Thermodynamics of Zinc, Cobalt and Arsenic Binding to the DNA Binding Domain of Glucocorticoid Receptor: The Good, the Bad and the Ugly

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Zinc(II) is essential for many biological functions, serving as a Lewis acid in metalloenzyme catalysis and playing a structural role in many transcription factor proteins. Glucocorticoid receptor (GR) is a member of the nuclear hormone receptor superfamily of transcription factors and plays a key role in hormone-regulated transcription of genes that are important for development and other biological processes. The DNA-binding domain (DBD) of GR is highly conserved among members of this superfamily and contains two structural Zn^{2+} binding sites with Cys_4 coordination. Because d^{10} Zn^{2+} is spectroscopically silent, it has been difficult to investigate its binding to GR or other proteins directly; however, Co^{2+}, which has similar coordination properties and coordination-dependent ligand field spectra, has been used as a probe for Zn^{2+} binding sites in metalloproteins.

In this study, the thermodynamics of Zn^{2+} and Co^{2+} binding to the DBD of GR and related peptides have been determined directly by isothermal titration calorimetry (ITC), a method that measures the heat flow during a binding titration. Analysis of ITC data, like that shown below for Co^{2+} titration into apo-GR-DBD, has provided thermodynamic values that can now be compared to spectroscopically-determined metal affinities and address the controversial question of cooperativity in Zn^{2+} binding to the DBD of nuclear hormone receptors. In addition, biological evidence exists for As^{3+} effects on GR-dependent gene expression, which may originate from the affinity of As^{3+} or methylated arsenic species for Cys thiols in target proteins, such as GR. Therefore, As^{3+} binding to GR-DBD, both in the absence and in the presence of Zn^{2+}, has also been quantified.