Biochemistry & Analytical Biochemistry
ISSN: 2161-1009

Hayashida, Biochem Anal Biochem 2015, 4:4
DOI: 10.4172/2161-1009.1000231

Review Article Open Access

Metallomics and Metal Effects in Vascular System

Naoki Hayashida*

Department of Bio-Signal Analysis, Yamaguchi University School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan

Abstract

All living things cannot maintain their homeostasis or survive without metals. For example, the homeostasis of metal ions; iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), potassium (K), sodium (Na), and calcium (Ca), is critical for many biological activities. On the other hand, the disorder of metal homeostasis leads to various human disorders including vascular system abnormalities. Metallomics is an analytic technology investigating the entirety of metals and metalloid species within a cell or tissues. Metallomics is an emerging field as well as metalloproteomics, however, the technological advances are very rapid and brings a lot of novel information. Metallomics consists of three main techniques; Atomic absorption spectrometry (AAS), Inductively coupled plasma (ICP), and X-ray fluorescence spectrometry (XRF). These novel techniques are recently applied to the studies of human diseases related to vascular system. Therefore, the significant information contributing to the development of novel therapy will be given us by metallomics in the near future.

Keywords: Metal; Metallomics; Atomic absorption spectrometry; Inductively coupled plasma; X-ray fluorescence spectrometry; Metalloprotein; Vascular system; Vascular cells

Introduction

Metals are critical components for living things and it is estimated that one third of all proteins require metal ions as vital cofactors for their functions and activities [1-3]. The homeostasis of metal ions including iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), potassium (K), sodium (Na), and calcium (Ca), is essential for many biological activities [4]. The surplus or deficit of these metal elements lead to various human diseases. Menkes disease is an example of genetic disorder of Cu metabolism caused by mutations of ATPase, Cu^{1+}-transporting, polypeptide (ATP7A). A lack of this protein or activity causes disorders in neuronal and vascular systems [5,6].

Metalloproteins are proteins that contain metal ion as cofactor. They belong to the most diverse classes of protein with intrinsic metal atoms providing a catalytic, regulatory and structural role that is essential to protein function [7]. Transition metals such as copper, iron, and zinc play important roles in survival of living things. For example, Zn is the most abundant cellular transition metal and plays vital roles for functions of more than 300 enzymes in DNA stabilization and gene expression [8]. Some metals are crucial for individual tissues or organs function, thus imbalance of homeostasis or deficiency of these elements can result in dysfunction causing serious disorders [9,10].

Recently, similar to genome and proteome, the 'metallome' become frequently used word in biology and medical science. The metallome is the distribution of inorganic species in cell. Moreover, metallomics and metalloproteomics appear as emerging fields addressing the role, uptake, transport and storage of the trace metals essential for living things. Metallomics is defined as the analysis of the entirety of metal and metalloid species within a cell or tissues, whereas metalloproteomics focuses on exploration of the function of metals associated with proteins [11].

This review introduces basic metallomics techniques and their progress, and also show the novel findings in vascular system by metallomics.

Technology in Metallomics

The metallomics is a novel experimental and bioinformatics approach that can identify the protein (metalloprotein), determine the concentration, condition, structure, and meta-metal distance [12-14]. Metallomics is mainly comprised of three quantitative technologies. These technologies are

1) Atomic absorption spectrometry (AAS), 2) Inductively coupled plasma, and 3) X-ray fluorescence spectrometry (XRF). These metallomics techniques are individually improved, and optimal metallomics techniques have been properly developed for the purposes.

Atomic absorption spectrometry (AAS): AAS can measure the metals using the optical light that is absorbed by these atoms in gas phase, thus determine the concentration of the objective metal. AAS can be used to determine over 70 different metals in solution.

There are two main types of atomic absorption instruments: flame-AAS (FAAS) and graphite furnace-AAS (GFAAS). In FAAS, the process of drying, pyrolysis and atomization occur at the same time, however, the same processes are separated in GFAAS. Owing to this property, GFAAS has higher sensitivity and can detect lower amount of metals than FAAS. In addition, FAAS is only able to analyze solutions, but GFAAS can analyze solutions, slurries and solids. GFAAS has strong points that FAAS does not have.

