Published in final edited form as:

Surf Interface Anal. 2014 November 1; 46(Suppl 1): 154–157. doi:10.1002/sia.5625.

Quantitative imaging of selenoprotein with multi-isotope imaging mass spectrometry (MIMS)

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Abstract

Multi-isotope imaging mass spectrometry (MIMS) allows high resolution quantitative imaging of protein and nucleic acid synthesis at the level of a single cell using stable isotope labels. We employed MIMS to determine the compartmental localization of selenoproteins tagged with stable isotope selenium compounds in human aortic endothelial cells (HAEC), and to compare the efficiency of labeling (to determine the ideal selenium source) from these compounds: [⁸²Se]-selenite, [⁷⁷Se]-seleno-methionine, and [⁷⁶Se]-methyl-selenocysteine. We found that all three selenium sources appear to be localized in the nucleus as well as in the cytoplasm in HAEC. Seleno-methionine appears to be a better source for (seleno)protein synthesis. For MIMS detection, we compared freeze-drying to thin layer vs. thin sectioning for sample preparation. MIMS provides a unique and novel way to dissect selenoprotein synthesis in cells.

Introduction

Selenium is an essential micronutrient in humans and animals, whose biological activity is mainly exerted through the functions of selenoproteins that contain selenocysteine (Sec), the 21st amino acid. Yet, selenoproteins synthesized from Sec are limited in number, and, with some key exceptions (the glutathione peroxidases), are largely of unknown function.

In selenium-deficient cells, supplementation with inorganic selenium (sodium selenite) or with organified selenium compounds (seleno-L-methionine [SeMet] and Se-methyl-selenocysteine [MeSeCys]) has been shown to enhance the synthesis of selenoproteins (1). These selenium sources are involved in selenoprotein synthesis via different selenide-producing pathways (Scheme 1) (2). Selenide, a common intermediary metabolite, is the precursor of selenophosphate used to synthesize Sec on its tRNA, a prerequisite step for selenium to be incorporated into selenoproteins. Although selenoprotein expression is known to be regulated by selenium, the ideal form of the selenium source has not been determined.

Multi-isotope imaging mass spectrometry (MIMS) facilitates quantitative imaging of the localization and turnover of biomolecules in mammalian cells with stable isotope-tagged tracers (3, 4, 5). In MIMS, the surface of a sample is sputtered with a primary Cs^+ beam, and the triggered secondary ions of interest are analyzed simultaneously with multiple detectors. Metabolism of a biomolecule will be represented by the stable isotope's incorporation, which can be measured as an increase in the ratio of the stable isotope to its common variant at the site over its ratio by natural occurrence. The measurement is quantitative because both ionic species are analyzed simultaneously from the sputtered microvolume.

In response to vascular oxidant stress, human aortic endothelial cells (HAEC) are regulated by endothelial cell antioxidant proteins among which are included the selenoprotein glutathione peroxidases. In this experiment, we employed MIMS using ^{82}Se -selenite, ^{77}Se -seleno-methionine, and ^{76}Se -methyl-selenocysteine to determine the compartmental localization of selenoproteins in HAEC, and to compare the efficiency of labeling from these selenium sources in order to identify the ideal source.

Materials and Methods

HAEC (Lonza) were propagated in EBM-2 medium/EGM-2 SingleQuot Kit containing 2% FBS, 100 IU/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin at 37°C in a 5 % CO_2 incubator, and used in passage 6 to 8. ^{76}Se -methylselenocysteine (99.9% purity), ^{77}Se -seleno-L-methionine (99.8% purity), and sodium ^{82}Se -selenite (98.9% purity) were synthesized by established methods (6).

HAEC were grown on Au-coated silicon chips ($5\text{mm} \times 5\text{mm}$) in a 48-well plate, and exposed to medium containing 300 nM ^{76}Se -methyl-selenocysteine, 600 nM ^{77}Se -seleno-methionine, or 150 nM ^{82}Se -selenite for four days. These chosen concentrations of selenium compounds were based on the optimal concentration for selenoprotein expression in prior work (1). As a control, HAEC were also grown in the absence of selenium isotopes. ^{15}N -thymidine (10 μM) was included to visualize the nucleus. At the end of the incubation, the gold chips were quickly rinsed with distilled water (3X) (7, 8), followed by freezing in liquid nitrogen and drying under vacuum (-80°C at 10^{-6} Torr). NanoSIMS50L (Cameca) was used for MIMS and set to detect simultaneously mass 12 ($^{12}\text{C}^-$), mass 26 ($^{12}\text{C}^{14}\text{N}^-$), mass 27 ($^{12}\text{C}^{15}\text{N}^-$), mass 28 ($^{28}\text{Si}^-$), mass 32 ($^{32}\text{S}^-$), mass 76 ($^{76}\text{Se}^-$), mass 77 ($^{77}\text{Se}^-$), mass 80 ($^{80}\text{Se}^-$), and mass 82 ($^{82}\text{Se}^-$). ImageJ and JMP 10 were used to analyze the MIMS data.

