Pure Appl. Chem., Vol. 80, No. 12, pp. 2565–2575, 2008.
doi:10.1351/pac200880122565

Challenges to metallomics and analytical chemistry solutions*

Sandra Mounicou[1] and Ryszard Lobinski[1,2,‡]

[1]Laboratory of Analytical, Bioinorganic, and Environmental Chemistry, CNRS UMR 5254, Hélioparc, 2, av. Pr. Angot, F-64053 Pau, France; [2]Department of Analytical Chemistry, Warsaw University of Technology, ul. Noakowskiego 3, 00-664 Warsaw, Poland

Abstract: Metal ions play a fundamental role in the chemistry of life. The understanding of the molecular bases of the living process requires the knowledge of the correlations existing between metal ions and the genome and the derived *-omes*: transcriptome, proteome, and metabolome. An indispensable step on this way is the characterization of the coordination environment of metal ions present and the identification and quantification of metal-containing chemical species. The ensemble of research activities related to metal ions in biological systems has been recently referred to as "metallomics" [1]. The progress in this field is largely dependent on the high-throughput acquisition of multielement and -species analytical data in biological samples. The paper gives a brief overview of the state of the art of analytical techniques and methods for the multielement quantitative analysis of biological microsamples, and for the detection, identification, and quantitation of metal-containing proteins and low-molecular-weight metabolites. The potential contribution of molecular biology techniques in terms of linking information on metals and metal-species to the genome of an organism is highlighted.

Keywords: metallomics; metalloprotein; analytical chemistry; mass spectrometry; coupled techniques.

INTRODUCTION

The second half of the 20th century was rich in achievements on the way to the understanding of the molecular mechanisms of the chemistry of life. The discovery of the structure of DNA [2], the deciphering of the amino acid coding mechanism [3], the first sequencing of the entire genome (*Haemophilus influenzae*) [4], and the completion of the human genome project [5,6] are just a few examples from a long list of ground-breaking discoveries. Whereas the knowledge of the genome of an organism allows the prediction of the entirety of the primary sequences of proteins that can be expressed, it does not say anything about which proteins are expressed nor what their functions are.

The function of one-third of the existing proteins is believed to depend on their interaction with a transition metal, such as, e.g., Cu, Fe, Zn, or Mo [7]. In this context, information about how an organism adjusts its post-translational machinery to manufacture metal-containing active sites as a function of environmental signals and stress is becoming crucial. Consequently, the chemistry of a cell needs

*Paper based on a presentation at the International Symposium on Metallomics 2007 (ISM 2007), 28 November–1 December 2007, Nagoya, Japan. Other presentations are published in this issue, pp. 2565–2750.

‡Corresponding author

to be characterized not only by its characteristic genome in the nucleus and the set of proteins present (proteome), but also by the distribution of the metals and metalloids among the different species and cell compartments, metallome.

The term "metallome" was first coined by Williams who referred to it as an element distribution, equilibrium concentrations of free metal ions, or as a free element content in a cellular compartment, cell, or organism [8]. The meaning of the term "metallome" was then proposed to be extended to the entirety of metal and metalloid species present in a cell or tissue type [9]. The research field dealing with the study of metallomes and their correlations with genomes and proteomes was referred to as metallomics [1]. Since then, metallomics has been the topic of a number of reviews [10,11] and journal special issues [12,13]. The terms "metallome" and "metallomics" have not only been used in different contexts, but also a number of related definitions proliferated, such as, for example, ionomics [14,15], hetero-atom tagged proteomics [16], or elementomics [17]. There is undoubtedly a need to clarify the terms and render their use systematic [18], but regardless of the accepted definition, two different approaches to the understanding of the metallome can be distinguished.

One approach is "metallome" to denote a set of element total concentrations in a cell or tissue. The metallome is then considered as a complement of the genome, and the term is an analogy to the genome in the sense that sequencing a genome is a finite problem with a specific endpoint, so is the determination of the trace element pattern in a cell. The term "ionome", coined by Salt et al. [14,15], may be more suitable to be used in this context.

The other way of understanding a metallome is as the set of concentrations of the species of elements present. The species can contain an element bound in a covalent way or as a coordination complex of certain thermodynamic stability and kinetic inertness. The different classes of metallospecies in a biological environment have been presented in Fig. 1. A metallome would therefore be a dynamic con-

