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Metallomics, elementomics, and analytical techniques*

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Abstract: Metallomics is an emerging and promising research field which has attracted more and more attention. However, the term itself might be restrictive. Therefore, the term "elementomics" is suggested to encompass the study of nonmetals as well. In this paper, the application of state-of-the-art analytical techniques with the capabilities of high-throughput quantification, distribution, speciation, identification, and structural characterization for metallomics and elementomics is critically reviewed. High-throughput quantification of multielements can be achieved by inductively coupled plasma-mass spectrometry (ICP-MS) and neutron activation analysis (NAA). High-throughput multielement distribution mapping can be performed by fluorescence-detecting techniques such as synchrotron radiation X-ray fluorescence (SR-XRF), XRF tomography, energy-dispersive X-ray (EDX), proton-induced X-ray emission (PIXE), laser ablation (LA)-ICP-MS, and ion-detecting-based, secondary-ion mass spectrometry (SIMS), while Fourier transform-infrared (FT-IR) and Raman microspectroscopy are excellent tools for molecular mapping. All the techniques for metallome and elementome structural characterization are generally low-throughput, such as X-ray absorption spectroscopy (XAS), NMR, and small-angle X-ray spectroscopy (SAXS). If automation of arraying small samples, rapid data collection of multiple low-volume and -concentration samples together with data reduction and analysis are developed, high-throughput techniques will be available and in fact have partially been achieved.

Keywords: metallomics; elementomics; analytical techniques; LA-ICP-MS; NAA; XRF; FTIRM.

INTRODUCTION

There are almost two million different "species" of living organisms on the Earth, however, besides their phenotypic and phylogenetical characteristics, only 11 elements appear to be approximately constant and predominant in all biological systems, which are called major elements. In the human body, these constitute 99.9 % of the total number of atoms present, but just 4 of them (C, O, H, and N) correspond to 99 % of the total and the other 7 elements (Na, K, Ca, Mg, P, S, and Cl) represent only about 0.9 % [1]. The other elements that are found in biological elements, no matter if they are essential or

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toxic, are correspondingly called trace elements. Trace elements, especially essential ones, have specific functions and are indispensable for life by acting as catalytic or structural components of larger molecules [2]. Since trace elements play an important role in life, total concentration analysis of the trace elements has been extensively established [3]. However, the biological effect of an element is not only dependent on the total concentration, but also highly related to their chemical species. For example, dialkylmercury derivatives are considered extremely toxic, while mercuric selenide has a relatively low toxicity and accumulates as an apparently benign detoxification product in marine animals [4], and methylmercury cysteine proves to be much less toxic than methylmercury chloride in a zebra fish larvae model system [5]. Therefore, the complete characterization of their properties regarding the chemical species like oxidation state, individual organometallic species, associated organic ligands and complex forms of a metal or nonmetal in biochemical systems is crucially required, which was defined as speciation analysis by IUPAC as "the analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample" [6].

Since it is found that most elements play a role in life processes and further, as Noddack proposed in 1932, all the elements in the periodic table are contained in the biological samples ("the Extended All Present Theory of the Elements"), consideration of the global role of all metals/metalloids in a system will give more comprehensive views than speciation analysis. Thus, the concepts and terms "metallomics" and "metallomes" for "integrated bio-trace element science" have been proposed, which were used by Williams in 2001 [7–8] for elemental distribution in organisms, and further elucidated by Haraguchi [9,10]. From the point of "metal-assisted function bioscience (or biochemistry)", metallomics is defined as the study of metals and metal species, and their interactions, transformations, and functions in biological systems and the full complement of metals and metal moieties (free and bound) are accordingly known as the "metallome" [11]. Although it is a rather new concept, metallomics has received great attention: the Royal Society of Chemistry-based *Journal of Analytical and Atomic Spectrometry* published two special issues on "metallomics" in 2004 and 2007 [11], and a new metallomics and biological elemental speciation web page (<http://www.rsc.org/Publishing/Journals/JA/News/biological_content.asp>) has been set up. An international symposium on metallomics has been held in Nagoya (<http://www.ism2007.org>), which attracted nearly 400 scientists from all over the world. Also, a Metallomics Center of the Americas (<http://www.che.uc.edu/metallomics/>) has been established at the University of Cincinnati, which is the first large-scale metallomics research center in the world. However, as the term "metallomics" itself reveals, it covers mainly the role of whole metal-binding species in a biosystem although to some extent, metalloid-binding ones are also considered. However, as aforementioned, it is the nonmetallic elements like C, N, O, H, S, and P that make the major parts of biological systems and play very important roles in them. Thus, it is necessary to study all the elements, regardless of metals, nonmetals, or metalloids, in biological systems. In this review, the extended "-omics" study such as "nonmetallomics", "metalloimics", and "elementomics" and the analytical techniques for their study will be discussed.

