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Research paper

Metallomics integrated with proteomics in deciphering metal-related environmental issues☆

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ABSTRACT

The present work shows the possibilities of metallomics to characterize metal-linking proteins in *Mus Musculus* that could be used in environmental assessment. The laboratory mouse *M. musculus* is used as reference of gene/protein sequence databases to address methodological approaches based on changes in transcripts regulation, proteins expression and metalloproteins profiles in the environmental bio-indicator *Mus spretus* that has been demonstrated to be genetically homologous to *M. Musculus*. A metallomic approach using size exclusion chromatography with inductively coupled plasma-mass spectrometry detection (SEC–ICP-MS) was applied to cytosolic extracts from different *M. musculus* organs: lung, liver, spleen, kidney, brain, testicle, hearth and muscle. The resulting profiles of metal-lobiomolecules revealed the presence of a Cu-binding fraction in the 7–10 kDa range which was not present in the other tissues, can be associated to low molecular mass metallothionein-like proteins. The application of reverse phase chromatography with ICP-MS detection to this fraction gives two peaks that have been isolated for later identification by tandem mass spectrometry. The mass balance of copper evaluated by ICP-MS analysis of the digested brain fractions isolated by SEC and RP chromatography reveals good recoveries of the separations. The application of 2-DE to both crude brain extract and SEC fraction (7–10 kDa) reveals the considerably reduction of the number of proteins confirming that a good purification has been attained by SEC. This integration of metallomics with proteomics and transcriptomics can be useful in further studies involving the free-living mouse *M. spretus* for assessment of environmental issues.

1. Introduction

The study of metals and metalloids in very complex biochemical systems involving cells and tissues from bioindicators (*Mus spretus* [1], *Scrobicularia plana* [2], *Procambarus clarkii* [3,4], and others) used in environmental pollution assessment is a very multifaceted task, which requires the integration of experimental approaches to summarize the multiple variables involved in the working ecosystems. Bioindicators generally can reflect the effect of contaminants on cellular metabolism and global homeostasis using different biomolecules related to environmental stress (biomarkers), which comprise many cytochromes P450 (CYPs) containing Fe, antioxidant enzymes such as superoxide dismutase (SOD), with Cu and Zn, metallothioneins (MT) that protect organisms against toxic metals, namely Cd and Hg [5], as well as small size metabolically or toxicologically significant metal-lobiomolecules: selenomethionine (SeMet) with Se, arsenobetaine (AsB) with As, and many others.

Abbreviations: CYPs, cytochromes P450; *Cyp*, transcripts corresponding to CYPs; 2-DE, two-dimensional gel electrophoresis; GSTs, glutathione-S-transferases, GSTs; *Gst*, transcripts corresponding to GSTs; HMM, high molecular mass (molecular mass separation range in a SEC column); LMM, low molecular mass (molecular mass separation range in a SEC column); MALDI-TOF-MS, matrix-assisted laser desorption/ionization with time of flight mass spectrometry; MALDI-TOF-PMF, MALTI-TOF for peptide mass fingerprinting; MT, metallothionein; PCR, real-time polymerase chain reaction; (RP)HPLC–ICP-MS, reverse phase chromatographic separation with ICP-MS detector; RT, reverse transcription; (RP)HPLC–ICP-MS, reverse phase chromatography–inductively coupled plasma mass spectrometry; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEC–ICP-MS, size exclusion chromatography–inductively coupled plasma mass spectrometry; SOD, superoxide dismutase.

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These conventional molecular markers represent good tools in pollution assessment, but a deeper insight into their toxicity mechanisms is necessary, which can originate biased interpretations of results. However, the advent of massive identification of proteins by proteomic approaches can provide a general appraisal about proteins altered under contaminants exposure [5]. Environmental proteomics represents a more comprehensive assessment of the toxic and defensive mechanisms triggered by pollutants without requiring any previous knowledge about the biological systems.

When the proteomic approach is applied to non-model sentinels organisms (bioindicators) some problems arise, because their genetic sequences are not included in databases, which difficult the identification of proteins using 2-DE/tryptic digestion of spots/MALDI-TOF-MS, since highly expensive and cumbersome *de novo* sequencing is necessary. This fact limits the applicability of the conventional proteomic approaches to the huge number of proteins and metalloproteins involved in these studies [5]. The use of information from sequenced model organisms genetically homologous to the bioindicators used in environmental studies can overcome this drawback. However, this homology has to be experimentally confirmed as it has been carried out in the Algerian mouse (*M. spretus*) in connection to the model laboratory mouse (*M. musculus*) [6,7]. The genetic template of *M. Musculus* has been used to design suitable primers for mRNA transcription and later retrotranscription and quantitative amplification by RT-PCR in *M. spretus*. Primers related to transcripts triggering key-proteins in environmental issues, such as cytochromes P450 (CYPs) and glutathione-S-transferases (GSTs), have been checked to produce optimal 100% amplification in the free-living mouse *M. spretus* [6]. This fact has been considered as evidence of genetic homology between *M. Musculus* and *M. spretus* [6,7].