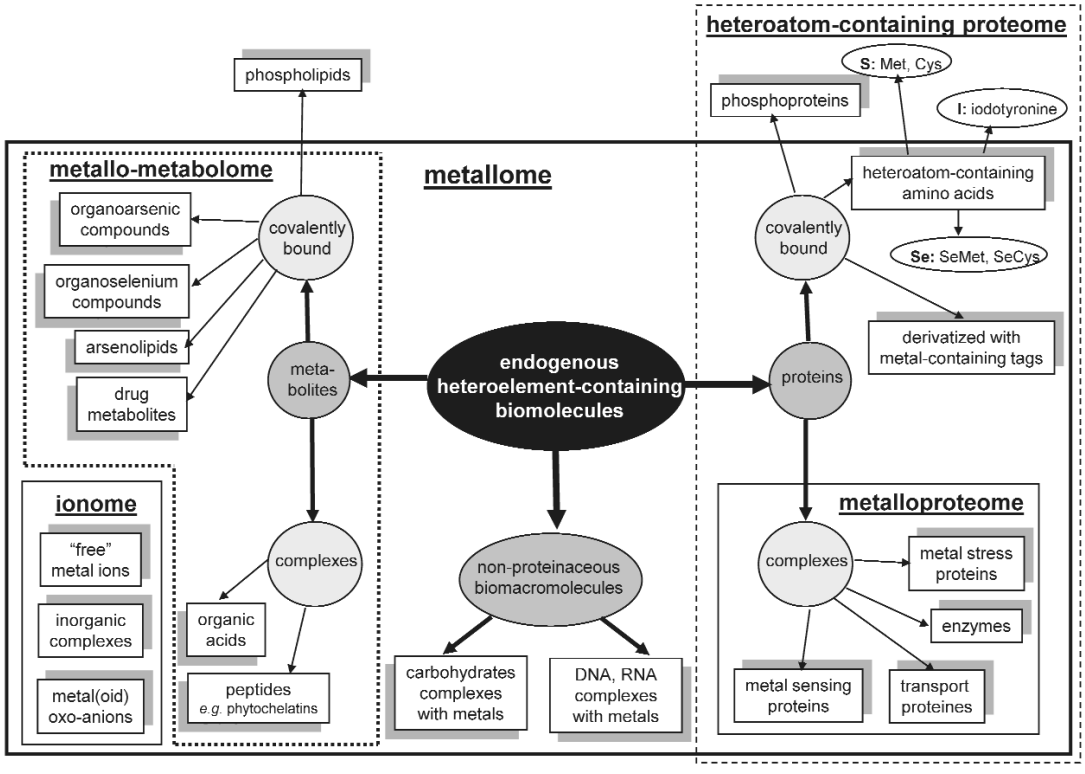

Fig. 1 Metallome chemically speaking: species of analytical interest in biology.

cept and could be determined only with a certain approximation. This would be an analogy to a proteome which responds in biologically important ways to environmental changes with an infinite number of possible variations. As there is no discrete proteome per se, even for simple single-cell organisms, there is no discrete metallome either.

ANALYTICAL TECHNIQUES FOR METALLOMICS

As a function of the adopted understanding of the term "metallome", two analytical approaches can be distinguished for metallomics. In terms of the total analysis, X-ray fluorescence spectrometry (XRF) and instrumental neutron activation analysis (INAA), typically used for the multielemental analysis of biological tissues, are giving way to inductively coupled plasma-mass spectrometry (ICP-MS). ICP-atomic emission spectroscopy (AES) is still the technique of choice for major elements and those for which ICP-MS suffers from isobaric interferences. The techniques are applied for bulk analysis, but the multielemental analysis with micrometer resolution is gaining popularity [19].

The in vivo determination of elemental species is a considerable challenge because of the lack of analytical techniques allowing the direct determination of the chemical forms of elements at the concentrations of concern in a biological matrix. On the other hand, the sheer extraction of the metal species from a biological matrix bears the risk of changing the original form. Therefore, only covalent and thermodynamically stable coordination complexes can be probed. This is ideally achieved with molecular (electrospray) MS but, despite the tremendous progress in terms of sensitivity in recent years, the signal of difficult-to-ionize metal species present at the trace level is severely suppressed in the presence of concomitant easy-to-ionize matrix compounds present at higher levels. Therefore, the probing of element species in biological extracts is usually achieved by a combination of a high-resolution chromatographic or electrophoretic separation technique with a sensitive element-specific detection, referred to as a hyphenated technique [20].

The impossibility of the in vivo speciation raises interest in molecular biology methods (e.g., gene purification, cloning, and heterologous expression) making possible the synthesis of a given metallospecies sufficiently pure and in quantity allowing the probing of the metal coordination environment by X-ray absorption spectroscopy (XAS) [21,22] or electrospray high-resolution MS [23,24].

Multielement analysis of biological samples by ICP-MS

ICP-MS is based on the conversion of all the forms of an element present in a sample into, usually singly charged, element ions which are separated in a mass analyzer and detected. The technique offers many advantages such as femtogram-level detection limits which are practically independent of the chemical from and the presence of matrix components, multielement and -isotopic measurement capacity, and a large dynamic range.

An inconvenience of the standard ICP-MS protocols following the biological sample digestion is considerable sample consumption (ca. 2 mL solution is required for a multielement analysis) which implies the need for at least 50–100 mg of material. This can be a problem in the case of high-throughput screening for metals in proteins in structural genomics. Hence, there is a need for micro- and nanoflow total consumption nebulizers, allowing the acquisition of an elemental fingerprint from as little as 1–5 μl sample. A set-up for a nanoliter flow-injection total consumption analysis was shown to allow a linear response for plutonium in the range below 1 fg with the detection limits in the attogram range [25]. A total consumption nanoflow nebulizer recently developed allows signal acquisition for a few minutes when less than 1 μl of sample is available [26].

The high sensitivity of ICP-MS makes it possible to acquire multielement concentration data in a very small amount of sample as, e.g., obtained using laser ablation (LA) sampling. Consequently, multielement images can be obtained with micrometer resolution as recently demonstrated for brain tissues [27,28]. The recent advances in the near-field LA sampling are harbingers of multielement subcellular

imaging [29]. Nanolocal analysis was shown to be possible in single-shot measurements of elements on biological samples with spatial resolution at the hundreds of nanometers range [29].

