SESSION 4
SELENIUM SPECIATION AND ANALYTICS

ANALYSIS OF Se SPECIES IN COMPLEX MATRICES

B. Gammelgaard

University of Copenhagen, Analytical Biosciences, Department of Pharmacy, Copenhagen, Denmark

Investigations on selenium absorption, distribution, metabolism and excretion (ADME) are highly dependent on sensitive and reliable analytical methods.

The present state of the art for quantitative measurement of the total concentration of selenium in body fluids and tissues, cell models and other biological samples is analysis by Inductively Coupled Plasma Mass Spectrometry (ICPMS). However, the complicated matrices of biological samples may result in interferences on measurements .

Distinguishing between different species demands efficient separation techniques like liquid chromatography and capillary electrophoresis and by coupling these separation techniques to the ICPMS detection technique, highly selective measurements can be performed. The selectivity and detection limits of such measurements are mainly dependent on the separation methods.

The main drawback of ICPMS is the loss of structural information. Thus, identity of species is often claimed based on co-elution with standards alone. In case of poor separation systems this has led to several wrong conclusions. The identity of new compounds should be established by molecular mass spect-rometry.

Analytical results are highly dependent on sample preparation. Hence, sample preparation protocols should be validated and mass balance studies performed to establish the fractions of each species.

Examples on identification and quantification of selenium species in complex matrices will be given.

SELENIUM SPECIATION IN SERUM AND CSF BY SAX-ICP-DRC–MS

B. Michalke

Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt GmbH,
Research Unit: Analytical BioGeoChemistry, Neuherberg, Germany

Se-speciation was performed in paired serum and cerebrospinal fluid (CSF) samples from neurologically healthy persons. Strong anion exchange (SAX) coupled to ICP-DRC-MS was used. Species identification was performed by 1. standard retention times, 2. standard addition and 3. 2-D techniques: SEC-SAX-ICP-DRC-MS, SAX-CZE-ICP-DRC-MS and SAX-ESI-FT-ICR-MS. LoD were from 0.01 – 0.018 µg/L. Quality control was performed by analyzing control materials. SePP was determined in a CSF sample within a laboratory comparison. The results fitted well to a reference values. In both sample types Se species were measured. Median values were (serum / CSF; µg Se/L): total Se: 58.39 / 0.86; SePP: 5.19, 0.47; Se-methionine (SeM): 0.23 / < LoD; glutathioneperoxidase (GPx): 4.2 /, 0.036; thioredoxinreductase (TrxR) 1.64 / 0.035; Se(IV) 12.25 / 0.046; Se-human serum albumin (Se-HSA) 18.03 / 0.068. Other Se-species were < LoD.

Linear relationships gave information about Se-species passage across neural barriers (NB). Strong correlation was found for $SePP\text{-}_{serum}$ and total $Se\text{-}_{serum}$, when total $Se\text{-}_{serum}$ > 65 µg/L. The previously described independence of total $Se_{\text{-}CSF}$ from total $Se_{\text{-}serum}$ and of $SePP_{\text{-}CSF}$ from $SePP_{\text{-}serum}$ was confirmed.

Linear relationships of Se-compounds between serum and CSF were found only for GPx and TrxR. Although, $HSA_{\text{-}CSF}/HSA_{\text{-}serum}$ values were in the normal range, the increased $TrxR_{\text{-}CSF}/TrxR_{\text{-}serum}$ and $GPx_{\text{-}CSF}/GPx_{\text{-}serum}$ ratios are not explained by molecular size dependence. It was concluded that there may be a facilitated diffusion across NB for anti-oxidative Se-enzymes or they might be also expressed in brain.

In a follow up study Se species in CSF from ALS patients were compared to a control group, matched on size, age and gender. Statistical analysis of Se speciation data revealed that higher concentrations of $selenite_{\text{-}CSF}$ and of $Se\text{-}HSA_{\text{-}CSF}$, but lower ones of $SePP_{\text{-}CSF}$ were associated with increased ALS risk. The associations were stronger among cases age > 50 years.

SELENOSUGARS TEN YEARS ON: KEY SELENIUM METABOLITES OR TRIVIAL EXCRETION PRODUCTS?

K.A. Francesconi, S. Kokarnig, D. Kuehnelt

University of Graz, Graz, Austria

Following the first report of selenosugars in rat urine in late 2002 and then in humans in 2003, these metabolites have been recognised as the major selenium urinary metabolites. Much of the data, however, have been obtained from experiments where selenium intake has been supplemented in some way, either by dietary supplements or medication. Under these conditions, selenosugars are dominant, and they become more clearly so when exposure to selenium is higher. However, the urinary excretion profile for selenium at lower selenium

intakes, at adequate or just below adequate levels, is far from clear. Here, selenosugars can constitute only 20% or less of the total urinary selenium, and most of the selenium is present in currently unidentified forms. This presentation will provide an overview of the changing status of selenosugars as possible key selenium metabolites since their identification ten years ago. The focus will be on the analytical methods developed to measure these metabolites, and what we might be missing by focussing on selenosugars.