Compared to GFAAS, AAS is relatively inexpensive and simple to use and has high precision with lower interferences. However, AAS can detect only one metal at a time and is not applied for isotope analysis. In addition, the light source of AAS is typically a hollow-cathode lamp of the analyte of interest, thus one hollow-cathode lamp is limited to single metal element detection. In order to improve the detection limits, optimizing techniques such as flow injection [15], preconcentration/matrix separation [16,17], as well as adding chemical modifiers to increase the signal-to-noise ratio is tried to be combined to AAS.

***Corresponding author:** Naoki Hayashida, Department of Bio-Signal Analysis, Yamaguchi University School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan, E-mail: hayasida@yamaguchi-u.ac.jp

Received: December 03, 2015; **Accepted:** December 08, 2015; **Published** December 11, 2015

Citation: Hayashida N (2015) Metallomics and Metal Effects in Vascular System. Biochem Anal Biochem 4: 231. doi:10.4172/2161-1009.1000231

Inductively coupled plasma (ICP): ICP is also commonly used for metal determination as well as AAS. This technique can be paired with atomic emission or mass spectrometric detection. Inductively coupled plasma-atomic emission spectrometry (ICP-AES) uses an argon (Ar) plasma to excite atoms and ions of analytes, and then detects the characteristic emission given off by these excited atoms and ions. The emission intensity is proportional to the concentration of the analyte of interest, thus ICP-AES makes it possible to measure the metals. The principle of Inductively coupled plasma-mass spectrometry (ICP-MS) is similar to that of ICP-AES, an Ar plasma is used to atomize and ionize the sample, and these charged species enter the mass spectrometer. The mass spectrometer separates the ions based on their mass-to-charge ratio, and determines the specific metals and metal species. Both ICP-AES and ICP-MS are capable of simultaneous detection of more metal elements, but the minimum detectable concentrations for ICP-MS are lower than those of ICP-AES. ICP-AES can handle a higher dissolved solid sample than ICP-MS, but it does not have the capability for isotope analysis as does ICP-MS. Although ICP requires a higher skill level to run the instrument, it is cost-effective and the detection benefits are well worth the effort.

X-ray fluorescence spectrometry (XRF): Basic XRF have been repeatedly improved, and a lot of modified XRF techniques are developed and used compared to AAS and ICP. For example, Synchrotron X-ray microscopy, X-ray absorption spectroscopy (XAS), Microfocused X-ray fluorescence (μ-XRF) spectrometry, Microprobe X-ray fluorescence elemental mapping (μSXRF), X-ray absorption near-edge spectroscopy (XANES), total-reflection X-ray fluorescence spectrometry (TXRF) [18], Extended X-ray absorption fine structure spectroscopy (EXAFS) [19], x-ray fluorescence microscopy (XFM) and Microfocused XAS (μ -XAS) [20] are already used.

These XRF techniques have their own strong points. EXAFS can evaluate the oxidation state of the metal, and can unveil the number, identity and distance to its neighboring atoms under certain condition. X-ray microprobe can be involved in XRF techniques, and this is used to resolve the quantitative and qualitative questions about metal bioavailability with minimal sample alteration [21,22].

As well as X-ray microprobe, other XRF techniques also have been applied to biological tissue analysis in the clinical medical sciences. For example, TXRF revealed that increased Cu concentration and decreased Ca and selenium (Se) concentrations in peripheral vascular disease patients compared to healthy control subjects [18]. The synchrotron radiation has been used to analyze the distribution and quantification of Zn and Ca in prostate cancer tissues [23]. This analysis revealed a role of Ca in the control of Zn transport into prostate tissues without the isolation and purification steps. The distribution and chemical form of Fe in brain tissue from Parkinson disease patients was investigated using similar techniques. Other XRF technique μ -SXRF was also used to examine the detailed cellular mechanisms of Parkinson's disease, and found Fe accumulation in specific structures that had not been histopathologically identified. This result indicates μ-SXRF has the micrometer-scale precision in samples that are too fragile for conventional dissection [24]. In this case, the comparison of the elemental concentration (μg cm-1) of individual neurons in healthy and diseased tissues was also succeeded.

