supported by the fact that the binding affinity of the Co^{2+}associated with the 567 nm peak is enhanced when the pH is increased from 7.5 to 9.0, consistent with having an imidazole ligand from a histidine amino acid residue. Based on the MCD intensities it is estimated that when the 5-coordinate site is fully occupied, 0.1 equivalent of cobalt is in the 6-coordinate site. Even when the cobalt concentration is very low, there is a small fraction of binuclear sites in *Ec*MetAP formed through cooperative binding between the 5- and 6-coordinate Co^{2+} ions. The magnetization behavior of the 6-coordinate Co^{2+} MCD peak is consistent with an isolated pseudo-Kramer's doublet ground state suggesting that the cobalt ions in the binuclear sites are not magnetically coupled.