Alternatively, HAEC were directly grown in 6-well plates and exposed to the selenium isotope-containing compounds. At the end of incubation, the cells were fixed with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M cacodylate buffer, embedded, and sectioned (500 nm in thickness) for MIMS analysis.

The cell preparations by freeze drying and PF-fixation are complementary. Freeze drying cells also allows for the analysis of the entire cellular volume in 3 dimensions but is relatively slow. Fixation retains selenium that has been incorporated into proteins. Analysis of PF-fixed sections is faster and allows us to analyze many cells.

Results

HAEC grew well on Au-coated chips (Fig. 1a). The inset in Fig. 1a demonstrates the image of a single cell grown on a Au-chip by light microscopy. Fig. 1c shows that the field for sputtering was $50\text{ }\mu\text{m} \times 50\text{ }\mu\text{m}$ and the acquisition time was 4 min scanning time per plane for 496 planes. The $^{12}\text{C}^{14}\text{N}$ derives from protein and nucleic acid fragments. The $^{12}\text{C}^{14}\text{N}$ image is the summed image of 496 planes, thus reflecting a dried HAEC cell having an unsmooth layer after freeze drying.

Next, we examined Se incorporation in HAEC after exposure to 300 nM ^{76}Se -methyl-selenocysteine, 600 nM ^{77}Se -seleno-methionine, or 150 nM ^{82}Se -selenite. Hue-saturation-intensity (HSI) of the image correlates with the increase in the ratio of two isotopes over the naturally occurring ratio using RGB color. The HSI shows that the ratio of $^{15}\text{N}/^{14}\text{N}$ in the nucleus of a HAEC cell was increased by a factor of 100- to 200-fold over its natural abundance ratio (0.00367), thus reflecting thymidine incorporation into the nucleus (Fig. 2a). Similarly, HSI $^{82}\text{Se}/^{80}\text{Se}$, HSI $^{77}\text{Se}/^{80}\text{Se}$, and HSI $^{76}\text{Se}/^{80}\text{Se}$ show that Se is incorporated in the cells after treatment with ^{82}Se -selenite, ^{77}Se -seleno-methionine, and ^{76}Se -methyl-selenocysteine, respectively. All three selenium isotope-containing compounds labeled HAEC in the nucleus as well as the cytoplasm. To analyze the hot spots, the cytoplasm of the cell was visually divided into 3 areas: cytoplasm 1* (Cyt 1), cytoplasm 2* (Cyt 2), and cytoplasm 3* (Cyt 3) (*defined by an increment of $5\text{ }\mu\text{m}$ away from the nucleus) (Fig. 2b). Treatment with ^{77}Se -seleno-methionine or ^{76}Se -methyl-selenocysteine led to a predominant distribution in the nucleus with a gradual decrease in intensity with distance from the nucleus, whereas treatment with ^{82}Se -selenite led to an even distribution in the nucleus and the cytoplasm (Fig. 2c). Based on the hot spot analysis in the cytoplasm, the order of labeling efficiency is 600 nM ^{77}Se -seleno-methionine > 150 nM ^{82}Se -selenite > 300 nM ^{76}Se -methyl-selenocysteine (= 10: 3: 0.5). The ratios of HAEC that were not treated with stable selenium isotopes were similar to natural ratios (data not shown.).

In the alternative preparation, which resulted in a uniform, thin layer of cells by sectioning, cell structure/morphology was maintained (Fig. 3a). HAEC were treated with 600 nM ^{77}Se -seleno-methionine for 2 to 4 days. HSI $^{77}\text{Se}/^{80}\text{Se}$ shows Se incorporation hot spots in the cell whereas its ratio of $^{82}\text{Se}/^{80}\text{Se}$ is, as expected, similar to the natural abundance. We compared Se incorporation at 2 and 4 days, and found that Se incorporation is time-dependent.

Conclusion

This study shows that MIMS is a promising technique for exploring the biosynthesis of selenoproteins in the cell. This specific work allowed us to determine the optimal analytical conditions. MIMS study will be combined with SEM analysis to ascertain protein synthesis localization within specific organelles in the cell.

Acknowledgments

C. Lechene is funded by the NIH grants (5P41EB001974-13, AG034641, R01 AG040019, R21AG034641-01, R01 AG040209), Human Frontier Science Program (RGP0048) and the Ellison Medical Foundation (AG-SS-2215-08). J. Loscalzo is funded by NIH grants (R37 HL61795, HL048743 and HL108630).

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Figure 1.

Figure 2.

Figure 3.

Scheme 1.