FROM METALLOMICS TO ELEMENTOMICS

As just mentioned above, it seems that "metallomics" might be a restrictive term. Therefore, using the term "elementomics" for the study of "biological elemental speciation" was proposed in the editorial of the first issue of the *Journal of Analytical and Atomic Spectrometry* in 2008 [12]. However, the word "elementomics" is not new. In 2006, Xiong et al. used this term to cover the study of the concentration of 55 elements in lung cancerous and pericancerous tissues and their correlations [13]. As an analogy to metallomics, elementomics may be defined as the study of elements of interest and element species and their interactions, transformations, and functions in biological systems, and elementome may thus be used for the full complement of studied elements and element moieties (free and bound). The elementomics study of nonmetal elements of interest will thus be regarded as "nonmetallomics", and the

study of metalloid elements will be regarded as "metalloimics". The relationship between these three terms as well as other -omics is illustrated in Fig. 1.

Since metals play a very important role in the function of some proteins, the term "metalloproteomics" was proposed by focusing on the distributions and compositions of all metalloproteins in proteome, and the characterization of their structure and function [14,15], which may be regarded as the overlap of metallomics and proteomics. Studies on metal-gene [16–18], metal-sugar [19–23], and metal-lipid [24,25] complexes and interactions have also attracted much attention, and the terms "metallogenomics" [26] and "metalloglycomics" [27] have been used and the term "metallolipidomics" may be used to cover the different overlap areas between metals with genes, sugars, or lipids as shown in Fig. 1.

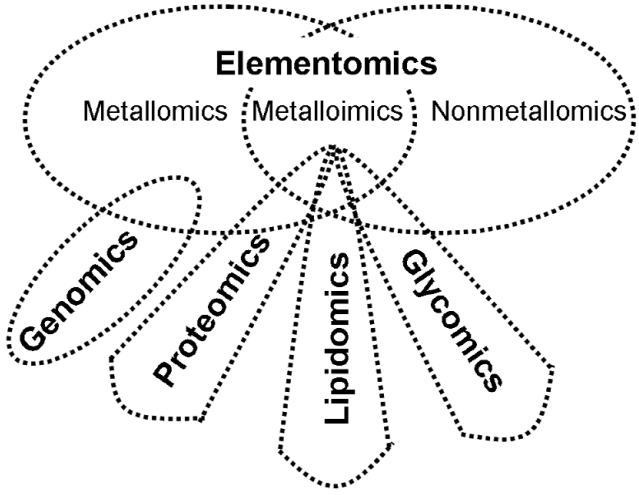

Fig. 1 An illustrative scheme for the relationship among elementomics, metallomics, metalloimics, nonmetallomics, genomics, proteomics, glycomics, lipidomics, and other -omics.

When considering nonmetallic elements, besides C, H, and O, many of them play very important roles in life processes. For example, phosphorylation is a very important step in the post-translational modification of a protein. Se is incorporated as selenocysteine at the active site of a wide range of selenoproteins such as glutathione peroxidase (GPx), thioredoxin reductase (TR), selenoprotein P, selenoprotein W, and iodothyronine deiodinase [28]. Phospholipids contained in sn-3 position with a phosphate residue are a component of membranes in all types of cells in plants, animals, and microorganisms [29]. Selenosugars are found to be the key urinary metabolites for Se excretion within the required low-toxic dose range in rats [30]. Arsenosugars have been found in seafood and human urine [31,32]. Thus, phosphoproteomics [33], selenoproteomics [34,35], and phospholipidomics [36,37] have been proposed, and selenoglycomics and arsenoglycomics may be used in the metalloimics study on all the selenosugars and arsenosugars in biological systems.

Therefore, by introducing the term "elementomics", all elements (free and bound) in biological systems can be studied in the -omics way like metallomics. Further, the research methods of -omics may be incorporated into other research fields. For example, nanomics may be used for studies of biological effects of all kinds of nanomaterials, which have received great attention in recent years [38–42]. Halogenomics may be used for the study of biological effects of persistent organic pollutants (POPs), since most of them are organohalogens like polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), polybrominated diphenylethers (PBDEs), and hexachlorobenzene (HCB) [43–45].

RESEARCH OBJECTS AND ANALYTICAL TECHNIQUES FOR ELEMENTOMICS

Just like metallomics, elementomics will study (1) quantification of elements of interest in biological systems; (2) distributions of studied elements in biological systems; (3) the speciation of given elements; (4) the structural analysis of the elementome; (5) the elucidation of reactions and related mechanisms of elementome; (6) metabolisms of elementome; (7) the specific element-assisted function biosciences in medicine, environment science, food science, agriculture, toxicology, biochemistry, and so on [10].

Corresponding to the objects of elementomics study, different analytical techniques can be used to reach these goals. The application of advanced nuclear analytical techniques on metalloproteomics study has been reviewed by Gao et al. [46]. In the following parts, the analytical techniques, both nuclear and non-nuclear, which can realize some of the above goals of metallomics and elementomics, especially analytical techniques for elemental quantification, elementome distribution, and speciation and structural analysis of elementome, will be reviewed.