The importance of metals in protein structure and function and metallochaperones

It is widely known that a lot of proteins (probably more than 30% of total proteins) require metals in order to maintain their structure and functions [25-27]. Metalloproteins play key roles in many biological processes, including respiration, nerve transmission, and defense against toxic agents. Metallochaperone is a protein that binds to metal ions and delivers them directory to target proteins, especially enzymes, via protein-protein interactions [27]. Therefore, metallochaperones are required for all metalloproteins and essential for cell survival, and metalloproteins cannot maintain their structure or function without metallochaperones.

The functions of metals in vascular system

The three pivotal metallomics technologies, Atomic absorption spectrometry (AAS), Inductively coupled plasma (ICP), and X-ray fluorescence spectrometry (XRF) are improved year by year and novel metallomics techniques have been produced. However, the prototype techniques of metallomics were used in the analysis of biological significance of metals before and gave us a lot of findings. Here, the findings related to vascular system produced by old and new generation metallomics techniques are summarized.

Calcium and vascular cells

In the history of the studies investigating the role of metals in vascular cells, calcium (Ca) was extensively examined compared to other metals using old techniques because Ca has essential roles in normal cardiovascular function and cardiovascular diseases including atherosclerosis (atherosclerotic vascular disease) and hypertension [28]. Importantly, vascular calcification is a prominent feature of atherosclerosis [29]. Atherosclerosis is critical in pathophysiology and clinical medicine, therefore, many researches of vascular calcification have been carried out.

Vascular calcification is caused by the deposition of calcium phosphate in the vessel wall. Also, calcification occurs in the intima and media of the vessel wall with aging and diabetes. Recently, Ca concentration in the calcified aortic and carotid plaques was quantified by ICP-AES [29]. The mean Ca concentration was 9.83 and 11.94 wt.%, respectively. The iron and zinc concentration was also investigated, however, iron (Fe) concentration was below the measurement limit and zinc (Zn) concentration was very variable among samples. Past clinical studies showed elevated circulating calcium, phosphate, and calcium phosphate product levels to be correlated with increased vascular calcification in patients with end-stage renal disease [30].

Under normal condition, Ca has essential roles in vascular smooth muscle cells and Ca channels are expressed in these cells [31]. Therefore, there is a possibility that metallomics approach can reveal Ca functions in normal and damaged vascular cells.

Copper and vascular cells

Copper (Cu) influences numerous proteins important for angiogenesis by nascent vasculature [32-34]. Copper metalloproteins are prevalent and important in all cells thus it was not easy to analyze Cu bioavailability specific to angiogenesis.

Recent developments in metallomics techniques succeeded these analysis. For example, in human microvascular endothelial cells, X-ray fluorescence (XFM) revealed relocalization of intracellular Cu across the cell membrane [35]. The authors also revealed Cu clustering in putative neoangiogenic areas of highly vasculalized human breast-infiltrating ductal carcinomas by XFM.

Total-reflection X-ray fluorescence spectrometry (TXRF) revealed the Cu concentration in peripheral vascular disease patients [18]. The authors obtained the Cu concentration of 20.3 μg/g in plasma. In this

study, they also measured plasma total homocysteine because there may be relationship between homocysteine level and atherosclerosis growth. This study also showed the strong correlation between Cu concentration and plasma total homocycteine levels in peripheral vascular disease patients [18].

Cu has other roles in vascular cells. Neovascularization requires the formation of new blood vessels, this process is very sensitive to Cu levels [32,36]. In addition, normal vascular cells secretes Cu ions to blood. As indicated above, copper metalloprotein has diverse functions. Thus more application of apparent metallomics approaches can give us novel information of Cu roles in angiogenesis, vascular cells, and total vascular system.

Other metals and vascular cells

In addition to calcium and copper, other metals have important function in vascular cells. Chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), zinc (Zn), cadmium (Cd), gadolinium (Gd), arsenic (As), lead (Pb) can target the vascular system. Especially, the toxic metals Cd, As, and Pb disturb the homeostasis of vascular cells in a variety of ways, ranging from hemorrhagic injury to subtle pathogenic remodeling and metabolic changes [37].