This homology has allowed the absolute measurement of mRNA levels from selected key genes from *M. spretus* dwelling at non-contaminated and contaminated areas. Therefore, transcripts of genes coding for different CYPs and GSTs were quantified using quantitative RT-PCR with primers design for *M. spretus* Cyp and Gst mRNA applying the known gene sequence from *M. Musculus* [6].

Similarly, cytosolic liver fractions from *M. spretus* collected in polluted sites were analyzed by 2-DE to search protein expression differences respect non-polluted ones. The protein map on gels showed over 2500 spots and image analysis yielded 36 spots with significant altered expression, of them 16 proteins were identified by MALDI-TOF-PMF and heterologous search against *M. musculus* databases [7].

On the other hand, metallomics [8] focus on metal-binding biomolecules which represent an important percentage of molecules involved in cell metabolism and cellular pathways [9–13]. Metallomics uses highly sensitive metal and metalloids detectors, namely ICP-MS, as key-tool to trace biomolecules tagged by any metal or metalloid. This "heteroatom" tag is used for chromatographic isolation of metal chemical species for latter identification by mass spectrometry. Metallomics is being successfully used for conventional environmental biomarkers analysis (Fig. 1), in massive metal-biomolecule analytical characterization [8–10,12,14], and quantitative proteomics [15].

In the present work a preliminary study about the metallome of the couple *M. musculus*/*M. spretus* is performed. The use of multidimensional chromatography with ICP-MS detection is considered for this purpose as well as the complementary application of the well established 2-DE proteomic approach to assist the complexity of successive subproteomes isolated during the metallomic research. This is a long a difficult task, because different tissues have to be considered both in *M. musculus* and *M. spretus* in order to characterize the corresponding metal-biomolecule profiles and latter successive purification to identify the isolated metal-containing molecules. Present study represents a starting point in this research that, in addition, intend the integration with proteomic and transcriptomic approaches in order to get in the future an

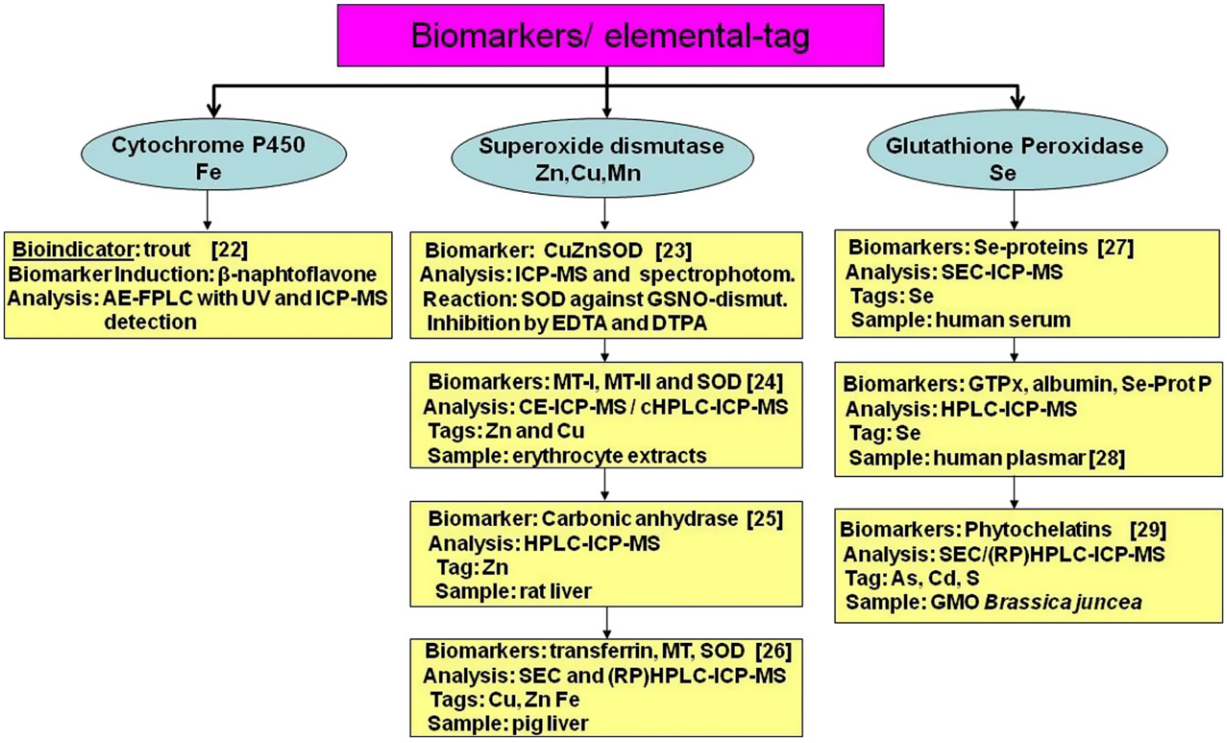

Fig. 1. Use of metallomic for traditional biomarkers analysis.