Analytical techniques for multielemental quantification

For -omics study, the most important requirement should be high-throughput. Plasma-based instruments like inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS), which can quantify multielements rapidly and simultaneously in one run, are ideal for this purpose. However, ICP-AES shows the low sensitivity and often the poor selectivity to accurately determine many of the less abundant but more important elements in biological systems. In contrast, ICP-MS is extremely sensitive, due to the efficient ionization from plasma coupled with the sensitive detection of the mass spectrometer. At its best, the parts per trillion detection limits are achievable that are generally 2–3 orders of magnitude lower than those in ICP-AES [47]. ICP-MS can detect most elements in biological systems, but sulfur, phosphorous, and halogens are not efficiently ionized by the ICP owing to their high ionization energies. Further, a number of polyatomic interferences also hinder the detection of S, P, and transition elements like Fe and V using ICP-MS [35]. Polyatomic interferences can be solved by either using high-resolution (sector field double focusing) mass spectrometer or in more commonly available quadrupole mass analyzer, the collision/reaction cell techniques [48,49]. Therefore S, P, Cl, Br, I, and even C have been detected by quadrupole ICP-MS [35,50]. Besides polyatomic interference effect, the memory effect of Hg, U, Os, Hf, and Pt, etc. is another important issue in precise ICP-MS detection. To solve this problem, complexing agents can be added to prevent the adherence of them to the walls of the spray chamber and the transfer tubing of the sample introduction system. For example, a series of complexing agents aiming to reduce the memory effect of Hg were compared and –SH containing reagents were found to be the best to eliminate it in the detection of Hg by ICP-MS [51].

High-resolution mass spectrometer is superior to the common quadrupole detector since it can provide extremely low instrumental background and very good detection limits (0.1–1 ng/mL) for nearly all elements, but the high cost may hamper its wide application in elementomics study.

For ICP-MS, liquid and gaseous samples can be directly introduced into system, but solid samples need pretreatment like digestion or ashing to make them into liquid solutions. The application of laser-ablation (LA) technique in ICP-MS has made the direct and online analysis of solid samples possible which have enhanced greatly the application of ICP-MS [52,53]. Its fast data acquisition, minimal sample preparation, and high sensitivity offer low detection limits for a wide range of major and trace elements in complex biological, environmental, and geological samples.

Neutron activation analysis (NAA) is also a multielement quantification technique which can simultaneously measure more than 30 elements in a sample. The detection limits of NAA range from 10^{-6}–10^{-13} g/g [54]. In typically instrumental NAA, stable nuclides (^{A}Z, the target nucleus) in the sample undergo neutron capture reactions in a flux of (incident) neutrons. The radioactive nuclides (^{A+1}Z,

the compound nucleus) produced in this activation process will, in most cases, decay through the emission of a beta particle (β^-) and gamma ray(s) with an inherent half-life. A high-resolution γ-ray spectrometer is used to detect these "delayed" γ-rays from the artificially induced radioactivity in the sample for both qualitative and quantitative analysis [54]. One of the principal advantages of NAA is that it is nearly free of any matrix interference effects as the vast majority of samples are completely transparent to both the probe (the neutron) and the analytical signal (the γ-ray). Moreover, because NAA can most often be applied instrumentally (no need for sample digestion or dissolution), there is little, if any, opportunity for reagent or laboratory contamination [3].

X-ray fluorescence analysis (XRF) is another multielement analytical technique which is based on the detection of characteristic fluorescence after excited by primary X-ray with sufficient energy [55]. If the exciting source is replaced by a high-energy proton beam, it will be called proton-induced X-ray emission spectrometry (PIXE) [56], which can provide better analytical sensitivity than XRF. If synchrotron radiation (SR) is used as exciting source, the SR-XRF will achieve an absolute detection limit of 10^{-12}–10^{-15} g and relative detection limit of several μg/g, even in ng/g levels [57]. XRF is basically a qualitative technique, but quantification can also be achieved [58,59].

In general, ICP-MS, NAA, XRF, and PIXE are all excellent techniques for multielement quantification. NAA, XRF, and PIXE can achieve nondestructive and in situ multielement analysis for solid samples while ICP-MS needs laser ablation for this purpose.

After getting the concentrations of different elements in samples, correlation studies using mathematical methods may lead to further understanding of the roles and their cross-talk of different elements. For example, positive correlation between Se and Hg contents has been found in fish [60,61], marine mammals and birds [62–65], animals, and workers exposed to inorganic mercury [66–69], which leads to the study of Hg and Se antagonism or synergism mechanism [70–72]. Other studies which measure multielements in human autopsy tissue [73] and cancerous lung [13] also found significant relationship among some elements.

Analytical techniques for elementome distribution

Analytical techniques for not only the element distribution but also the molecular distribution will be reviewed in this section.

