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# The thermodynamics of protein interactions with essential first row transition metals☆

Fadi Bou-Abdallah *, Thomas R. Giffune

*State University of New York at Potsdam, Potsdam, NY 13676, United States*

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ABSTRACT

*Background:* The binding of metal ions to proteins is a crucial process required for their catalytic activity, structural stability and/or functional regulation. Isothermal titration calorimetry provides a wealth of fundamental information which when combined with structural data allow for a much deeper understanding of the underlying molecular mechanism.
*Scope of review:* A rigorous understanding of any molecular interaction requires in part an in-depth quantification of its thermodynamic properties. Here, we provide an overview of recent studies that have used ITC to quantify the interaction of essential first row transition metals with relevant proteins and highlight major findings from these thermodynamic studies.
*General significance:* The thermodynamic characterization of metal ion–protein interactions is one important step to understanding the role that metal ions play in living systems. Such characterization has important implications not only to elucidating proteins' structure-function relationships and biological properties but also in the biotechnology sector, medicine and drug design particularly since a number of metal ions are involved in several neurodegenerative diseases.
*Major conclusions:* Isothermal titration calorimetry measurements can provide complete thermodynamic profiles of any molecular interaction through the simultaneous determination of the reaction binding stoichiometry, binding affinity as well as the enthalpic and entropic contributions to the free energy change thus enabling a more in-depth understanding of the nature of these interactions. This article is part of a Special Issue entitled Microcalorimetry in the BioSciences — Principles and Applications, edited by Fadi Bou-Abdallah.


## 1. Introduction

Metalloproteins represent one of the most diverse classes of proteins that play important roles in key biological processes from DNA synthesis to energy production. Approximately one third of all proteins have a bound metal and almost half of all enzymes require the presence of a specific metal ion to function (1,2,3). The binding of metal ions to proteins is a crucial process required for the protein's catalytic activity, structural stability and functional regulation. Metal coordination by proteins essentially occurs via the proteins' side chain functional groups which include histidine, methionine, cysteine, tyrosine, aspartic and glutamic acids. Amino acids with simple hydroxo or amino functions, such as serine, threonine, lysine and tryptophan are less well suited for metal-ion coordination.

While the affinity and selectivity of a protein to a particular metal ion is the result of unique protein features in addition to metal coordination chemistry, metalloproteins are dynamic molecules and incorporation of a correct metal ion is rather a question of biology. According to the Irving-Williams series (4), the affinity of divalent metal ions to ligands follows the order of $Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} < Zn^{2+}$ but holds generally well for metal binding sites in proteins although deviations from this affinity trend can occur (5). In animals and humans, $Mn^{2+}$ and $Fe^{2+}$ bind rather weakly to proteins whereas tighter binding is often observed with $Cu^{2+}$ and $Zn^{2+}$ although redox-active metal ions (i.e. Mn, Fe, Cu, Ni) can modulate binding affinities. The undisputable role of metal ions in the folding and mis-folding of proteins and the interplay between metal homeostasis and protein dynamics, structural and conformational changes is illustrated by their relevance in numerous neurodegenerative diseases including Menke's syndrome, Wilson's disease, Alzheimer's disease and Parkinson's disease. These diseases are characterized by elevated levels and mis-compartmentalization of either iron, copper or zinc or a com'bination of them (6). The focus of this review concerns the thermodynamics of protein interactions with the first row transition metals or the d-block elements which occupy the central block of the periodic table. Of those elements, only the most essential metal ions in humans (i.e. V, Mn, Fe, Co, Ni, Cu, and Zn) will be considered. The occurrence of these trace metals and some of the essential functions and symptoms associated with their deficiency and overdose in humans is shown in Table 1. Because too much or too

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* Corresponding author.
*E-mail address:* bouabdf@potsdam.edu (F. Bou-Abdallah).

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**Table 1**
Essential transition metal occurrence in humans with important functions and characteristic symptoms (7,8,9).

| Element | Fraction of total body mass | Mass (for a 70 kg adult) | Functions | Deficiency symptoms | High exposure symptoms |
|---|---|---|---|---|---|
| Vanadium (V) | 2 ppb | 0.14 mg | Insulin-like effects, halo-peroxidases in seaweeds, electron redox mediator | Impaired metabolism and/or growth | Severe eye, nose and throat irritation |
| Manganese (Mn) | 0.2 ppm | 14 mg | Glucose metabolism, vitamin B1 function, Photosystem II complexes | Infertility, impaired skeletal growth | Fatigue, anorexia, impotence |
| Iron (Fe) | 60 ppm | 4.2 g | DNA synthesis, oxygen transport | Anemia, immune system disorders, fatigue, impaired mental development | Iron overload, organs damage |
| Cobalt (Co) | 20 ppb | 1.4 mg | Vitamin B12 function | Pernicious anemia | Dermatitis, thyroid and heart damage, suspected carcinogenic |
| Nickel (Ni) | 0.2 ppm | 14 mg | Growth, hydrogenases | Impaired growth/depression | Dermatitis, lung and nasal cancer |
| Copper (Cu) | 1 ppm | 70 mg | cytochrome oxidases, superoxide dismutases | Secondary anemia, liver disorder, energy deficiency, inadequate oxygen transport | Liver cirrhosis (in enfant), death |
| Zinc (Zn) | 33 ppm | 2.3 mg | Metabolic conversion, growth, development and fertility, enzymes and transcription factors | Low sperm count, impaired immune system, lack of appetite | Fume fever, vomiting, stomach cramps, diarrhea, skin irritant |

little is often harmful and even lethal, the concentrations and distributions of metals are tightly regulated by proteins and enzymes in cells so as to provide appropriate amount of metal ions while preventing toxicity.

