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TRENDS

Joanna Szpunar

Metallomics: a new frontier in analytical chemistry

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Introduction

Metal ions are utilised by biological systems in fundamental processes such as signalling, gene expression, and catalysis. The absence of some metals results in disease, whereas the presence of others has frequently been evoked in the context of carcino- (As, Cr, Pt), immuno- (Au, Co, Cr, Ni, Pt), embryo-/terato- (Hg), spermio- (Cd, Pb, Tl), nephro- (Cd, U) or neurotoxicity (Al, Hg, Mn). The essential transition metals (e.g. Cu, Fe, Se, Zn) are used by cells as cofactors of enzymes, but they can also catalyze cytotoxic reactions [1]. The molecular bases of many of the metal-dependent biochemical processes remain elusive. The mechanisms by which the metal is sensed, stored or incorporated as a cofactor in a cell are often unknown [2].

The function of a metal is determined by its bioligand environment. Intracellular metals are available in a cytosolic pool in the micromolar or picomolar concentration range. They contribute to protein stability through their presence in the centre of large molecules, often with enzymatic activity (metalloenzymes) [3]. The activity of intracellular metal ions is controlled by several families of proteins, either detoxifying, protecting or simply involved in cell cycle, proliferation and apoptosis [1]. The availability of the complete sequence of several genomes offers unprecedented opportunities to identify all metalloproteins and their enzymatic metabolites and to allow a comprehensive look at the role of essential and toxic metals in health and disease.

J. Szpunar (✉)
Equipe de Chimie Analytique Bio-inorganique,
CNRS UMR 5034,
Hélioparc, 2, av. Pr. Angot, 64053 Pau, France
e-mail: joanna.szpunar@univ-pau.fr

Concepts of metallome and metallomics

An organism must regulate transcription, translation, proper assimilation and incorporation of the necessary metal to function. Therefore, the chemistry of a cell needs to be characterized not only by its characteristic genome in the nucleus and a protein content, a proteome, but also by the distribution of the metals and metalloids among the different species and cell compartments, *metallome*. The latter term would therefore refer to the entirety of individual metal species in a cell and encompass the inorganic (free and complexed) element content and the ensemble of its complexes with biomolecules, and especially with proteins, *metalloproteome*. This definition of the metallome: the entirety of metal and metalloid species within a cell or tissue type [4], seems to be a much better analogy with genome and proteome than the reference to the "free metal content of a cell" only, as proposed elsewhere [2].

Recently, Haraguchi and Matsura suggested the term "metallomics" to denote metal-assisted function biochemistry and postulated it to be considered at the same level of scientific significance as genomics or proteomics [5]. The metallomic information will comprise the identities of the individual metal species (*qualitative metallomics*) and their concentrations (*quantitative metallomics*). As such, metallomics can be considered as a subset (referring to cellular biochemistry) of speciation analysis understood as the identification and/or quantification of elemental species [6]. Species of interest for metallomics will include complexes of trace elements and their compounds (e.g. metal probes) with endogenous or bio-induced biomolecules such as organic acids, proteins, sugars or DNA fragments [7].

Deciphering a metallome will thus inform us of: (i) how an element (metal or metalloid) is distributed among the cellular compartments of a given cell type, (ii) its coordination environment; in which biomolecule it is incorporated or by which bioligand it is complexed, and (iii) the concentrations of the individual metal species present. Monitoring the changes of the metallome as a function of time and exposure to external stimuli can be referred to as *comparative metallomics*.

Analytical techniques for metallomics

The analytical challenges to metallomics include reaching a specificity of analytical response with regard to a particular element species, and sensitivity allowing an analysis to be carried out with microsample volumes (several picolitres or several thousands individual cells equivalent of a vacuole volume, or of a human biopsy, respectively) [7]. The linchpin analytical technique for metallomics appears to be inductively coupled plasma mass spectrometry (ICP MS) applied as a detector in chromatography or electrophoresis (capillary or gel). Its incontestable advantage is the capability of discrimination (with the isotopic resolution) between metal (heteroelement)-containing species and metal-free species in an HPLC or capillary electrophoresis effluent or, via laser ablation, in a two-dimensional gel spot.