Multielement distribution

Static distribution

Besides total concentration of multielements, the spatial distribution of them in samples is also very important in understanding their bioavailability, trophic transfer, and environmental risk. A number of complementary analytical techniques exist for the mapping of elemental distributions in biological tissues including SR-XRF with microbeam (SR-μXRF), microscopic EDX (energy-dispersive X-ray fluorescence), microscopic WDX (wavelength-dispersive X-ray fluorescence), microscopic PIXE, LA-ICP-MS, microscopic SIMS (secondary ion mass spectrometry).

SR-μXRF has been used widely in elemental distribution study. For example, cellular distribution of arsenic and other elements in arsenic hyperaccumulator *Pteris nervosa* and their relations to arsenic accumulation has been studied using line-scanning of XRF, and the transport route for arsenic in *P. nervosa* was found [74]. By regulating with a slit or focusing system, such as Kirkpatrick–Baez mirror system [75], refractive lenses [76], or with a Fresnel zone plate [76], the beam size can be made in micron or even submicron level. Raster scanning of the specimen and acquisition of the entire X-ray spectrum yields quantitative topographical maps for a wide range of elements [77]. Different cells [78,79], tissues [80,81], and organ slices [82] have been successfully studied using this microbeam 2D SR-μXRF [83,84]. However, either line-scanning or 2D elemental mapping performed are along the sample surface. Therefore, the information retrieved is a product of the incident X-rays, penetration capability, and the self-absorption correction depending on both the energy of the exciting radiation and the energy of

the fluorescence radiation and has the disadvantage that it is not explicitly depth-sensitive. To perform depth analysis, the confocal arrangement, which consists of X-ray optics in the excitation as well as in the detection channel has been set up by several groups with a depth resolution from 10 to 40 μm and minimum detection limits are sub-mg/kg level [85–89]. 3D micro-XRF investigations have been made successfully in biological samples like rice-grain [90], duckweed [91], and human joint bone [92].

Besides the above-mentioned 3D SR-XRF, the SR-XRF tomography measurement is also possible to perform 3D elemental analysis by measuring a series of projected distributions under various angles and back-projected using the appropriate mathematical algorithms [93,94]. Since this method involves rotation of the sample over 180º or 360º relative to the primary beam, it is limited to the investigation of relatively small objects. The spatial resolutions for XRF tomography are situated at the 1–2 μm level while routinely a resolution of 5 μm is being employed [95].

Other XRF-based techniques like EDX have been coupled to microscopes like TEM (transmission electron microscopy) or SEM (scanning electron microscopy) to map elemental in roots and leaves of *Arabidopsis thaliana* [96], mice liver tissue [97], and pyramidal neurons [98], etc. Although both SEM-EDX and TEM-EDX can provide very good spatial resolution at about 10 nm, the detection limits are at about g/kg level [99] which may hold back their application in trace element detection in biological samples.

Just like SR-XRF, PIXE with proton microprobe has also been developed [100–102] and has been applied to elemental distribution in plant and animal tissues [103–105] and human blood cells and tumors [106]. Further, 3D micro-PIXE also has been developed to perform depth analysis recently [107,108] and has the spatial resolution of 4 μm by using characteristic Ti-K-X rays (4.558 keV) produced by 3 MeV protons with beam spot size of ~1 μm [109].

With the microprobe technique, LA-ICP-MS has became one of the more powerful tools in the quantitative analysis of major and minor element in situ owing to the very high sensitivity of ICP-MS and direct laser sampling to obtain more information from small samples in the micro area. LA-ICP-MS is well established for elemental mapping in the geological sciences [110,111] and has been applied to biological tissues such as plant leaves [112], snake tails [113], tree rings [114], rat brains [115], pig liver [116], human brains [117], and human teeth [118]. The spatial resolution of LA-ICP-MS is about 10 μm with a detection limit of sub mg/kg level [99]. Accurate quantitative analysis by LA-ICP-MS is a challenge because of the lack of suitable certified solid standards. Various calibration procedures have been used, and the most accurate approach is to have true matrix matching of standards and samples. Commercially available certified reference materials have also been used for calibration [116,119].

SIMS operates on the principle that bombardment of a material with a beam of ions with high energy (1–30 keV) results in the ejection or sputtering of atoms from the material. A small percentage of these ejected atoms leave as either positively or negatively charged ions, which are referred to as secondary ions. The collection of these sputtered secondary ions and their analysis by mass-to-charge spectrometry gives information on the composition of the sample, with the elements present identified through their atomic mass values. Counting the number of secondary ions collected can also give quantitative data on the sample's composition [120]. SIMS can be used for practically all elements of the periodic table (only the noble gases are difficult to measure because they do not ionize easily) with a detection limit of ng/kg level and lateral resolution at 10 μm [121]. Since ions of different mass are measured separately, SIMS is ideally suited for the study of isotopic compositions of small samples [122]. SIMS works by analyzing material removed from the sample by sputtering, and is therefore a locally destructive technique, which may not be a suitable technique for live biological samples but still has been used in soybean root [123], grape seeds [124], animal tissues, and cells [125]. During a SIMS measurement, the sample is slowly sputtered (eroded) away and depth profiles can be obtained, which is the 3D measurement [126].

