

Metals and biomolecules - bioinorganic analytical chemistry

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Bioinorganic trace analytical chemistry is a rapidly developing field of research at the interface of trace element analysis and analytical biochemistry. It targets the detection, identification and characterization of substrates and products of reactions of trace metals and metalloids with the components of living cells and tissues. Hyphenated techniques based on the coupling of a separation technique (HPLC or CZE) with ICP-MS or ESI-MS/MS are becoming a fundamental tool for the functional characterization of trace elements or otherwise inconspicuous metal ions in biological systems.

The great efforts made by the living organisms to take up, accumulate, transport, and store essential or toxic metals and metalloids are realized by surrounding the metal ion by electron-pair donating biological ligands. The evolution of a metal in a living organism happens by its interaction with the highly complex bioligand coordinating environment. Species of inorganic elements had been known in the chemistry of life long before the term "chemical speciation" was born. Iron-containing hemoglobin, magnesium-containing chlorophyll, and arsenic-containing arsenobetaine and arsenosugars have been the landmark "bioinorganic" compounds issued from the field of the chemistry of natural products. Importance of zinc as a vital component of many enzymes has been known since the 30ties whereas the 60ties and 70ties marked the birth of a new interdisciplinary research area referred to as bioinorganic chemistry. Its major center of gravity has been the study of the influence of metal ions, ligands, and metal complexes on human health [1-3].

Work of an analyst has always been the hardly ever noticed fundament of bioinorganic chemistry. It is more true for this discipline than for any other one that its progress has been limited by the continuous inadequacy of the analytical methodology. Indeed, already analysis for total metals at the biological naturally occurring levels is difficult and the state of the art techniques can still hardly cope with many problems [4]. The trace or ultratrace quantity of the metal of interest is divided among several species in which the contribution of the metal to the total structure, however vital, is minute in terms of weight. The species has also the second component - a macromolecular ligand of the biological origin that needs a characterization. The often low thermodynamic stability of the complex makes the analytical task even more complex.

The recent progress in microscale isolation and purification techniques, such as, especially HPLC, CZE and capil-

lary electrochromatography, on one hand, and the increasing sensitivity of trace analysis by ICP-MS on the other hand, were at the origin of a new generation of analytical methodology based on the coupling of a high performance separation technique with an ultrasensitive atomic spectrometric detector [5]. This analytical approach, still perceived as a curiosity by chromatographers who see in ICP-MS another element selective detector, and by spectroscopists, who see in chromatography just another sample introduction technique, is becoming a fundamental tool for the functional characterization of trace elements or otherwise unaccounted for metal ions in biological systems. Bioinorganic analytical chemistry is a rapidly developing field of research at the interface of trace element analysis and analytical biochemistry which targets the detection, identification and characterization of complexes of metals (metalloids) with molecules of natural origin (biomolecules) by coupled (hyphenated) techniques.

Targets of the bioinorganic analytical chemistry

Bioligands complexing metals are schematically shown in figure 1. Species of interest from the point of view of the bioinorganic analytical chemistry can be divided in five major categories:

a) biosynthesized molecules with the "true" metal(metalloid) - carbon bond.

This category includes selenoaminoacids and their higher analogues: selenogluthathione and selenoproteins. They can coordinate metals, especially Hg, using the Se atom as the coordination center. Another important class includes organoarsenic compounds, e.g. arsenobetaine and arsenosugars.

b) complexes with aminoacids, oligopeptides and polypeptides (proteins).

Metal complexes with proteins, including enzymes, are carriers of biochemical function. Whereas the carboxamide function itself of peptide bonds $-C(=O)-N(-H)-$ is only a poor metal coordination site, peptides contain several functional groups in the side chains that are particularly well suited for metal coordination. They include especially cysteine ($-CH_2SH$) and methionine $-CH_2CH_2SCH_3$, which bind metals with sulfur affinity (Cd, Cu, Zn) in compounds such as glutathione, phytochelatin, and metallothioneins; and histidine of which both nitrogen atoms become available for coordination after metal-induced deprotonation (e.g. Cu, Zn in superoxide dismutase).