Concerning Cd, acute Cd exposure results in hemorrhagic injury to the testis vasculature endothelium. Chronic Cd exposure is associated with various cardiovascular disorders such as hypertension and cardiomyopathy. In addition, Cd can inhibit chemotaxis and tube formation of vascular endothelial cells at non-cytotoxic concentrations of 10-100 nM. These angiostatic effects may be mediated through disruption of vascular endothelial cadherin, a Ca^{2+} -dependent cell adhesion molecule.

With regard to As, ingestion of water containing disease-promoting concentrations of As promotes capillarization of the liver sinusoidal endothelium. This capillarization is a hallmark for liver fibrosis and also contributes to an imbalance of lipid metabolism in liver.

Cadmium (Cd), arsenic (As), and lead (Pb) pose serious risks to human health. The importance of these metals as environmental health hazards is readily evident from the fact that all three are ranked in the top 10 on the current Agency for Toxic Substances and Disease Registry Priority List of Hazardous Substances [38]. Toxicity of these three metals most intensively appear in kidney, testis, and liver [37].

Because all metals are not biodegradable, they can persist in the environment and produce a variety of adverse effects. Exposure to these metals can result in damage to a variety of organ systems [39-41].

Metals contained in cigarette smoke and vascular cells

Smoking is a significant risk factor for development of atherosclerosis. The toxicity and influences of the metals on the human health contained in cigarette are well analyzed. It is revealed that cigarette smoke extracts (CSE) contain aluminium (Al), silicon (Si), titanium (Ti), vanadium (V), chromium (Cr), iron (Fe), nickel (Ni), cobalt (Co), copper (Cu), manganese (Mn), zinc (Zn), strontium (Sr), lead (Pb), cadmium (Cd), and barium (Ba) in the range from nM to μ M concentrations by ICP analysis [42]. In addition, it is well known that some metals catalyze protein oxidation by oxidants and the concentrations of metals increased in smokers' blood [43]. Oxidation of cellular proteins causes a loss of microtubule function, and this loss of function results in a contraction of vascular endothelial cells, in other words, leakiness of the vascular endothelium is induced by the metals contained in cigarette smoke [42]. Cigarette smoke may similarly affect every person of all generations, but ICP-MS analysis in young smokers' serum revealed the increased Cd and Sr levels [44]. Moreover, the mRNA level of intermediate filament protein vimentin that is crucial for the maintenance of cell shape was significantly reduced. This result reflects the damages on the structure of the vascular endothelium in young smokers [44]. Metallomics approach is useful to prove the smoking risk by showing the toxic metals concentration in smokers' serum and influence on the vascular cell shape.

Conclusion

It is already known that a variety of metals including transition metals affect vascular system, however, metallomics techniques demonstrated novel findings between metals and vascular system. The main metallomics techniques are classified to three categories, however, the improvement of metallomics techniques is very rapid, now that many types of techniques are available for individual purposes. Metallomics are already applied to not only vascular system but also brain and kidney, thus it is promising that metallomics will inform us entirely novel data in biology. Most of old and recent information are related to the toxicity of metals, but there are no doubts that metalloproteins are required for the maintenance of homeostasis in all cells. In the near future, metallomics will bring us the essential information of specific role of all metals that contributes to our health.

Acknowledgements

This work was supported by JSPS KAKENHI Grant-in-Aid for Scientific Research (C) (25430090 N.H.), and Takeda Science Foundation (23009 N.H.). Thanks to Drs. Shigeru Kakuta, Ken-ichiro Otsuyama, Takeshi Honda, Hiromi Yamane, Rumi Kitamura, Kaoru Hayashida, Akiko Moriyama, Junko Ikenaga, Kaoru Yamamoto, Hideshi Yokoyama, and Shuji Noguchi for encouragement and helps with the experiments. I would like to thank the Yamaguchi University Center for Gene Research, Dr. Yo-ichi Mizukami and the Center staff for their support of my research. Special thanks to Drs. Eisuke Enoki and Yoshiharu Kamosaki for research assistance and encouragement.