Taken together, the techniques that can thus be used for multielemental distribution are summarized in Table 1. SR-μXRF, PIXE, LA-ICP-MS, and SIMS are all very good multielement imaging

analysis techniques with spatial resolution of at least μm level and detection limit of at least mg/kg level. Although TEM-EDX and SEM-EDX have much better spatial resolution, the sensitivity is much lower for trace elements than SR-μXRF, PIXE, LA-ICP-MS, and SIMS. When comparing with LA-ICP-MS and SIMS, the beam time limitation for SR-μXRF and PIXE may hinder their wide application.

Table 1 Selected techniques that can be used for simultaneous multielement distribution study.

Techniques	Excitation source	Elements can be detected	Isotopes detection?	Dimension?	Detection limit	Resolution	Ref.
SR-μXRF	SR X-ray	From oxygen on in periodic table	No	1D, 2D, 3D	~mg/kg	~μm	[84]
XRF Tomography	SR X-ray		No	3D	~mg/kg	1–2 μm	[95]
EDX	X-ray tube		No	1D, 2D	~g/kg	nm	[99]
PIXE	Proton beam	From Na on in periodic table	No	1D, 2D, 3D	~mg/kg	4 μm	[109]
LA-ICP-MS	Plasma	Nearly all elements	Yes	1D, 2D	sub mg/kg	10 μm	[99]
SIMS	Ion	Nearly all elements	Yes	1D, 2D, 3D	ng/kg	10 μm	[121]

Dynamic distribution

Besides determination of the static distribution of elements, monitoring the absorption, distribution, transportation, storage, retention, metabolism, excretion, and toxicity of elements is very important. Isotopic tracing is one of the techniques that may realize the above goals [127]. The commercialized PET (positron emission tomography) and SPECT (single photon emission computed tomography) are indeed such kinds of isotopic tracing techniques since ^{18}F and ^{99m}Tc are generally used. The major concern for isotopic tracing is the radioactivity of these isotopes. Therefore, application of stable isotopes tracing has been proposed combined with the application of NAA and ICP-MS [128,129]. ICP-MS measurement is being exploited for the use of enriched stable isotopes for multitracer investigations, which can also help to avoid spectral and background interference problems [130].

Molecular distribution

Another aspect of elementome distribution is molecular distribution. Vibrational-based techniques like FTIRM (Fourier transform infrared microspectroscopy) and Raman microspectroscopy are such excellent tools that are suitable for molecular imaging [131].

FTIRM was developed over 20 years ago using a conventional thermal infrared (IR) light source and a single-element IR detector which has been used to identify and spatially resolve the chemical makeup of many diverse materials, including various plant and animal tissues, forensics materials, and pharmaceuticals [132]. By coupling to a synchrotron IR source, FTIRM provides chemical information on the organic components of a tissue such as proteins, lipids, nucleic acids, and carbohydrates, at a diffraction-limited spatial resolution of 2–10 μm in the mid-IR region [133,134]. Functional group maps or "chemical images" can be generated pixel by pixel for each individual absorption band from a series of spectra collected in a grid pattern. The relative amounts of these groups are determined by the magnitude of absorption at each wavelength. These chemical maps can be superimposed onto photomicrographic images, adding a microchemical dimension [131]. A recent review work on chemical imaging of biological tissue using FTIRM has been done by Miller et al. [133,135].

In many disease states and environmental contaminations, both the organic and metal ion compositions are altered. Thus, simultaneous monitoring of the change is important and meaningful. Combining application of SR-μXRF and FTIRM on mapping of both molecular and elemental distribution may realize this goal, and a new sample substrate for imaging and correlating organic and trace metal composition in biological cells and tissues has been successfully developed recently [136].

A disadvantage of FTIR is that samples must be made very thin, therefore, not all materials will fit into this device. A second difficulty is that identification of individual molecules may be challenging because a spectrum represents a combination of all absorbing molecules in the IR path [137].

The only competing technology that truly gives the same type of microbeam molecular information is Raman microspectroscopy, which can give molecular vibrational information that complements but does not overlap with that obtained from FTIR [131].

Using Raman spectroscopy in biological investigations presents several advantages, especially when compared to IR technique: (1) water causes very little interference; (2) as acquisitions are obtained with a reflection optical arrangement, there are no limitations regarding the transparency or thickness of the samples; (3) the major bands in tissue spectra that correspond to mineral and organic constituents are clearly separated and both phases can therefore be simultaneously investigated in a nondestructive way [138].

Comparing with IR, Raman has an advantage because of its ease of sampling, and the possibility for confocal measurements [139]. Confocal Raman microspectroscopy is the usual technique of choice when dealing with microscopic samples like cells [140,141]. It has been experimentally found that line mapping is the fastest method for acquiring spectral information at a reasonable spatial resolution (approximately 1 μm). Even with that method, it takes several hours to acquire a Raman image of cells and tissues, which can pose a severe drawback for the study of dynamic living systems [142].