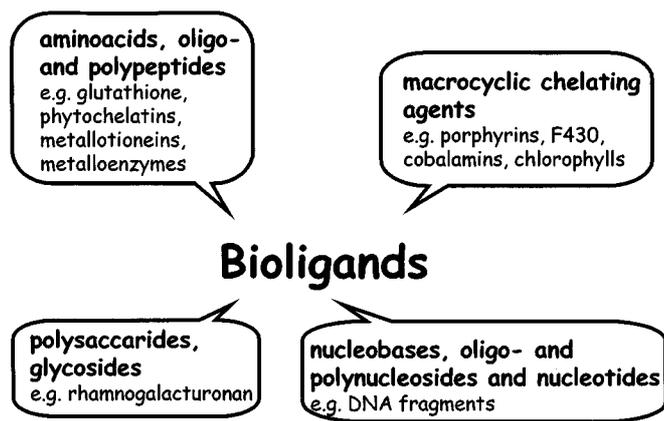


Figure 1. The principal bioligands complexing metals in biological samples.

c) complexes with nucleobases, oligo- and polynucleotides, and –nucleosides.

Heterocyclic nucleobases, alone or as constituents of nucleosides or nucleotides offer several different coordination sites for metal ions. Of particular interest is the coordination of metal ions, e.g. CrO_4 or inert metal complexes to DNA because of the specificity with regard to certain base-pair sequences in the double helix.

d) complexes with biosynthesized macrocyclic chelating agents.

The most important group is the analogues of tetrapyrrole which in their deprotonated form can tightly bind even relatively labile divalent metal cations. The best known compounds of this group include chlorophyll and products of its degradation, cobalamins (the coenzymatically active forms of vitamin B_{12}), and porphyrins including the heme group found in hemoglobin, myoglobin, cytochromes and peroxidases.

e) complexes with other biomacromolecules (polysaccharides, glycoproteins).

Relatively little is known about the relevance of metal coordination to lipids and carbohydrates, although the potentially negatively charged oxygen functions can bind cations electrostatically and even undergo chelate coordination via polyhydroxy groups.

Analytical tools in bioinorganic chemistry

In the classical speciation analysis the analyte is usually precisely defined and calibration standards are generally available. This fact allows the development of an analytical procedure for the standards and... makes a lot of work end there without the need for a real sample.

A virtually different situation is faced by an analyst interested in naturally occurring species in biological tissues. The first problem is to understand what species is actually the analytical target. Some metal-protein complexes have been

identified, but the majority, especially those at lower trace and ultratrace levels have remained yet undiscovered. To date, the applications of hyphenated techniques to bioinorganic chemistry have been rather exploratory (looking for new species) than confirmatory (determining an expected-to-be-found compound). The two acute problems associated with the reliability of the biochemical speciation analysis include (i) the question whether a signal produced by the detector belongs to one particular compound, and (ii) the identification of this compound. In order to reach these objectives a special strategy, shown schematically in figure 2 should be adapted. It includes the following steps: (i) investigation whether there are stable species of the interesting element in the sample, (ii) verification whether the detected signal corresponds to one or more species, (iii) isolation of the species of interest, and (iv) characterization of this (these) species.

For this purpose, a dual strategy based on (i) the application of orthogonal (complementary) separation techniques to assure the purity of the compound arriving at the detector, and (ii) the application of complementary (targeting the metal and the organic moiety) detection techniques, is necessary. This issue is illustrated in figure 2 on the example of speciation of metallothionein (MT)-bound cadmium (MT) in rabbit liver.

Detection of species

The ease of coupling size-exclusion chromatography (SEC) with ICP-MS (the compatibility of the mobile phase in terms of composition and flow rate with the nebulizer) has made it a primary technique able to detect stable metal complexes with macromolecules (cf. Makarov and Szpunar, this issue). Metal ions are retained on the residual ion-exchange sites of the support and very labile complexes do not pass through the column. The information obtained by SEC-ICP-MS allows (i) the detection of clusters of compounds with roughly similar molecular mass, and (ii) making a balance of all the formes present in comparison with the total concentration of element. The purity of peaks can theoretically be verified by SEC-ESI-MS but the separation from a complex sample matrix is usually insufficient which affects negatively the sensitivity and selectivity of the ESI-MS signal. Fractions containing the analytical peak are heart-cut and preconcentrated by lyophilization.