References

1. Szpunar J (2000) Bio-inorganic speciation analysis by hyphenated techniques. Analyst 125: 963-988.
2. Blackstock WP, Weir MP (1999) Proteomics: quantitative and physical mapping of cellular proteins. Trends Biotechnol 17: 121-127.
3. Rosenzweig AC (2002) Metallochaperones: bind and deliver. Chem Biol 9: 673-677.
4. Mertz W (1981) The essential trace elements. Science 213: 1332-1338.
5. Poulsen L, Møller LB, Plunkett K, Belmont J, Tümer Z, et al. (2004) X-linked Menkes disease: first documented report of germ-line mosaicism. Genet Test 8: 286-291.
6. Kaler SG, Holmes CS, Goldstein DS, Tang J, Godwin SC, et al. (2008) Neonatal diagnosis and treatment of Menkes disease. N Engl J Med 358: 605-614.
7. Shi W, Zhan C, Ignatov A, Manjasetty BA, Marinkovic N, et al. (2005) Metalloproteomics: high-throughput structural and functional annotation of proteins in structural genomics. Structure 13: 1473-1486.
8. Easter RN, Qilin Chan, Lai B, Ritman EL, Caruso JA, et al. (2010) Vascular metallomics: copper in the vasculature. Vasc Med 15: 61-69.
9. Maret W (2013) Zinc and the zinc proteome. Met Ions Life Sci 12: 479-501.
10. Cerchiaro G, Manieri TM, Bertuchi FR (2013) Analytical methods for copper, zinc and iron quantification in mammalian cells. Metallomics 5: 1336-1345.
11. Shi W, Chance MR (2008) Metallomics and metalloproteomics. Cell Mol Life Sci 65: 3040-3048.
12. Chance MR, Fiser A, Sali A, Pieper U, Eswar N, et al. (2004) High-throughput computational and experimental techniques in structural genomics. Genome Res 14: 2145-2154.

13. Hasnain SS (2004) Synchrotron techniques for metalloproteins and human disease in post genome era. J Synchrotron Radiat 11: 7-11.

14. Szpunar J (2004) Metallomics: a new frontier in analytical chemistry. Anal Bioanal Chem 378: 54-56.

15. Hill SJ, Chenery S, Dawson JB, Fisher A, Price WJ, et al. (2000) Advances in atomic emission absorption and fluorescence spectrometry, and related techniques. J Anal At Spectrom 15: 763-805.

16. Yebra-Biurrun MC, Cespón-Romero RM (2007) Fast ultrasound-assisted extraction of copper, iron, manganese and zinc from human hair samples prior to flow injection flame atomic absorption spectrometric detection. Anal Bioanal Chem 388: 711-716.

17. Gong R, Zhang D, Zhong K, Feng M, Liu X (2008) Determination of trace copper in water samples by flame atomic absorption spectrometry after preconcentration on a phosphoric acid functionalized cotton chelator. J Serb Chem Soc 73: 249-258.

18. Mansoor MA, Bergmark C, Haswell SJ, Savage IF, Evans PH, et al. (2000) Correlation between plasma total homocysteine and copper in patients with peripheral vascular disease. Clin Chem 46: 385-391.

19. Punshon T, Jackson BP, Lanzirotti A, Hopkins WA, Bertsch PM, et al. (2009) Application of synchrotron X-ray microbeam spectroscopy to the determination of metal distribution and speciation in biological tissues. Spectroscopy Lett 38: 343-363.

20. Langner P, Mikutta C, Suess E, Marcus MA, Kretzschmar R (2013) Spatial distribution and speciation of arsenic in peat studied with Microfocused X-ray fluorescence spectrometry and X-ray absorption spectroscopy. Environ Sci Technol 47: 9706-9714.

21. Howe JA, Loeppert RH, DeRose VJ, Hunter DB, Bertsch PM (2003) Localization and speciation of chromium in subterranean clover using XRF, XANES, and EPR spectroscopy. Environ Sci Technol 37: 4091-4097.

22. Arai Y, Lanzirotti A, Sutton S, Davis JA, Sparks DL (2003) Arsenic speciation and reactivity in poultry litter. Environ Sci Technol 37: 4083-4090.

23. Ide-Ektessabi A, Fujisawa S, Sugimura K, Kitamura Y, Gotoh A (2002) Quantitative analysis of zinc in prostate cancer tissues using synchrotron radiation microbeams. X-ray Spectrometry 31: 7-11.