The combining application of both FTIRM and Raman microspectroscopy can provide valuable information about the biochemical composition since FTIR spectra are due to polar functional groups while the bands in Raman spectra are due to nonpolar functional groups, which has been used in bone [143], pork muscle [144], bacterial strains [145], and human tissues [146].

Analytical techniques for speciation and structural analysis of elementome

The scheme for elementome speciation and structural analysis is shown in Fig. 2. The general route for elemental speciation is as follows: different species in samples are first extracted using acid, base, enzyme or just diluted, then the extracted solution is separated by powerful selective separation techniques like high-performance liquid chromatography (HPLC), gas chromatography (GC), gel electrophoresis (GE) or electro-chromatography (EC) and monitored by a sensitive and element-specific detector like ICP-MS, and NAA [35,147–150]. The sample pretreatment is extremely important to keep the original species intact during the operating processes.

In the point of elementomics speciation and structure characterization, the high throughput in extraction, separation, detection, identification, and structural analysis is needed. As aforementioned, ICP-MS and NAA are all multielement detectors, therefore, the process of sample pretreatment, identification, and structural analysis may be the bottlenecks for elementomics study.

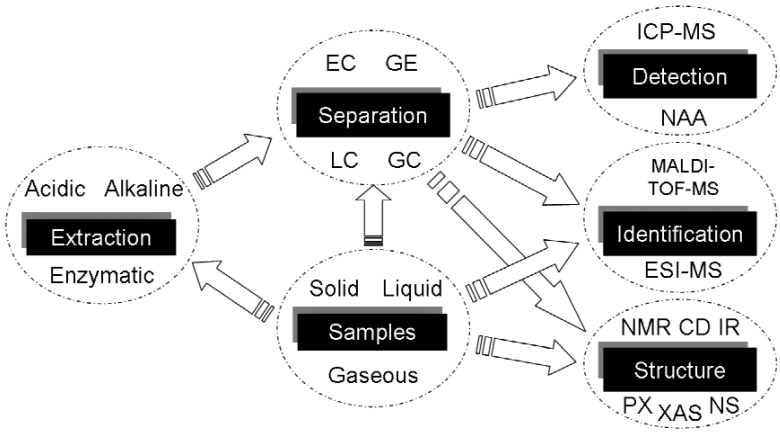

Fig. 2 Hyphenated scheme for speciation and structure analysis. Note: techniques that can be used in separation, detection, identification, and structure analysis are also shown in the circle.

Sample pretreatment

For the extraction of different species in biological samples, wet ashing like acid digestion/leaching, alkaline extraction, and enzymatic hydrolysis are commonly used. However, since molecular information in vitro or in vivo is required in speciation, it is crucial to keep the original species intact since the transformation among different species may happen. For example, some studies have shown that artificial methylmercury is prone to form during sample preparation in the presence of inorganic mercury [151,152]. Ultrasound-assisted extraction is a milder way than microwave-assisted digestion and has been found to have the same efficiency in the study of plant samples [153], however, significant transformation still took place during ultrasound-assisted alkaline digestion of biological samples [154]. Thus, a mild mercaptoethanol extraction method for determination of mercury species in biological samples by HPLC-ICP-MS has been proposed by Wang et al. [155].

Separation

After extraction, the separation of different species is needed before detection. The separation can be performed through further extraction, electrophoresis, or other techniques. The further extraction process can be done by (1) liquid–solid extraction like HPLC, solid-phase extraction (SPE), solid-phase microextraction (SPME); (2) liquid–liquid extraction like solvent extraction, pressurized liquid extraction (PLE, or accelerated solvent extraction, ASE), subcritical water extraction (SWE), supercritical fluid extraction (SFE); and (3) liquid–gas extraction like GC and purge-trap techniques [156,157]. Electrophoresis techniques for elementome separation include 1D or 2D GE, capillary electrophoresis (CE), capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micelle electrokinetic capillary electrophoresis (MEKC, or micelle electrokinetic capillary chromatography, MECC) and capillary array electrophoresis (CAE) [158]. The combination of the merit of both HPLC and CE leads to capillary electrochromatography (CEC) which has also been widely used in separation steps [159,160].

Electrophoresis-based separation techniques have been widely used in proteomics study. The most commonly used electrophoresis-based techniques is GE, which is a procedure for separating a mixture of molecules through a stationary material (gel) in an electrical field [161]. The GE, especially 2D GE, is a powerful tool for protein separation but is hard to be connected online with the detectors. Although LA-ICP-MS can be used for detection, the process is difficult to be automated and thus is a low-throughput process in general. On the other hand, CE is easily automated, which makes high-throughput separation possible. CE is a family of techniques used to separate a variety of compounds. These analyses, all driven by an electric field, are performed in narrow tubes and can result in the rapid separation of many hundreds of different compounds. The versatility and number of ways that CE can

be used means that almost all molecules, and even whole organisms can be separated using the powerful methods [162].