Purity of the species

Another separation mechanism should be used to verify whether the SEC peak contains one or more element containing compounds and to remove the matrix which should enable an analysis by mass spectrometry. The techniques are ion-exchange and reversed phase chromatography and capillary zone electrophoresis (CZE). ICP-MS is commonly used for detection because of its unmatched sensitivity.

Identification and characterization of the species

Electrospray MS allows the determination of the molecular mass and, in the case of small molecular mass compounds, at least partial elucidation of the structure. This technique can be applied on-line in microbore, usually reversed-phase, chromatography but preparative isolation of the compound of interest may be necessary.

1. Detect the presence of stable metal or metalloid species in a sample by SEC HPLC with a sensitive element selective detector (ICP-MS or HR ICP-MS)



2. Check whether your signal contains only one species by using orthogonal separation techniques (IEC or RP HPLC, CZE) with ICP-MS detection



3. Scale up the separation procedures to isolate and purify the target species



4. Complete its characterization by ESI tandem MS, NMR, etc.

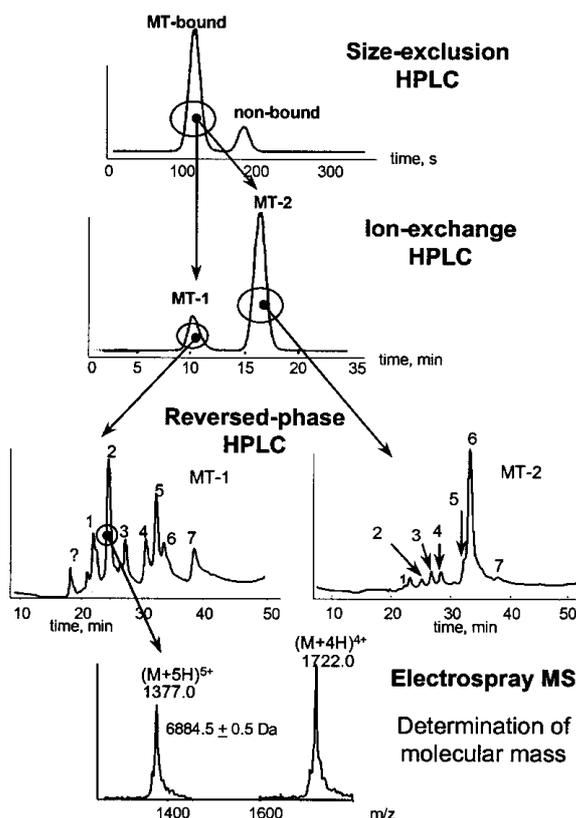


Figure 2. Analytical strategies in bioinorganic chemistry.

Bio-inorganic analytical chemistry - fields of economic and social interest

The high interdisciplinarity of bioinorganic chemistry has been responsible for the variety of fields that have benefited from the development of species-selective analytical methodology.

Ecotoxicology and ecophysiology

Homeostatic control, metabolism and detoxification of a number of essential (Zn, Cu) and toxic (Cd, Hg, As) trace elements by environmental biota has been in the focus of interest because of environmental and economic consequences. The most famous area is research on phytochelatins (cadystins) and metallothioneins (MTs). Phytochelatins are short metal-induced sulfhydryl-rich peptides possessing the general structure $(-GluCys)_n-Gly$ with $n = 2 - 11$. Metallothioneins are a group of non-enzymatic low molecular mass (6 – 7 kDa), cysteine-rich metal-binding proteins, resistant to thermocoagulation and acid precipitation. The bioconcentration of metals by plants and animals is an area of research that should be able to provide answers to the fundamental questions of plant and animal biochemistry, nutrition and stress physiology. Methylation of mercury and the formation of arsenobetaine and arsenosugars in marine fauna have been two other major fields of intense bioinorganic speciation-related research.

Environmental chemistry. Waste management

The use of specially engineered metal-accumulating plants to remove metals from the contaminated environment (phytoextraction) has been attracting considerable attention. The optimization of the processes of phytoextraction (the use of metal-accumulating plants to remove toxic metals from soil), rhizofiltration (the use of plant roots to remove toxic metals from polluted waters), and phytostabilization (the use of plants to eliminate the bioavailability of toxic metals in soils) is dependent on the better understanding of the biological mechanisms of toxic metal uptake, translocation and resistance that are controlled by metal speciation.