Probing metal coordination environment by XAS

XAS is based on the analysis of oscillations in X-ray absorption vs. photon energy that are caused by interference. The spectra are displayed as graphs of the absorption coefficient of a given material vs. energy, typically in a 500–1000 eV range beginning before an absorption edge of one of the elements in the solid. The first part of the spectrum referred to as X-ray near-edge spectroscopy (XANES) is characteristic to the oxidation state of an element, whereas the part of the spectrum at higher energies referred to as extended X-ray absorption fine structure (EXAFS) allows the determination of the coordination number, radial distances, and gives an insight in the type of ligands present. The technique requires an energetic X-ray source and is gaining in popularity owing to the democratization of access to synchrotrons [7].

XAS is an attractive technique for the characterization of the metal oxidation state and coordination environment in the proteins generated by gene purification and cloning in structural genomics studies [7,21]. It is used with increasing success for in situ element speciation in hyperaccumulating plants [30,31], when the element of interest occurs at a high concentration and is preferentially bound by a single ligand. However, the lower is the concentration and the higher is the complexity of speciation of a metal of interest, the smaller is the chance to obtain meaningful information.

In vivo species-specific screening by hyphenated techniques

The high sensitivity and element specificity of ICP-MS make it an attractive detector in chromatography and electrophoresis, these separation techniques conferring the molecular specificity to analysis. As a consequence, a number of hyphenated techniques (summarized in Fig. 2) have been developed; they

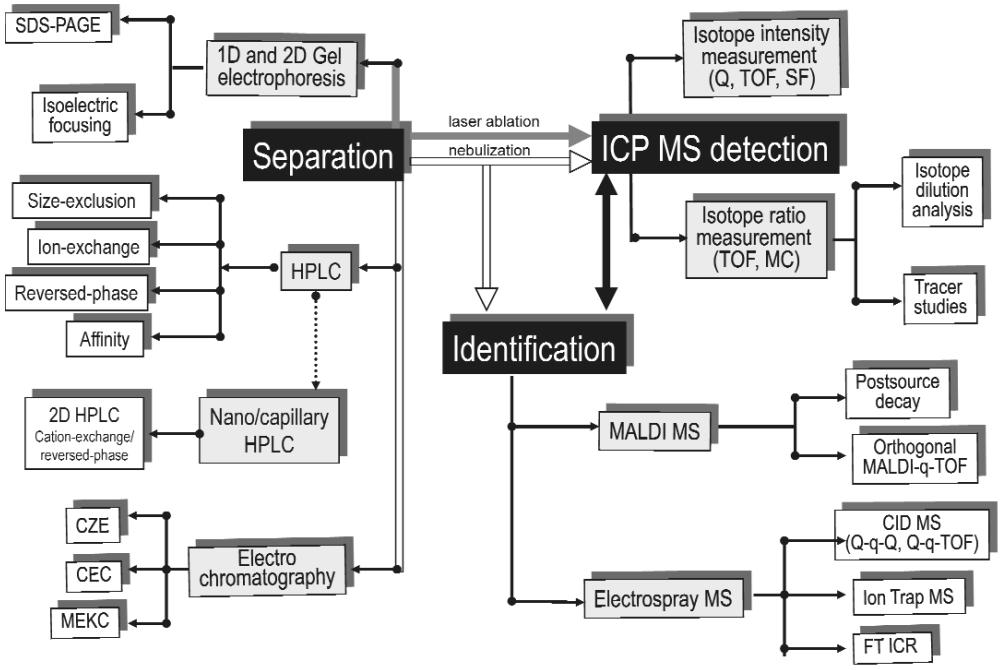

Fig. 2 Hyphenated techniques for metallomics.

allow the monitoring, and sometimes characterization in terms of stoichiometry, of metal complexes in biological cytosols. The critical issue in the developed protocols is the control of the stability of the species of interest on the time scale of the separation.

The coupling of the separation technique of choice with ICP-MS can be realized on-line, as in column chromatography or capillary electrophoresis (CE), or by means of LA sampling, as in isoelectric gel focusing (IEF), 1D sodium dodecyl sulfate (SDS) gel electrophoresis (GE), and 2D GE [20].

High-performance liquid chromatography (HPLC)-ICP-MS coupling

The coupling is carried out by means of a nebulizer operating in the flow range that is optimum for the separation. Nebulizers operating at flow rates ranging from 0.1 to 1000 μL min^{-1} are now available. The presence of salts up to 100 mM is generally not a problem. Organic solvents can be introduced provided that a desolvation system is used and oxygen is added to the plasma to facilitate the combustion.

The concerns of avoiding the metal-ligand denaturation and achieving quantitative recovery make the size-exclusion (SE) liquid chromatography (LC) mechanism the most frequent choice. The polymer stationary phases accept fairly concentrated samples (e.g., 3–5 times diluted cytosol). Elution conditions can usually be found for the quantitative recovery of a number of metallospecies. The size-exclusion chromatography (SEC)-ICP-MS coupling was demonstrated to allow rapid screening and fractionation according to the molecular mass of metal-containing species in biological materials (for reviews, see refs. [10,32]).