ICP MS has long been used for the on-line detection of metals in chromatography, usually size-exclusion and ion-exchange, of biological cytosols [8, 9]. The selectivity of these approaches has been very low and the peaks observed corresponded to metal-containing fractions defined by specific criteria (size, charge, hydrophobicity), rather than to individual species. Such approaches, termed as fractionation according to IUPAC [6], should not be referred to as metallomics or metalloproteomics, but rather as sample preparation procedures for the latter.

The recent key developments in ICP MS of potential significance for metallomics have included:

i) the advent of collision/reaction cell instruments, extending the measurable elements to phosphorous and sulphur, and improving the isotope ratio measurements of such vital elements as selenium, chromium, vanadium and iron [10],

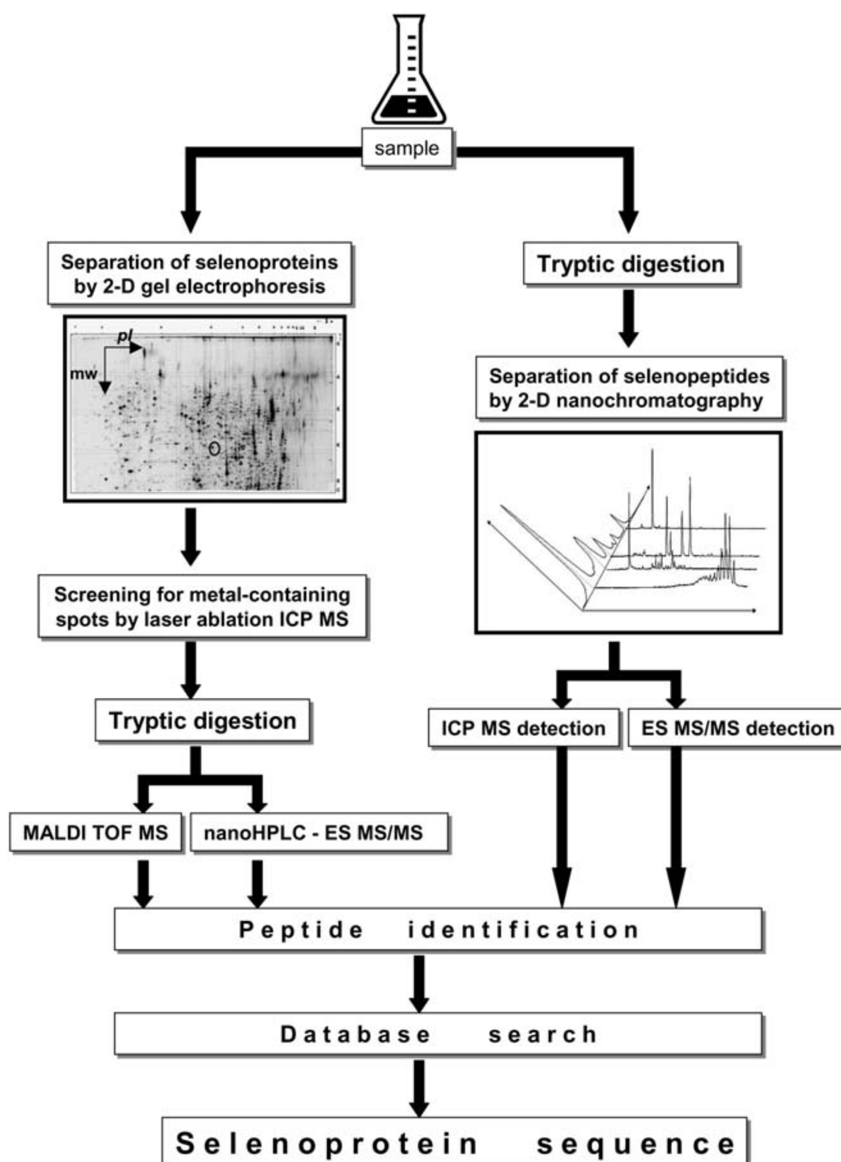

Fig. 1 A generic strategy for metallomics exemplified for selenoproteomics

(ii) the development of interfaces allowing the introduction of effluents from capillary (300-μm i.d.) and soon from nanocapillary (<150-μm i.d.) columns via a direct injection or total consumption nebulizer resulting in subfemtogram detection limits in dry plasma conditions [11],

