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Separative techniques for metalloproteomics require balance between separation and perturbation

Agnès Hagège [a,b,*], Thi Ngoc Suong Huynh [b], Marc Hébrant [c,d]

[a] CNRS, UMR 7265, CEA/DSV/iBEB, 13108 St., Paul Les Durance, France
[b] Laboratoire d'Etude des Protéines Cibles, CEA/DSV/iBEB/SBTN, BP 17171, 30207 Bagnols sur Cèze Cedex, France
[c] LCPME, Université de Lorraine, UMR 7565, 405 rue de Vandoeuvre, 54600 Villers-lès-Nancy, France
[d] CNRS, LCPME, UMR 7565, 405 rue de Vandoeuvre, 54600 Villers-lès-Nancy, France

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ABSTRACT

Metal-binding proteins play a crucial role in biological systems and associated metals display many critical functions, so many analytical tools have been developed to identify metal-protein complexes. However, identification of metal-protein complexes remains challenging since it addresses non-covalently bound species that may dissociate during analysis, due to their separation from their medium or to the interactions implicit in the separation. Many strategies, usually involving separation techniques coupled with elemental detection, have been investigated to overcome technical issues that hamper the identification and the characterization of metal-protein complexes. This review focuses on the separation techniques used in this field and their impact on dissociation of metal-protein complexes. We discuss metal losses induced by both the separation system and disequilibrium effects.

Contents

1. Introduction

It has been estimated that more than one third of proteins are associated with metals in living organisms [1] and their identification is a key step for elucidation of the metal-dependent biological mechanisms.

Metals, when involved in proteins, display many critical functions (structural, regulatory or functional). Multi-electron reactions or oxygen transport often require metals that undergo redox changes (e.g., Cu and Fe). Other processes, such as hydrolytic reactions, are realized in the presence of metalloproteins where the metal acts as a Lewis acid catalyst (e.g., Zn). Metals may also contribute to the three-dimensional structure of proteins, which is critical for

Abbreviations: AN-PAGE, Anodal native polyacrylamide gel electrophoresis; BN-PAGE, Blue native polyacrylamide gel electrophoresis; BSA, Bovine serum albumin; CA, Carbonic anhydrase; CE, Capillary electrophoresis; DCT1, Divalent cation transporter 1; ESI/MS, Electrospray ionization/mass spectrometry; HILIC, Hydrophilic interaction liquid chromatography; HSA, Human serum albumin; HSAB, Hard and soft acids and bases principle; ICP-AES, Inductively coupled plasma/atomic emission spectroscopy; ICP-MS, Inductively coupled plasma/mass spectrometry; IEC, Ion-exchange chromatography; IEF, Isolelectric focusing; IPG, Immobilized pH gradient; LA, Laser ablation; PAGE, Polyacrylamide gel electrophoresis; PIXE, Particle-induced X-ray emission; RPLC, Reversed-phase liquid chromatography; SDS, Sodium dodecylsulfate; SEC, Size-exclusion chromatography; SOD, Superoxide dismutase; Tf, Transferrin; UHPLC, Ultra-high-performance liquid chromatography.

* Corresponding author. Tel.: +33 (0) 4 66 79 19 88; Fax: +33 (0) 4 66 79 19 05.
E-mail address: agnes.hagege@cea.fr (A. Hagège).

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substrate recognition. A well-known example is the role of Zn in Zn fingers, whose geometry allows the formation of folded parts of proteins that are essential for recognition by DNA. As a consequence, besides these proteins, a large number of proteins are devoted to the uptake, the transport and the storage of metals. All these proteins play a crucial role in biological systems, so the binding of an unexpected metal can have dramatic consequences. While *in-vivo* metal misincorporation in an enzyme site appears to occur rarely [2], the transport of undesirable metals seems to be reported more often [3,4].

Metal trafficking is a very important function for living organisms. How a protein selects the right metal, especially a weakly-binding one, is indeed the result of highly controlled processes.

Coordination chemistry is of course central to understand this selection [5,6]. It obviously depends on the intrinsic properties of the metal (i.e., charge, ion radius, electronic structure and the resulting Lewis acid character). Metals are classified as hard, borderline and soft acids (Fig. 1) according to their polarizability, radius, and oxidation state. As predicted by the hard and soft acids and bases principle (HSAB) developed by Pearson [7], hard acids prefer hard bases and soft acids prefer soft bases. The affinity of a hard acid for a hard base is mostly electrostatic, whereas the interaction between a soft acid and soft base is mostly covalent.

For transition metals, the Irving–Williams series ($Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} < Zn^{2+}$), which describes the selectivity sequence, irrespective of the nature of the coordinated ligand, is largely evoked to explain the affinities of proteins for divalent metals [8]. This statement is often illustrated by the complex formation with 1,2-diaminoethane, essentially due to electrostatic effects and variations of ligand-field-stabilization energy [9].

Selectivity also depends on the protein-binding site, as largely exemplified by Dudev and Lim [10]. Selectivity is affected by numerous factors, such as binding site geometry, nature of the binding ligands, and localization (solvent exposed or buried sites). Moreover, as in the well-known case of the Fe^{3+}-transferrin complex stabilized by carbonate ions [11], some metal-protein complexes are known to imply more than two partners.