The purity of peaks in SEC is usually poor. Even if a single species of a given element is present, thousands of matrix compounds may co-elute. They are transparent to the element specific detector used but render direct electrospray MS, which is essential for identification difficult. Matching the elution volume with a standard cannot be considered as a definitive proof of the species identity because of the small number of theoretical plates in SEC. Therefore, the SEC separation is usually followed by ion-exchange, reversed-phase, or hydrophilic interaction LC prior to electrospray MS. The role of multidimensional chromatography in speciation analysis of metal complexes with biomolecules was discussed [33].

Capillary zone electrophoresis ICP-MS coupling

CE offers a number of incontestable advantages for metal species analysis such as a small sample size (in the nanoliter range) required, high resolution, and the absence of stationary phase, and hence, the possibility of analysis of labile complexes. The development and commercialization of a dedicated CE-ICP-MS interface eliminated most of the problems of the earlier laboratory-made designs [34].

The rapidly developing application areas of CE-ICP-MS include metal-binding studies with pure compounds of biological origin, e.g., recombinant proteins [35,36], the determination of stoichiometry of metal-protein complexes [37–39], and the use of CE for fine (2^{nd} or 3^{rd} dimension) separations of metal-containing fractions isolated by chromatography [40,41].

Gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE), employed either in the monodimensional (IEF blue native, BN, or SDS) or 2D mode (IEF x SDS) is considered the most adequate technique for the separation of proteins. Metal-specific detection in the gels has enjoyed considerable interest for a long time, the principal techniques including autoradiography with its inherent use of radioactive isotopes (e.g., ^{75}Se), synchrotron radiation XRF, and proton-induced X-ray emission (PIXE) with the need for hardly available facilities.

LA-ICP-MS imaging, pioneered by Nielsen et al. [42], offers a competitive alternative for the in situ probing of the protein spots for the presence of metals and metalloids. The technique consists in guiding a laser beam over the gel surface within an electrophoretic lane, from spot to spot, or within a raster. The ablated analytes are swept into the ICP by a continuous stream of argon, and the ions are analyzed by MS. As a result, an electropherogram is obtained in which the quantity of a given element is a function of its position in the gel. Detection by LA-ICP-MS is a potentially fast and fairly robust tech-

nology, because no further reaction or derivatization step is involved, and the signal is, theoretically, directly proportional to the quantity of the analyte element in the gel [43,44]. The principal difficulty in the use of GE for metallomics is the preservation of the metal-protein bond. Many metal-complexes with proteins are labile and can be destroyed by exchange with the metal impurities of the gel.

The current areas of developments include a decrease in detection limits and metal imaging in 2D gels. The use of a 100–150-μm laser beam (nanosecond pulsed) results in a relatively small amount of protein (ca. 1 % of the protein contained in a typical 10–15-mm large band) effectively used for analysis. In LA-ICP-MS sampling can be optimized separately from the detection, and the generated signal depends on the ablated mass. Consequently, the more material can be brought to the plasma at the time scale of mass detection, the higher is the sensitivity. A recent development proposed a fast (up to 280 mm s^{-1}) scanning with a femtosecond pulsed laser beam at a high repetition rate (10 kHz) parallel to the protein band when the gel advanced slowly in the orthogonal direction in order to increase the amount of ablated protein per unit of time [45]. The observed gain in limits of detection was ca. 40-fold.

The high stability and precision of LA-ICP-MS makes it possible to scan a gel in raster mode and thus to acquire element images. The analysis in the imaging mode is time-consuming. Scanning a gel of 5 cm^2 with a 100-μm resolution takes about 15 h, but the technique allows the identification of areas with increased metal concentrations regardless of the presence of intense protein spots.

Identification of metal-species by electrospray MS

Electrospray ionization (ESI) is able to generate ions of metal complexes in the gas phase which can be then analyzed by MS. The accurate mass determination should allow the determination of the empiric formula (and thus the metal-ligand stoichiometry), whereas the collision-induced dissociation (CID) mass spectra should deliver information on the structure of the ligand [46]. Paradoxically, the number of reports on the successful use of ESI-MS for the identification of metal-complexes has been remarkably scarce [10].

The problem is the vulnerability of ESI-MS to the presence of concomitant ions generated by matrix components which suppress the ionization of the species of interest. This concerns in particular the currently used quadrupole time-of-flight (QTOF) mass spectrometers for which the intrascan dynamic range does not usually exceed two orders of magnitude. Therefore, purification of species by 2D LC and their introduction by nanoHPLC into the ion source in order to avoid the simultaneous arrival of the analyte ion and more abundant ions from other species at the source is necessary [47,48]. A progress in the use of ESI-MS for identification of metal-bioligand complexes is expected with the wider availability of Fourier transform mass spectrometers, using either ion cyclotron resonance or electrostatic orbital traps which offer a larger intrascan range and the possibility of the multistage fragmentation and MS facilitating the species identification.