Extraction-based separation techniques are generally low-throughput. For example, in both HPLC and GC separation, only one injection is possible for one run. However, although it is still one injection for one run, the application of a number of SPE columns at the same time may realize batch separation. Column-switching is another technique that can improve separation efficiency by reducing sample preparation time [163–165]. 2D HPLC has been proposed by combing application of two columns for separation of proteins and POPs like PCBs, PCDD/Fs, and PAHs [166–168]. Gradient elution 2D LC is most important to peptide and protein separation, and is used as an alternative to 2D GE for use in proteomics [169]. The greatest impediment to the widespread use of 2D LC is its long analysis time ranging up to days per analysis, but this problem can be resolved by improving the speed of gradient separations such as using high-temperature, ultrafast HPLC conditions [170]. Besides 2D HPLC, automated multidimensional LC has also been proposed with enhanced resolution [171–173]. However, 2D and multidimensional HPLC are in fact also column-switching methods.

Membrane separation is based on applying a driving force across a membrane and transporting matter from one phase to the other, including dialysis, electrodialysis, filtration, and membrane extraction [174]. Therefore, membrane separation is indeed a phase–phase extraction process. In membrane separation techniques, membrane extraction has been paid much attention since these techniques require very little solvent and provide remarkable clean-up efficiency, and can also provide very high concentration enrichment factors of hundreds or more. Further, the readily automating and connecting online to chromatography systems and other instruments also make high-efficiency separation possible.

Identification

The general procedure of identification of known and stable compounds like methylmercury [175], selenium [176,177], arsenic [178], or organotin species [179], is generally done by comparing the retention time between standards and samples. However, only the same retention time does not guarantee that they are the same species. Further, owing to the complexity of biological samples, unknown chromatographic peaks are often found that cannot match with any known standard species in practice. Thus, it is often required to use molecular mass spectrometry like matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry (MALDI-TOF-MS) or electrospray ionization (ESI)-MS to confirm the species or to get the molecular information of the unknown species [180,181]. MALDI-TOF-MS is hard to connect with separation techniques like HPLC. Therefore, it is generally offline identification of species. ESI-MS can be connected to separation techniques like HPLC, which makes online identification of unknown species possible [182,183], and the hyphenated techniques using HPLC-ICP-MS online with ESI-MS have been done in the identification of a number of Se-containing compounds in selenized yeast aqueous extracts [184].

Although MALDI-TOF-MS or ESI-MS can be used in the identification steps, it should be noted that the detection sensitivities for organic molecules obtained by organic mass spectrometry are much lower than the inorganic mass spectrometer like ICP-MS. Therefore, sensitivity-matching in the hyphenated system, for example, using ESI-MS and ICP-MS, is required [10].

Structural analysis

A series of techniques can be used for elementome structure characterization like ray-based, magnetic resonance-based, and spectroscopy-based techniques, etc. [185].

Ray-based techniques can characterize the structure on the atomic level. The rays that can be used for structure analysis include X-ray, γ-ray, or neutron beam. In the X-ray-based techniques, X-ray crystallography is the most powerful tool for the determination of macromolecular 3D structure at a resolution of 0.15–2 nm, but the requirement for single crystal will greatly limit its application to numerous biological samples. X-ray absorption spectroscopy (XAS), especially extended X-ray absorption fine structure (EXAFS), may provide an alternative tool for the local structure around certain atoms at a resolution of 0.0001–0.001 nm without the requirement for crystalline samples [186]. For example,

Hg in human hair and blood samples from long-term, mercury-exposed populations have been studied using EXAFS, and the structural information like bond distances and coordination numbers of Hg was obtained [187,188]. Further, EXAFS can give a refinement of the structure obtained from X-ray crystallography since EXAFS has a higher spatial resolution than X-ray crystallography especially in local structures [189–191]. Neutron beam-based techniques like single-crystal neutron diffraction spectroscopy (SCND) can provide complementary structural information at about 20 nm obtained from X-ray-based techniques since the light atoms like hydrogen can be easily detected even with the existence of heavier ones [192]. Both small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) are structural characterization tools of solid and fluid biological samples at relatively lower resolution (ca. 100 nm) than single-crystal X-ray or neutron diffraction spectroscopy but again does not need crystalline samples [193].

Just like XAS, Mössbauer spectroscopy is also a kind of local structure characterization tool, but is based on the γ-ray absorption or emission rather than X-ray absorption [194]. The major limitation for the application of Mössbauer spectroscopy in biological samples is that only a few isotopes can be used. The most commonly used isotope is ^{57}Fe, which has been used for the study of ferritins in biological samples, but the low content and natural abundance of Fe may hinder its application [195].

Nuclear magnetic resonance (NMR) spectroscopy is a potentially powerful alternative to X-ray crystallography for the determination of macromolecular 3D structure at the same resolution of 0.15–2 nm [196]. NMR has the advantage over crystallographic techniques in that experiments are performed in aqueous solution as opposed to a crystal lattice. Besides, NMR can be used to determine protein secondary structure content empirically [197]. The major difference of the structure of single crystals obtained through NMR or X-ray crystallography is that groups (10–50) of structures each satisfy the experimental constraints equally well for one unit cell from NMR while only one, or at most a few structures, will be obtained from X-ray crystallography. Therefore, for NMR, the potential problem is either the entire ensemble of structures are evaluated or a mean conformation is produced and then evaluated [198]. Mean structures from ill-defined portions of the polypeptide chain will have nonstandard geometries and may cause problems in analyses.

