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**13. ABSTRACT** *(Maximum 200 words)*

Specific metal ions bind to particular sites within proteins. The factors that might influence the thermodymanics of metal ion binding to proteins include metal ion radius, hard-soft acid-base effects, and ligand field stabilzation energy changes. We have probed the contributions of these effects through the use of a series of peptides based on naturally occuring "zinc finger" domains. The metal ion binding sites studied include $Cys_2His_2$, $Cys_3His$, $Cys_4$, and $Cys_2His(X)$ where X = $OH_2$, $Cl^-$, N-methylimdazole, and $^-SCH_2CH_2OH$. Metal ions tested included Zn(II), Co(II), Cd(II), Fe(II), Ni(II), and Mn(II). We found that ligand field stabilization energy changes quantitatively account for preferences for Zn(II) over Co(II). For Cd(II), hard-soft acid-base effects are dominant with a greater than 100-fold increase in affinity for Cd(II) over Zn(II) for each Cys for His replacement. For other metal ions, multiple factors clearly contribute. These studies provide components for a rational basis for the design of specific metal binding sites for biosensors and other applications.

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N00014-90-J-1700

# Ligand Field Stabization Energy Control of Metal Ion Binding

## (a) Summary

The goal of this project has been to investigate the factors that contribute to metal ion binding specificity to peptides. The studies have been performed using a series of derivatives of a consensus zinc finger peptide. The parent peptide has the sequence ProTyrLys**Cys**ProGlu**Cys**GlyLysSerPheSerGlnLys-AspSerLeuValLys**His**GlnArgThr**His**ThrGly where the residues involved in direct interactions with the metal ions are shown in boldface. Our studies have led to three major results described in separate publications.

(i) Electronic effects can account for the metal binding binding preferences for selected metal ions. Thus, the preferences of a series of peptides with tetrahedral binding sites of the form $Cys_nHis_{4-n}$ for zinc(II) over cobalt(II) can be accounted for in terms of changes in ligand field stablization energy accompanying the transition from an octahedral site in aqueous solution to a tetrahedral site within a peptide. In addition, for this same series of peptides, the affinity for cadmium(II) markedly increases as the number of cysteine ligands increases. This result can be rationalized in terms of hard-soft acid-base effects.

Beth Allyn Krizek, Denise L. Merkle, and Jeremy M. Berg, "Ligand Variation and Metal Ion Binding Specificity in Zinc F ıger Peptides", Inorganic Chemistry, 32, 937-940 (1993).

(ii) These zinc finger peptides will bind other metal ions including iron(II), nickel(II), and manganese(II) to produce paramagnetic species with distorted tetrahedral geometries. For these metal ions, metal binding specificity is not as easily rationalized in a quantitative sense. Thus, for manganese(II), the binding affinity is much lower than that expected based on ligand field stabilization energy effects. The strong preference of this ion for oxygen rather than nitrogen or sulfur ligands predominates in this case. For nickel(II), the affinity is much higher than that expected from ligand field stabilization energy effects. This is presumably due to the fact that small distortions of the metal binding site away from tetrahedral geometry can significantly reduce the ligand field stabilzation energy penalty upon binding.

Beth Allyn Krizek and Jeremy M. Berg, "Complexes of Zinc Finger Peptides with $Ni^{2+}$ and $Fe^{2+}$, Inorganic Chemistry, 31, 2984-2986 (1992).

(iii) A truncated peptide that lacked one of the normal metal binding residues was prepared. This peptide binds metal ions with one coordination site available for the binding of exogenous ligands such as water, chloride, imidazole, or thiolates. The binding affinities of these monodentate ligands were determined and were found to be in the millimolar range. Attempts to

use these peptide-metal complexes as catalysts for model reactions executed by zinc-containing enzymes were not successful.

Denise L. Merkle, Michael H. Schmidt, and Jeremy M. Berg, "Design and Characterization of a Ligand-Binding Metallopeptide", Journal of the American Chemical Society, 113, 5450-5451 (1991).

(b) Publications

(1) Denise L. Merkle, Michael H. Schmidt, and Jeremy M. Berg, "Design and Characterization of a Ligand-Binding Metallopeptide", Journal of the American Chemical Society, 113, 5450-5451 (1991).

(2) Beth Allyn Krizek and Jeremy M. Berg, "Complexes of Zinc Finger Peptides with $Ni^{2+}$ and $Fe^{2+}$, Inorganic Chemistry, 31, 2984-2986 (1992).

(3) Beth Allyn Krizek, Denise L. Merkle, and Jeremy M. Berg, "Ligand Variation and Metal Ion Binding Specificity in Zinc Finger Peptides", Inorganic Chemistry, 32, 937-940 (1993).

(c) Patents

None

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