CORRELATING GENOTYPE WITH THE METALLOME: THE CONCEPT OF IONOMICS

High-throughput ICP-AES and ICP-MS were the principal analytical techniques for the comprehensive trace element profiling with an objective to get an insight into gene networks involved in mineral-ion accumulation in plants. The biological significance of connections between a genome of an organism (*Arabidopsis thaliana* [14] and *Saccharomyces cerevisiae* [15]) and its elemental profile could be demonstrated. The range of trace elements investigated included well-known enzymatic cofactors: Mn, Fe, Co, Ni, Cu, Zn (and Mo for *A. thaliana*), element with dual (essentiality/toxicity) behavior (Se) and, in the case of *A. thaliana* common toxic elements, such as As, Cd, and Pb. Relative quantification of the element concentration was achieved by average signal normalization. Different elemental profiles were demonstrated for 51 out of 6000 mutagenized *A. thaliana* plants [14] and for 212 out of 4385 investigated yeast mutant strains [15]. The necessity for the global approach was corroborated by changes observed in levels of not only one but of multiple elements in most mutants [15]. The observation that

only 6 of the 50 *Arabidopsis* ion-profile mutants show changes in only one element was considered to support strongly the existence of regulatory networks in the ionome of plants [15].

The precision of this approach as a functional genomics tool can be improved by integrating speciation-relevant information or by studies at cellular or subcellular resolution. In particular, the use of LA sampling holds promise for development of high-resolution ionomics imaging. If developed in living plant tissue, such technology is likely to open up a completely new window onto the ionome, allowing changes in the total shoot seed or root ionomes to be mapped to specific tissues and cell types. Such ionomic imaging would also allow colocalization of in vivo gene expression and protein localization patterns with ionomic changes, providing spatial linkage between gene, protein, and ionomic function [15].

METALLOPROTEOMICS

The study of the identity, metal-binding sites, and stoichiometry of metal-protein complexes (metalloproteomics) is an important area of research in metallomics [22,49].

Metalloproteins and structural genomics

The structural genomics initiatives have resulted in the purification to homogeneity of several thousands of proteins, with an emphasis on proteins of unknown structure. The typical procedure involves separation of genes by cloning with purification tags, followed by high-throughput heterologous expression and affinity purification [22]. High-throughput quantification of transition-metal content in these proteins is essential to identify the proteins which are rendered functional by the presence of a metal. The bioinformatic analysis of the protein sequence (by correlation with the relevant gene) allows the finding of the conserved metal-binding motifs and provides important clues to function and metal site structure [49].

XRF has been used to screen for the metal-binding proteins [22,49] to annotate the transition-metal content but ICP-MS fitted with the microliter sample introduction set-up is a valid alternative. The availability of a relatively large amount of relatively pure metalloproteins with the known primary sequence allows the determination of the metal-protein stoichiometry, usually by the measurement of the metal to sulfur ratio in CE-ICP-MS [37–39] and an insight into the metal-binding site by XANES and EXAFS [7,22].

Development of bioinformatics tools is making it possible to predict full sets of trace element-containing proteins in organisms for which complete genomic sequences are available, although methods for their identification are still in development [50–52]. Genome browsing in the field of metalloprotein analysis cannot be limited to the analysis of amino acid identity or similarity in sequences; the nature and spacing of amino acids acting as metal ligands and the nature of amino acids present in the metal-binding regions must also be taken into account [53].

In vivo metalloprotein detection and identification

The above-discussed in vitro approach allows the construction of a database of metal-binding sequence motifs, but the biological information about relative expression levels, post-translation processing, and modifications is lost. An alternative is the separation and analysis of metalloproteins in vivo; the metal sites are expected then to retain biological relevance. The two principal approaches include non-denaturating (native) GE and multidimensional LC.

During native GE, metal-protein complexes are expected to remain associated and folded as they would be in the cell. On the other hand, the complexes cannot move through the polyacrylamide gel as easily as individual, denatured proteins and may not separate cleanly or predictably. An attempt to detect and characterize multiple metal (Zn, Cd)-binding proteins in complex bacterial (*E. coli*) cyto-

plasmic fractions was reported by Binet et al. [54]. The approach was validated with a well-characterized, heterogenuously expressed Zn- and Cd-binding MT from the cyanobacterium [54]. A direct microlocal technique for protein gel spots was developed for the simultaneous determination of P, S, Si, Cu, Zn, and Al in the brains of Alzheimer's disease patients [55]. In order to study the formation of proteins containing Cu, Zn, and Fe in a human brain sample, isotopic-enriched tracers (^{54}Fe, ^{65}Cu, and ^{67}Zn) were doped to 2D gels of separated Alzheimers-diseased brain proteins prior to the isotope ratio measurement by LA-ICP-MS [56].

Whereas the detection of metal-containing proteins in the gel by LA-ICP-MS is relatively straightforward, a question remains how much of the originally present metalloprotein is detected. Mass balance of the metal introduced onto the gel is a critical parameter but is hardly ever reported. Also, the recovery of the protein for its identification is problematic. The identification of singular protein spots was attempted by matrix-assisted laser desorption/ionization (MALDI)-Fourier transform ion cyclotron resonance (FTICR)-MS after excision from the 2D gel and tryptic digestion [56]. The protein ligand can certainly be sequenced in this way, but the proof that the metal detected by LA-ICP-MS and the protein identified belonged together is often speculative.

The preservation of the metal-protein bond and the recovery of the metalloprotein can be monitored easier in multidimensional HPLC because of the ease of the on-line ICP-MS quantification. A proof of principle of the multidimensional HPLC was demonstrated for the isolation of the metallothioneins from animal tissue cytosols by SE, anion-exchange LC, and reversed-phase HPLC [57]. ESI-MS detection used in parallel allowed the determination of the molecular weight of the complex. A 10-fold gain in the sensitivity could be achieved, and the metal stoichiometry could be determined following an on-line acidification resulting in the removal of the metals and allowing the measurement of the molecular mass of the ligand.