Agricultural and nutritional sciences. Food industry

Both essential and non-essential trace elements are taken into the body with foodstuffs and some of these elements may be biologically incorporated in food itself. Classic speciation analysis puts strong emphasis on the evaluation of risk induced by the contamination of foodstuffs (oysters and mussels by organotin, fish by methylmercury, wine by automotive lead emissions). Fish, shellfish and wine are important commodities and legislation based on species will be more appropriate than one based on the total element analysis. Large arsenic contents in foodstuffs no longer frighten the consumers since it was proven that practically all the arsenic is present as the non-toxic arsenobetaine.

Another important aspect is the bio-availability of a trace element incorporated in a food material that may be markedly different to a simple ion. Therefore a diet that, on the basis of a total element analysis, appears to provide adequate amounts of a particular element may in fact be quite inadequate because a large fraction of the metal is present in a highly insoluble fraction. And conversely, the bioavailable form can be a particular species that must be present in the food itself (e.g. Co^{2+} and vitamin B_{12}).

The use of Se and Fe in supplements is increasingly popular; the risks and benefits to the consumer are, however, not known, especially in view of the narrow range between toxicity and essentiality for these elements.

Clinical biochemistry

Clinical chemistry is apparently the most prospective field for speciation analysis which is still unexplored due to inadequate sensitivity of the instrumentation available, and often low thermodynamic stability of the analytes. Metalloproteins have a number of vital (regulatory, storage, catalytical, transport) functions in humans. The largest interest is attracted by essential elements which include some transition metals such as Fe, Cu and Zn associated with ferritin (Fe, Cu, Zn), α -amylase (Cu), alcohol dehydrogenase (Cd, Zn) and carbonic anhydrase (Cu, Zn) and other proteins. The oxidation states of these elements (redox speciation) are of concern as well. The interest in elemental speciation in breast milk is stimulated by the species-dependent difference in bioavailability of trace elements by formula-fed and by breast-fed children.

Medecine and pharmacology

Metals are components of many therapeutic drugs. A wide range of Tc compounds (e.g. Tc-labeled antibodies, Tc-mercaptoacetyl glycine complex) are used for diagnostic imaging of renal, cardiac and cerebral functions and of various forms of cancer. Platinum (cisplatin, carboplatin), Ru^{3+} (*fac*- $[\text{RuCl}_3(\text{NH}_3)_3]$) and gold (auranofin) compounds are well-known in cancer therapy whereas some other gold compounds (aurithiomalate, aurothioglucose) are important

antiarthritic drugs. Another area of concern is speciation of Co, Cr, Fe, Se, Zn in solutions for parental nutrition and oral supplements. On one hand, the essential trace element should be in a chemical form that guarantees its optimal bioavailability in the drugs and intravenous solutions. On the other hand, the consequences of trace element contamination of intravenous solutions by impurities from the ingredients can be reinforced by the chemical forms of these elements. For example, Al and Cr bound to small molecules were found to accumulate in dialysis patients.

The need for interdisciplinarity

The evaluation of risks posed by metals and metalloids to the environment and, consequently, to human health can be properly done only by the knowledge of their speciation in the environment, foodstuffs and human tissues. To be effective, research in these fields has to be multidisciplinary and must not be carried out in isolation. Bioinorganic analytical chemistry being at the crossroads of interest of many disciplines can profit from the interdisciplinarity in the same degree that it can suffer from the lack of it. The need for an interface between analytical chemists and eco- and clinical toxicologists, nutritionists, biochemists and pharmacologists is urgent and is the prerequisite to fertilize the speciation-related research with novel breaking ideas.

References

1. Williams, R. J. P. Bioinorganic chemistry: Its conceptual evolution, *Coord. Chem. Rev.* **1990**, *100*, 573.
2. Lippard, S. J.; Berg, J. M. Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, California, 1994.
3. Kaim, W.; Schwederski, B. Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life, Wiley, Chichester, 1994.
4. Lobinski, R.; Marczenko, Z. Spectrochemical Trace Analysis for Metals and Metalloids, Elsevier, Amsterdam, 1996.
5. Lobinski, R. Elemental Speciation and Coupled Techniques, *Appl. Spectr.* **1997**, *51*, 260A.