Isothermal titration calorimetry (ITC) has been widely used to study the thermodynamics of metal ion–protein interactions. Like with most binding events, enthalpic (ΔH) and entropic (ΔS) changes provide insights into the structure and dynamics of this recognition event. Thus, unraveling the thermodynamic driving forces that govern the interactions of metal ions with proteins is critical to understanding metal trafficking pathways, metal homeostasis and metal detoxification. This review briefly discusses the chemical and biochemical properties of essential first row transition metals and then provides a thermodynamic quantification (binding stoichiometry, binding affinity and enthalpic and entropic contributions to the binding free energy) of their interactions with representative proteins using isothermal titration calorimetry. A thorough and comprehensive review paper by N. Grossoeheme on metal-based ITC experiments highlighting common complications and solutions to unavoidable metal ions solution chemistry and different linked equilibria is included in this special volume and will not be revisited here. In that study, the author reviewed ITC experimental concerns and pitfalls and offered guidance to scientists wishing to perform ITC titrations that involve metal ions.

## 2. Chemical, biochemical and binding properties of essential first row transition metals

### *2.1. Zinc (Zn)*

Zinc is the second most abundant trace element in the human body, following iron, and is generally thought of as non-toxic. It is an essential element for humans and animals and required growth, development and fertility. It is also important in digestion, nucleic acid synthesis and function of the immune system. It is known to activate parts of the brain that govern taste and smell and has been proposed to have therapeutic benefits against infectious diseases including a shortening of the common cold in humans. Although a trace element, it is hard to underestimate the impact of zinc on human physiology given that an estimated 10% of the human genome encodes zinc proteins amounting to at least 3000 proteins (10). There are several hundred kinds of zinc-containing enzymes where zinc plays a catalytic and/or a structural role (8–10). The six fundamental classes of enzymes where zinc is found include oxidoreductases, hydrolases, transferases, lyases, ligases, and isomerases. These enzymes catalyze the metabolic conversion or degradation of proteins, nucleic acids, lipids, porphyrin precursors and other bioorganic compounds. Additionally, there exist numerous superfamilies of zinc finger domains which are ubiquitous structural elements known to bind DNA, RNA and protein whereby zinc plays a critical role in the regulation of the transcription and translation of the genetic message (8–10).

From a chemical point of view, in the active sites of proteins and enzymes, zinc is usually a Lewis acid that lowers the pK of coordinated water (from about 10 for free $[Zn(H_2O)_6]^{2+}$ to less than 7 in enzymatic systems) and/or activates substrates (10,11). Because it is redox inactive, zinc does not have d-d transitions (therefore no absorption spectroscopy) and lacks the ligand field stabilization phenomenon which means that zinc can, in principle, adopt flexible coordination number and geometry (from tetrahedral to distorted trigonal bipyramidal) that is dictated by the size and charge of its ligands. Two examples of zinc interaction with small peptides (i.e. zinc-fingers) and proteins (i.e. human carbonic anhydrase), whereby zinc serves a structural role in one instance and a catalytic role in the other, will be discussed in this section.

Zinc finger transcription factors are one of the largest classes of eukaryotic proteins that require one or more $Zn^{2+}$ ions to stabilize a protein structure. They are generally recognized for their DNA/RNA binding ability and use different combinations of Cys and His residues to coordinate the zinc ions (12). Zinc finger proteins are composed of several modular zinc finger domains, each containing a single zinc-binding site that recognizes a specific base pair segment of DNA (or RNA). Zinc fingers are typically unfolded in the absence of zinc (apo state) and folded into biologically active three-dimensional structures upon Zn binding (13–15). The coordination of the zinc ion to the cysteine and histidine residues is essential to drive the folding process of the zinc finger structure which otherwise would not be obtained if these ligands were switched within the sequence (16). Carbonic anhydrases (CAs) are a family of zinc dependent metalloenzymes that catalyze the hydrolysis of carbon dioxide to bicarbonate ion. Depending on the class type (α, β or γ), the catalytic zinc ions in CAs are bound by either three histidine residues in the α- and γ-classes or by one histidine and two cysteine residues in the β-class (17–18). Interestingly, zinc fingers and carbon anhydrases represent the extremes of metalloprotein design in terms of protein-folding thermodynamics due to the fact that the former are unfolded while the latter are folded in the absence of zinc. In the case of carbonic anhydrase, the measured zinc ion binding free energy represents the actual free energy of metal-ligand binding because the protein is already in the folded state in the absence of zinc (i.e. binding of zinc occurs between the apo-folded state and the holo-folded state). With zinc fingers, the apo-protein is unfolded which means that the apo-folded protein has a higher energy (i.e. a positive free energy cost to protein folding). Thus, the actual free energy of metal ion binding is the sum of the measured thermodynamic contribution of zinc binding plus the free energy due to protein folding.

#### *2.1.1. ITC of zinc binding to zinc finger domains*

As reported earlier, zinc plays a major role in stabilizing protein structures and the best known example is the $Cys_2His_2$ zinc-finger