Indirect insight into metal-dependent proteins

A common feature of the above-discussed approaches is an attempt to preserve the integrity of the metal-protein bond and the detection of the complex. An alternative may be a selective isolation of the group of proteins having affinity to bind metals. This can be achieved by immobilized metal affinity chromatography (IMAC). All proteins having the capacity of binding the metal immobilized on the stationary phase can be retained from a biological sample. The identification is then carried out by the classical bottom-up proteomics protocol based on GE followed by the spot excision, tryptic digestion, and MALDI-MS or nanoHPLC-ESI MS/MS [58]. In this approach, not only quantitative information is lost, but also evidence about the occurrence of a metal-protein complex should be regarded with care unless strongly supported by bioinformatics.

The above approach was successfully used to identify hepatic proteins with copper-binding ability. Forty-eight cytosolic proteins and 19 microsomal proteins displaying copper-binding ability were identified; 80 % of them were shown to contain putative metal-binding domains [59,60].

METALLO-METABOLLOMICS

The interactions of metals with low-molecular-weight ligands (referred to as the cellular metabolome) are an important topic of metallomics studies. The mechanisms of resistance of many organisms to toxic elements include a high turnover of organic acids (phytate, malate, citrate, oxalate, succinate) [61], metallophores of mugineic acid family [62], and the expression of metal-binding peptides [63,64]. Of particular interest are hyperaccumulating plants which have developed efficient metal homeostasis mechanisms that allow them to live and reproduce in metal-rich environments [65]. The complexation of metals leads to a number of relatively poorly characterized metal complexes. The understanding of the mechanisms that control detoxification can benefit from the identification of the species formed.

Electrospray MS-MS is the ultimate analytical technique, but, as discussed above, purification of the metal-species prior to analysis is necessary. Although a simple isolation of the molecule of interest, e.g., Al-citrate complex [66], or Ni-NA complex nicotianamine as a nickel-binding ligand in *Thlaspi caerulescens* [67] by SELC is sufficient in some cases, a further purification using another HPLC mechanisms is necessary. Hydrophylic interaction chromatography (HILIC) seems to be particularly attractive for this purpose [68] because of the compatibility of the mobile phases used with the ESI.

The parallel detection by ICP-MS is recommended in order to determine the column recovery; it also allows the determination of the number of species present and quantification. The knowledge of the accurate mass of the metal-species of interest or development of software facilitating the search of mass spectra for relevant data is required. The field of metallo-metabollomics can largely benefit from the FT-MS offering a larger intrascan dynamic range and thus increasing probability of the detection of metal-complexes using on-line HPLC sample introduction following little purification.

CONCLUSIONS AND OUTREACH

Metallomics, understood as a holistic high-throughput metal speciation analysis in a biological system correlated with the genomics, proteomics, and metabolomics data, is taking shape as a research field at the interface of analytical chemistry. While the performance of analytical techniques is steadily progressing in terms of identification and quantification, the correlation of data on the identity and quantity of metal species in a biological system with the information present in the genome is becoming important [67]. The wider use of molecular biology methods is expected to complement the in vivo bioanalytical data with in vitro molecular genetic data and lead to an understanding of metal functions at the molecular level.

REFERENCES

1. H. Haraguchi. *J. Anal. At. Spectrom*. **19**, 5 (2004).
2. J. D. Watson, F. Crick. *Nature* **171**, 737 (1953).
3. F. H. Crick, L. Barnett, S. Brenner, R. J. Watts-Tobin. *Nature* **192**, 1227 (1961).
4. R. D. Fleischmann, M. D. Adams, O. White, R. A. Clayton, E. F. Kirkness, A. R. Kerlavage, C. J. Bult, J. F. Tomb, B. A. Dougherty, J. M. Merrick, K. McKenney, G. Sutton, W. FitzHugh, C. Fields, J. D. Gocayne, J. Scott, R. Shirley, L.-I. Liu, J. C. Venter. *Science* **269**, 496 (1995).
5. IHGSC. *Nature* **409**, 860 (2001).
6. J. C. Venter, M. D. Adams, E. W. Myers, P. W. Li, R. J. Mural, G. G. Sutton, H. O. Smith, M. Yandell, C. A. Evans, R. A. Holt, J. D. Gocayne, P. Amanatides, R. M. Ballew, D. H. Huson, J. R. Wortman, Q. Zhang, C. D. Kodira, X. H. Zheng, L. Chen, M. Skupski, G. Subramanian, P. D. Thomas, J. Zhang, G. L. Gabor Miklos, C. Nelson, S. Broder, A. G. Clark, J. Nadeau, V. A. McKusick, N. Zinder, A. J. Levine, R. J. Roberts, M. Simon, C. Slayman, M. Hunkapiller, R. Bolanos, A. Delcher, I. Dew, D. Fasulo, M. Flanigan, L. Florea, A. Halpern, S. Hannenhalli, S. Kravitz, S. Levy, C. Mobarry, K. Reinert, K. Remington, J. Abu-Threideh, E. Beasley, K. Biddick, V. Bonazzi, R. Brandon, M. Cargill, I. Chandramouliswaran, R. Charlab, K. Chaturvedi, Z. Deng, V. Di Francesco, P. Dunn, K. Eilbeck, C. Evangelista, A. E. Gabrielian, W. Gan, W. Ge, F. Gong, Z. Gu, P. Guan, T. J. Heiman, M. E. Higgins, R.-R. Ji, Z. Ke, K. A. Ketchum, Z. Lai, Y. Lei, Z. Li, J. Li, Y. Liang, X. Lin, F. Lu, G. V. Merkulov, N. Milshina, H. M. Moore, A. K. Naik, V. A. Narayan, B. Neelam, D. Nusskern, D. B. Rusch, S. Salzberg, W. Shao, B. Shue, J. Sun, Z. Y. Wang, A. Wang, X. Wang, J. Wang, M.-H. Wei, R. Wides, C. Xiao. *Science* **291**, 1304 (2001).
7. S. S. Hasnain. *J. Synchrotron Radiat.* **11**, 7 (2004).
8. R. J. P. Williams. *Coord. Chem. Rev*. **216–217**, 583 (2001).
9. J. Szpunar. *Anal. Bioanal. Chem*. **378**, 54 (2004).