24. Szczebowska-Boruchowska M, Lankosz M, Ostachowics J, Adamek D, Krygowska-Wajs A, et al. (2004) Topographic and quantitative microanalysis of human central nervous system tissue using synchrotron radiation. X-ray Spectroscopy 33: 3-11.

25. Wernimont AK, Huffman DL, Lamb AL, O'Halloran TV, Rosenzweig AC (2000) Structural basis for copper transfer by the metallochaperone for the Menkes/ Wilson disease proteins. Nat Struct Biol 7: 766-771.

26. Rosenzweig AC (2002) Metallochaperones: bind and deliver. Chem Biol 9: 673-677.

27. Rosenzweig AC (2001) Copper delivery by metallochaperone proteins. Acc Chem Res 34: 119-128.

28. Tomera JF, Lilford K, Friend KD, Kukulka SP, Harakal C (1995) Calcium accumulation in experimental hypertension. Food Chem Toxicol 33: 579-590.

29. Joh JH, Kim DI (2014) Quantitative analysis of vascular calcification. Exp Ther Med 7: 23-26.

30. Block GA, Hulbert-Shearon TE, Levin NW, Port FK (1998) Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic hemodialysis patients: a national study. Am J Kidney Dis 31: 607-617.

31. Marks AR (1992) Calcium channels expressed in vascular smooth muscle. Circulation 86: III 61-67.

32. Ziche M, Jones J, Gullino PM (1982) Role of prostaglandin E1 and copper in angiogenesis. J Natl Cancer Inst 69: 475-482.

33. McAuslan BR, Reilly WG, Hannan GN, Gole GA (1983) Angiogenic factors and their assay: activity of formyl methionyl leucyl phenylalanine, adenosine diphosphate, heparin, copper, and bovine endothelium stimulating factor. Microvasc Res 26: 323-338.

34. Alessandri G, Raju K, Gullino PM (1984) Angiogenesis in vivo and selective mobilization of capillary endothelium in vitro by heparin-copper complex. Microcirc Endothelium Lymphatics 1: 329-346.

35. Finney L, Mandava S, Ursos L, Zhang W, Rodi D, et al. (2007) X-ray fluorescence microscopy reveals large-scale relocalization and extracellular translocation of cellular copper during angiogenesis. Proc Natl Acad Sci USA 104: 2247-2252.

36. Raju KS, Alessandri G, Ziche M, Gullino PM (1982) Ceruloplasmin, copper ions, and angiogenesis. J Natl Cancer Inst 69: 1183-1188.

37. Prozialeck WC, Edwards JR, Nebert DW, Woods JM, Barchowsky A, et al. (2008) The vascular system as a target of metal toxicity. Toxicol Sci 102: 207-218.

38. ATSDR (2005) CERCLA priority list of hazardous substances. Available at: http://www.atsdr.cdc.gov.cercla.

39. Järup L, Berglund M, Elinder CG, Nordberg G, Vahter M (1998) Health effects of cadmium exposure--a review of the literature and a risk estimate. Scand J Work Environ Health 24 Suppl 1: 1-51.

40. Hughes MF (2002) Arsenic toxicity and potential mechanisms of action. Toxicol Lett 133: 1-16.

41. Ibrahim D, Froberg B, Wolf A, Rusyniak DE (2006) Heavy metal poisoning: clinical presentations and pathophysiology. Clin Lab Med 26: 67-97, viii.

42. Bernhard D, Csordas A, Henderson B, Rossmann A, Kind M, et al. (2005) Cigarette smoke metal-catalyzed protein oxidation leads to vascular endothelial cell contraction by depolymerization of microtubules. FASEB J 19: 1096-1107.

43. Mortada WI, Sobh MA, El-Defrawy MM (2004) The exposure to cadmium, lead and mercury from smoking and its impact on renal integrity. Med Sci Monit 10: CR112-116.

44. Bernhard D, Rossmann A, Henderson B, Kind M, Seubert A, et al. (2006) Increased serum cadmium and strontium levels in young smokers: effects on arterial endothelial cell gene transcription. Arterioscler Thromb Vasc Biol 26: 833-838.