Spectroscopy-based techniques, including UV–vis absorption spectroscopy; UV–vis luminescence spectroscopy such as molecular fluorescence spectroscopy, molecular phosphorescence spectroscopy, and chemiluminescence spectroscopy; circular dichroism spectroscopy (CD); and the aforementioned FTIR and Raman spectroscopy can give structural information on a molecular level. For example, CD spectra between 260 and approximately 180 nm can be analyzed for the different secondary structural types like alpha helix, parallel, and antiparallel beta sheet, turn, and other [199].

All these techniques that use structure characterization are generally low-throughput, considering the number of samples treated at one time and the time used for one sample, but attempts on high-throughput techniques like high-throughput XAS by automation of arraying small samples, rapid data collection of multiple low-volume low-concentration samples, and data reduction and analysis have been proposed [200].

Attempts on simultaneous speciation of species of different elements in biological samples have been performed. Some selected papers studying simultaneous multielemental speciation are listed in Table 2 [201–205]. Since selenium plays important roles in detoxification of both arsenic and mercury, much effort has been expended on the simultaneous speciation of both selenium and mercury species or both selenium and arsenic species by HPLC-ICP-MS [206–208]. Solvent extraction with NAA detection for different organohalogen species in biological samples like milk, human tissues, and pine needles has been studied [209–213].

Table 2 Selected papers studying simultaneous multielemental speciation in biological samples.

Sample	Elements	Method	Detection limit	Ref.
Tuna fish	As and Se	HPLC-ICP-MS	7–12 ng/mL	[208]
Oyster tissue	Hg and Sn	CGC-ICP-ID-MS	Hg: 0.11–0.24 g/kg Sn: 0.15–0.30 g/kg	[204]
Oyster tissue	Hg and Sn	GC-ICP-MS		[205]
Oyster tissue	Hg, Sn and Pb	SPME-MCGC-ICP-TOF-MS	Sn, Pb: <pg/g Hg: 1.3–2.0 pg/g	[202]
Human urine	Hg, Pb, and Sn	SPME-GC/MS-MS	7–22 ng/L	[201]
Human urine	Hg and Se	HPLC-ICP-MS	Se, 0.05–0.3 μg/L Hg, 1.48–2.0 μg/L	[207]
Human urine	Ge, Se, Sn, Sb, Hg	HG/LT-GC/ICP-MS	2–12 pg/L	[203]
Human urine	Se and As	HPLC-ICP-MS	As, 0.1–0.4 μg/L Se, 0.7–2 μg/L	[206]
Human tissue	EO-Cl, -Br, -I	NAA	0.1 μg/g	[210]
Pine needles	EO-Cl, -Br, -I	NAA	Cl 50 ng, Br 8 ng, I 3.5 ng	[209]

CONCLUSION AND PERSPECTIVES

As an integrated biometal science, metallomics has received more and more attention. However, if taking the interaction of various elements into consideration, the term itself may be restrictive. Elementomics may therefore be considered as extended concepts of metallomics in which both metal and nonmetal elements will be studied in biological systems. The complexity of metallomics and elementomics in living organisms results in significant analytical challenges. With the same objects as metallomics, the comprehensive quantification, distribution, speciation, identification, and structural characterization of elementome require high-throughput and powerful analytical techniques. Thus, some of the analytical techniques which can or at least have promise to be high-throughput in elementomics study have been reviewed. In general, high-throughput quantification of multielements can be achieved by ICP-MS and NAA while the high-throughput multielement distribution mapping can be done by fluorescence-detecting techniques like SR-XRF, XRF tomography, EDX, PIXE, and ion-detecting-based SIMS. LA-ICP-MS can also realize multielement distribution mapping. For molecular distribution, FTIRM and Raman microspectroscopy are excellent tools. When it comes to identification and structural characterization of elementome, all the techniques are generally low-throughput. Therefore, this should be the bottleneck in elementomics study. However, by automation of arraying small samples, rapid data collection of multiple low-volume low-concentration samples, and data reduction and analysis, high-throughput techniques may be and in fact have partially been achieved.

Bioinformatics uses computer software tools for database creation, data management, data warehousing, data mining, and global communication networking, which is the recording, annotation, storage, analysis, and searching/retrieval of nucleic acid sequence (genes and RNAs), protein sequence, and structural information [214]. With the development of metallomics and elementomics, especially high-throughput detection, distribution, speciation, and characterization of metallome and elementome, more and more data will be obtained. Therefore, bioinformatics tools will also be highly needed to treat such a huge amount of data and perhaps the databases of metallome and elementome quantification, distribution, speciation, and structural information as well as methods to access, search, visualize, and retrieve the information.

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