10. J. Szpunar. *Analyst* **130**, 442 (2005).
11. J. Lopez-Barea, J. L. Gomez-Ariza. *Proteomics* **6**, S51 (2006).
12. N. Jakubowski, R. Lobinski, L. Moens. *J. Anal. At. Spectrom.* **19**, 1 (2004).
13. D. W. Koppenaal, G. M. Hieftje. *J. Anal. At. Spectrom*. **22**, 855 (2007).
14. B. Lahner, J. Gong, M. Mahmoudian, E. L. Smith, K. B. Abid, E. E. Rogers, M. L. Guerinot, J. F. Harper, J. M. Ward, L. McIntyre, J. I. Schroeder, D. E. Salt. *Nat. Biotechnol.* **21**, 1215 (2003).
15. D. J. Eide, S. Clark, T. M. Nair, M. Gehl, M. Gribskov, M. L. Guerinot, J. F. Harper. *Genome Biol.* **6**, R77 (2005).
16. A. Sanz-Medel. *Anal. Bioanal. Chem.* **381**, 1 (2005).
17. N. Jakubowski, G. M. Hieftje. *J. Anal. At. Spectrom*. **23**, 13 (2007).
18. R. Lobinski, H. Haraguchi, B. Sarkar, J. S. Becker. *Chem. Int.* **29**, 24 (2007).
19. R. Lobinski, C. Moulin, R. Ortega. *Biochimie* **88**, 1591 (2006).
20. J. Szpunar, R. Lobinski, A. Prange. *Appl. Spectrosc*. **57**, 102A (2003).
21. I. Ascone, R. Fourme, S. Hasnain, K. Hodgson. *J. Synchrotron Radiat*. **12**, 1 (2005).
22. R. A. Scott, J. E. Shokes, N. J. Cosper, F. E. Jenney, M. W. W. Adams. *J. Synchrotron Radiat.* **12**, 19 (2005).
23. K. P. Taylor, B. A. Parks, D. M. Kurtz Jr., J. I. Amster. *J. Biol. Inorg. Chem*. **6**, 201 (2001).
24. K. A. Johnson, M. F. J. M. Verhagen, P. S. Brereton, M. W. W. Adams, I. J. Amster. *Anal. Chem*. **72**, 1410 (2000).
25. D. Schaumloffel, P. Giusti, M. V. Zoriy, C. Pickhardt, J. Szpunar, R. Lobinski, J. S. Becker. *J. Anal. At. Spectrom.* **20**, 17 (2005).
26. P. Giusti, R. Lobinski, J. Szpunar, D. Schaumloffel. *Anal. Chem*. **78**, 965 (2006).
27. J. S. Becker, M. V. Zoriy, C. Pickhardt, N. Palomero-Gallagher, K. Zilles. *Anal. Chem.* **77**, 3208 (2005).
28. J. S. Becker, M. Zoriy, J. S. Becker, J. Dobrowolska, A. Matusch. *J. Anal. At. Spectrom.* **22**, 736 (2007).
29. J. S. Becker, A. Gorbunoff, M. Zoriy, A. Izmer, M. Kayser. *J. Anal. At. Spectrom*. **21**, 19 (2006).
30. J. L. Gardea-Torresdey, J. R. Peralta-Videa, G. De La Rosa, J. G. Parsons. *Coord. Chem. Rev.* **249**, 1797 (2005).
31. K. Bluemlein, A. Raab, A. A. Meharg, J. M. Charnock, J. Feldmann. *Anal. Bioanal. Chem.* 1 (2007).
32. J. Szpunar. *Analyst* **125**, 963 (2000).
33. J. Szpunar, R. Lobinski. *Anal. Bioanal. Chem*. **373**, 404 (2002).
34. D. Schaumlöffel, A. Prange. *Fresenius' J. Anal. Chem.* **364**, 452 (1999).
35. K. Polec, J. Szpunar, O. Palacios, P. Gonzalez-Duarte, S. Atrian, R. Lobinski. *J. Anal. At. Spectrom.* **16**, 567 (2001).
36. K. Polec-Pawlak, O. Palacios, M. Capdevilla, P. Gonzalez-Duarte, R. Lobinski. *Talanta* **57**, 1011 (2002).
37. K. Polec-Pawlak, D. Schaumlöffel, J. Szpunar, A. Prange, R. Lobinski. *J. Anal. At. Spectrom.* **17**, 908 (2002).
38. A. Hagege, T. Baldinger, M. Martin-Jouet, F. Zal, M. Leroy, E. Leize, A. V. Dorsselaer. *Rapid Commun. Mass Spectrom*. **18**, 735 (2004).
39. V. Van Lierde, K. Strijckmans, M. Galleni, B. Devreese, J. Van Beeumen, F. Vanhaecke, L. Moens. *LC-GC Europe* 617 (2003).
40. D. Schaumlöffel, L. Ouerdane, B. Bouyssiere, R. Lobinski. *J. Anal. At. Spectrom.* **18**, 120 (2003).
41. S. Mounicou, S. McSheehy, J. Szpunar, M. Potin-Gautier, R. Lobinski. *J. Anal. At. Spectrom.* **17**, 15 (2002).
42. J. L. Neilsen, A. Abildtrup, J. Christensen, P. Watson, A. Cox, C. W. McLeod. *Spectrochim. Acta B* **53**, 339 (1998).
43. R. Ma, C. W. McLeod, K. Tomlinson, R. K. Poole. *Electrophoresis* **25**, 2469 (2004).