COMPREHENSIVE SPECIATION OF SELENIUM ON THE PROTEOMIC AND METABOLOMIC LEVEL IN CEREALS BY ELEMENTAL AND MOLECULAR MASS SPECTROMETRY

K. Bierła, J. Bianga, L. Ouerdane, J. Szpunar, R. Łobiński

CNRS/UPPA UMR 5254, Laboratory of Bioinorganic Analytical and Environmental Chemistry, Pau, France

Cereals are an important source of dietary Se for humans and animals. Selenium taken up from soil is transformed into a variety of selenium species, including bioactive compounds of importance for human nutrition and health. The existence of a large number of this species has been largely demonstrated by the coupling of high performance chromatography with inductively coupled plasma mass spectrometry (ICP MS) but most of them could not be identified because of the insufficiency of analytical methodology. There is hardly any information on then identity of proteins accumulating selenium in cereals, even if it is known that protein-bound selenium accounts for more than 70% of selenium.

A breakthrough in the de novo identification of selenium species constituting the selenium metabolome and proteome became possible with the arrival of high resolution electrospray mass spectrometry. It allowed the determination of accurate mass of the selenium species at levels compatible with those present in non-accumulating plants in extracts and enzymatic digest after chromatographic separation. A further possibility of direct on-line multistage fragmentation of the selenium species allows the structure elucidation of even fairly complex species and protein sequencing. Particularly attractive is the combination of these approaches with selenium specific quantitative detection by ICP MS delivering information on the relevant abundances of the individual species and the mass balance of the selenium in a sample and extracts.

This presentation discusses the analytical methodology for the comprehensive characterisation of the selenium metabolome and proteome in two cereals: wheat and rice.

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QUANTIFICATION OF PLASMA SELENOPROTEINS – THE NEED FOR REFERENCE METHODS AND STANDARDS

H. Goenaga-Infante [1], M.E. del Castillo [2], P. Fisicaro [2], A. Raab [3]

[1] LGC Limited, Teddington, United Kingdom
[2] Laboratoire National de Métrologie et d'Essais (LNE), Paris, France
[3] TESLA University of Aberdeen, Department of Chemistry, Aberdeen, United Kingdom

The lack of reference methods and well characterised calibration standards for plasma selenoprotein quantification is a remaining challenge, limiting the achievement of comparable results between laboratories, in particular, for selenoprotein quantification in samples from supplementation trials.

An attractive approach to the entire protein quantification is the use of species-specific isotope dilution calibration, which requires of synthesis and characterisation of plasma selenoprotein isotopically enriched with stable Se and or C isotopes. This would enable protein accurate quantification using either elemental and/or molecular MS detectors. Overproduction of selenoproteins of different species in micro-organisms is difficult. Due to this, the production of isotope enriched selenoprotein(s) suitable for standard production is and will be for some time (at least) difficult. A suitable alternative is protein quantification down to the peptide level (e.g. IDMS on specific peptides in tryptic digests of plasma). This would require the chemical synthesis of specific peptide sequences containing Se-cysteine with enriched Se identical to natural occurring Se-containing peptides from.

This work aims to produce isotopically enriched Se species namely peptides containing ^{76}Se enriched Se-cysteine and/or ^{76}Se-enriched SEPP1 (plasma Selenoprotein P, P49908) and to evaluate their feasibility to perform accurate selenoprotein quantification using species-specific isotope dilution mass spectrometry. The importance of having reference methods in place for provision of reference values to clinical supplementation trials and for the production of reference materials, needed for method validation, will be discussed.

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SELENIUM SPECIES ANALYSIS REVEALS DICHOTOMOUS BEHAVIOR IN THE GERMAN GENERAL POPULATION TO GENERATE TRIMETHYLSELENIUM ION

T. Jäger, S. Dorner, H. Drexler, T. Göen

University Erlangen-Nuremberg, Institute for Occupational, Social and Environmental Medicine, Erlangen, Germany

Selenium is an essential trace element for humans with a small therapeutic index. The different toxic potential of the resorbed or metabolized selenium species demands a differentiated assessment to examine both adverse and beneficial health effects. The aim of the study was to determine background values of inorganic, methylated and organic selenium species in human urine of the general population.

Three analytical methods based on different liquid chromatography separation techniques coupled to an inductively coupled plasma-mass spectrometer were used for the analysis of nine selenium species in human urine: selenate (SeVI), selenite (SeIV), trimethylselenium ion (TMSe), methylselenoglutathione (SeMeG), methyl-2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside (SeSug1), methyl-2-amino-2-deoxy-1-seleno-β-D-galactopyranoside (SeSug3), selenomethionine (SeMet), selenoethionine (SeEt) and methylselenocysteine (SeMCys). The procedures were applied to 45 urine samples of individuals of the general population (28 females/17 males, aged 19 – 69 years).

SeSug1 was detected in 100% (median: 1.79 µg/g creatinine; range: 0.47 – 8.97 µg/g creatinine) and SeSug3 (0.80; 0.07 – 3.53 µg Se/g creatinine) in 80% of the study population. SeSug1 and SeSug3 were strongly correlated. In 20% of the samples other species were detected which could be related to SeVI (0.12; 0.03 – 1.12 µg Se/g creatinine) and SeMCys (0.13; 0.03 – 0.41 µg Se/g creatinine). TMSe was detected in 18% of the samples.

But whenever TMSe was detected it was found in considerable concentrations (2.91; 2.72 – 4.42 µg se/g creatinine). The species SeMet, SeEt, SeMG and SeIV were in all samples below the limit of detection. The present study indicates a dichotomous distribution in the German population concerning the ability to build TMSe. Furthermore, the study results are consistent with the literature in which the selenium containing sugars play a fundamental role in selenium meta-bolism.