However, in many cases, a protein alone is unable to select the right metal and prevent adventitious displacements by other metals. It has been pointed out that selectivity cannot be explained only by binding constants and coordination geometries but is also achieved by the fine control of metal availability [12]. Mechanisms used by cells to regulate metal concentrations have been discussed by several authors [3,13–17]. The free-metal concentration is adapted through the action of both metal transporters and storage proteins. These proteins are regulated in turn by metal sensors controlling the transcription of their coding genes.

Moreover, in some cases, the selectivity of metal insertion can be enhanced by protein-protein interactions (e.g., in ligand-exchange reactions involving chaperones) [18].

Apart from disorders caused by essential metals, such as copper in Menkes and Wilson diseases, interactions of metal ions with proteins have also been studied in the context of increasing metal exposure due to anthropogenic activities. Essential metal-transport proteins, such as DCT1, may be used by toxic metals to enter cells [4]. Other proteins, usually not devoted to receiving metals, have the capacity to bind metal ions (e.g., to cysteine-containing sites, leading to an inhibition of their activity). As an example, the inhibition of DNA-decatenation activity of topoisomerase II was recently attributed to cadmium binding to cysteines [19]. These issues were recently reviewed by Ba et al. [3].

More recently, metal-containing compounds emerged in medicine, not only as drugs (e.g., compounds for chemotherapy based on Ru or Pt) but also in medical imaging (e.g., gadolinium complexes used as contrast agents in magnetic resonance imaging), as recently reviewed [20].

Interactions of proteins with metallodrugs (e.g., based on Pt or Ru) have been investigated to provide information about the transport route or deactivation pathways for these drugs. The importance of such studies in the design of "next generation" metal-based drugs, targeting a specific pathway was even mentioned by Groessl and Hartinger [21].

Metal speciation in biological media can be regarded as the result of numerous competitive reactions involving not only proteins but also small ligands (e.g., carbonate, citrate, phosphate, and lipids) and other essential metals (Fig. 2). This statement has opened a new field of investigation, dedicated to metals associated with proteins, also called metalloproteomics. In other words, metalloproteomics should be understood as the description of the whole set of metal-containing proteins of an organism at a given time.

Both bottom-up and top-down strategies have been used in the metalloproteomics field [22]. In bottom-up strategies, analysis of both metal content and function of metalloproteins is conducted after a purification step. Several approaches have been considered for the determination of metal-protein interactions from the point of view of structure, thermodynamics or kinetics. The methods often involve the use of UV and fluorescence spectroscopies or

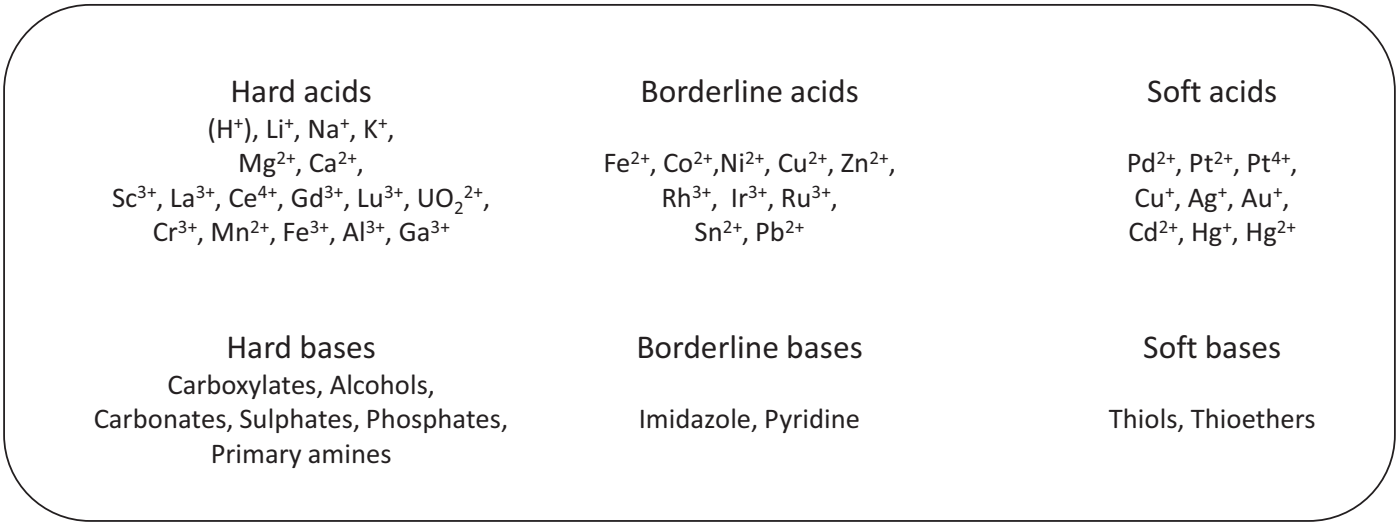

Fig. 1. Classification of common metals and ligands according to the hard and soft acids and bases principle (HSAB).