(iii) scanning two-dimensional gels for the presence of metal-containing spots by laser ablation ICP MS [12] offering a cheaper alternative to synchrotron radiation XRF [13] for this purpose.

In parallel to detection of metal species, techniques for the identification of the protein ligands are rapidly progressing. This concerns in particular high-throughput proteomics approaches based on two-dimensional gel electrophoresis, in situ tryptic digestion of spots for MALDI TOF MS fingerprinting of peptides followed, if necessary, by nanoelectrospray MS/MS sequencing [14].

A generic strategy for metallomics is exemplified in Fig. 1 for selenoproteomics**.** A cytosol sample is submitted, by analogy to proteomics, to a two-dimensional SDS gel which is scanned for the presence of selenium by laser ablation ICP MS. The Se-containing spots are excised, tryptically digested and submitted to peptide mapping by MALDI TOF MS and HPLC–electrospray tandem MS prior to database searching for protein sequence. The success of this approach is critically dependent on the stability of the initial metal–protein complex. However, whereas selenoproteins and selenium-containing proteins in which selenium is covalently bound in selenocysteine and selenomethionine, respectively, are relatively stable, metal–protein complexes may dissociate in the presence of SDS. Therefore, separations in non-denaturating conditions (often at the expense of resolution) should be considered. An alternative may be an approach based on two-dimensional PAGE of a demetalated proteome followed by a high-throughput screening of the reactivity of the isolated protein spots with selected metals (e.g. by capillary electrophoresis–ICP MS) to identify the targets for structural analysis.

An alternative to two-dimensional PAGE which is particularly useful for smaller genetically encoded proteins, such as metallothioneins, metal probes (e.g. anti-cancer drugs) or enzymatic metabolites (e.g. phytochelatin) is multidimensional HPLC [15]. The identification of the metallocompounds can be carried out directly on-line, or after demetalation, by electrospray MS/MS. In the example shown in Fig. 1, a mixture of selenopeptides produced by tryptic digestion of selenoproteins (without their prior separation) is resolved by two-dimensional nanoflow HPLC. The selenium-containing peaks, selected owing to the ICP MS detection, are identified by ES MS/MS. Multidimensional HPLC offers a still unexplored potential for the separation of metal complexes with high molecular weight proteins as an alternative to two-dimensional gel electrophoresis.

Validation of metallomic data requires the demonstration of their being linked to the genome and the proteome of a cell. This issue was recently addressed by combining in vivo bioanalytical data with in vitro molecular genetic data [16]. A complementation screen on a toxic nickel medium allowed the cloning of a gene responsible for metal transport in a hyperaccumulating plant. This allowed the reproduction in yeast of the synthesis of the relevant enzyme (nicotianamine synthase) and the metabolite (nicotianamine) identified earlier in vivo by a metallomics approach. The wider use of molecular biology methods is expected to complement the analytical metallomics and lead to an understanding of metalloprotein functions at the molecular level.

The identification of metals in structural genomics targets is a burgeoning research field which can largely benefit from recent advances in analytical chemistry. To date, effort has been widely dissipated across disciplines, in groups which often do not communicate. The current advances in hyphenated techniques [7] and cross-fertilization with other "-omics" sectors [17] allow a holistic approach to the metal speciation in individual cells or cell types. A complete fingerprint of the metal-binding components in a cell is likely to provide a new insight into the role of metal ions in biochemistry.

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