44. G. Ballihaut, C. Pecheyran, S. Mounicou, H. Preud'homme, R. Grimaud, R. Lobinski. *Trends Anal. Chem*. **26**, 183 (2007).
45. G. Ballihaut, F. Claverie, C. Pecheyran, S. Mounicou, R. Grimaud, R. Lobinski. *Anal. Chem.* **79**, 6874 (2007).
46. H. Chassaigne, V. Vacchina, R. Lobinski. *Trends Anal. Chem*. **19**, 300 (2000).
47. M. Dernovics, R. Lobinski. *J. Anal. At. Spectrom*. **23**, 72 (2007).
48. M. Dernovics, T. Garcia-Barrera, K. Bierla, H. Preud'homme, R. Lobinski. *Analyst* **132**, 439 (2007).
49. W. Shi, C. Zhan, A. Ignatov, B. A. Manjasetty, N. Marinkovic, M. Sullivan, R. Huang, M. R. Chance. *Structure* **13**, 1473 (2005).
50. C. Andreini, I. Bertini, A. Rosato. *Bioinformatics* **20**, 1373 (2004).
51. W. Maret. *J. Anal. At. Spectrom.* **19**, 15 (2004)
52. I. Bertini, A. Rosato. *Eur. J. Inorg. Chem*. 2546 (2007).
53. I. Bertini, A. Rosato. *Proc. Natl. Acad. Sci.* **100**, 3601 (2003).
54. M. R. B. Binet, R. Ma, C. W. McLeod, R. K. Poole. *Anal. Biochem.* **318**, 30 (2003).
55. J. S. Becker, M. Zoriy, U. Krause-Buchholz, J. S. Becker, C. Pickhardt, M. Przybylski, W. Pompe, G. Rodel. *J. Anal. At. Spectrom*. **19**, 1236 (2004).
56. J. S. Becker, M. Zoriy, M. Przybylski, J. S. Becker. *Int. J. Mass Spectrom*. **242**, 135 (2005).
57. R. Lobinski, J. Szpunar. *Anal. Chim. Acta* **400**, 321 (1999).
58. P. P. Kulkarni, Y. M. She, S. D. Smith, E. A. Roberts, B. Sarkar. *Chem.—Eur. J.* **12**, 2410 (2006).
59. S. D. Smith, Y.-M. She, E. A. Roberts, B. Sarkar. *J. Proteome Res.* **3**, 834 (2004).
60. Y. M. She, S. Narindrasorasak, S. Yang, N. Spitale, E. A. Roberts, B. Sarkar. *Mol. Cell. Proteomics* **2**, 1306 (2003).
61. M. N. V. Prasad, J. Hagemeyer. *Heavy Metal Stress in Plants: From Molecules to Ecosystem*, Springer, Heidelberg (1999).
62. M. S. Wheal, L. I. Heller, W. A. Norvell, R. M. Welch. *J. Chromatogr., A* **942**, 177 (2001).
63. C. Cobbett, P. Goldsbrough. *Annu. Rev. Plant Biol.* **53**, 159 (2002).
64. M. Mejare, L. Bulow. *Trends Biotechnol*. **19**, 67 (2001).
65. D. E. Salt, U. Krämer. In *Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment*, I. Raskin, B. D. Ensley (Eds.), John Wiley, New York (2000).
66. T. B. Polak, R. Milacic, B. Mitrovic, M. Benedik. *J. Pharm. Biomed. Anal.* **26**, 189 (2001).
67. V. Vacchina, S. Mari, P. Czernic, L. Margues, K. Pianelli, D. Schaumloeffel, M. Lebrun, R. Lobinski. *Anal. Chem.* **75**, 2740 (2003).
68. L. Ouerdane, S. Mari, P. Czernic, M. Lebrun, R. Lobinski. *J. Anal. At. Spectrom.* **21**, 676 (2006).