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Detection of immunolabels with multi-isotope imaging mass spectrometry (MIMS)

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Abstract

We have developed a method that combines the use of stable isotopes, MIMS and antibody. We began with using well-established antibodies, anti-actin and anti-synaptophysin, in mouse intestinal cells. We extended the method to an immunogold assay to specifically localize Ribeye, a major protein component of retina synaptic ribbons, or to localize a synaptic vesicle-containing protein, synaptophysin. Both are localized in presynaptic nerve terminal of photoreceptors cells in retina. Our results show that by MIMS analysis of the Au signal we can directly identify antibodies tagged with non amplified 1.4 nm gold nanoparticles. They also demonstrate that the gold nanoparticle-tagged antibodies do not dilute the ¹⁵N/¹⁴N signal used for measuring protein turnover. Thus we can simultaneously and directly use MIMS to measure protein turnover and to identify cell type or specific protein.

Keywords

Multi-Isotope Imaging Mass Spectrometry; Secondary Ion Mass Spectrometry; Stable isotopes; Gold-Nanoparticle; Immunoassay; Synaptic ribbon

Introduction

MIMS combines tracer methods, intensive quantitative image analysis, and a novel type of secondary ion mass spectrometer, the Cameca NanoSIMS50L, which has the unique capability of simultaneously recording several quantitative atomic mass images at high spatial resolution and mass resolution at high transmission. Secondary ion mass spectrometry (SIMS) is based upon the sputtering of a few atomic layers from the surface of a sample, induced by a 'primary ion' bombardment. Images are obtained by stepping the primary ion beam across the sample. For each step location on the sample, the number of secondary ions sputtered is recorded. MIMS images represent the variation in intensity of each selected secondary ion species across the pixels of the area scanned. We locate and measure the experimentally induced enrichment of a specific stable isotope in a sample by deriving a ratio image from the pixel-wise division of individual masses (e.g. ratio ¹²C¹⁵N / ¹²C¹⁴N). This ratio method compensates for any matrix effect. MIMS allows one to simultaneously image the distribution and measure the accumulation within or between cells, of molecules labeled with any isotopes.¹⁻⁵ Stable isotopes are an integral part of the animate and inanimate composition of earth, they do not alter biochemical reactions

and are not harmful to the organism. This allows the use of MIMS for studies in humans.³ Here we present a method which allows us simultaneously measure stable isotope ratios and gold nanoparticle immuno-reporter tags.

Method

Adult mice were fed a ¹⁵N-leucine diet for two days. Mouse intestine was extracted, fixed in 4% paraformaldehyde, embedded in LRWhite, sectioned at 100 nm thick and mounted on silicon chips. Retinal tissue was extracted from unlabeled mice and prepared in the same manner.

Immunofluorescence

We used the method described by Micheva et al⁶. Silicon-mounted intestinal samples were incubated in 50 mM glycine (Sigma) in TBS for 5 min at room temperature, then blocked with a solution containing 0.05% Tween (Sigma) and 0.1% BSA (Sigma) in TBS. Actin antibody (Millipore, CA, USA) was purified from a 1 mg/mL solution by buffer exchange using a spin column (Abcam, MA, USA) to remove tris-glycine. Dehydrated synaptophysin antibody (Abcam) was reconstituted in 100 µL of deionized water. Primary antibody solutions were diluted to 1:10 in blocking solution containing 0.05% Tween (Sigma, MO, USA) and 0.1% BSA (Sigma) in TBS (Sigma) and incubated with the tissue sections for 1h at room temperature and then overnight at 4°C. Sections were washed five times with TBS, incubated with alexa fluor-conjugated secondary anti-mouse antibody for one hour at room temperature, washed five times with TBS, and then rinsed with distilled water. Alexa fluor-conjugated antibody binding was verified by fluorescence reflection microscopy (Nikon E800 microscope).

Immunoassay using fluorescence microscopy was performed on the tissue section after cesium primary ion beam (Cs+) bombardment; the cellular immunoreactivity, on the same section, was imaged using an immune complex to detect antigen of interest.

Immunogold labeling

Primary antibody solutions were prepared (described above). Sulfo-N-Hydroxysuccinimido nanogold particles (1.4 nm; Nanoprobes, NY, USA) were dissolved in 200 mL of deionized water. This solution, 3.3 nmol, was mixed separately with 0.66 nmol of each primary antibody at pH=7.5-8. The reaction mixtures were incubated overnight at 4°C. Non-reacted AuNp were removed with Micro Bio-Spin chromatography columns containing tris buffer (Bio-Rad, CA, USA) according to manufacturers' instructions. AuNp-conjugated antibodies were then diluted 1:10 in blocking solution (described above).

For intestinal samples, silicon chips with LR-embedded samples were incubated at room temperature for one hour and overnight at 4°C with each gold-conjugated primary antibody, then washed five times with TBS and rinsed with distilled water. MIMS was performed as described previously by Lechene et al⁷. In this approach, the nanogold-conjugated antibody was used as a targeting reagent. Immunogold detection and MIMS measurement were performed simultaneously in a single experiment.

For LRWhite-embedded retinal samples, alexa Fluor fluoronanogold-Fab' conjugates (Nanoprobes) were used to reveal, indirectly, both ribeye and synaptic vesicle proteins in the outer plexiform layer of mouse retina. Immunogold detection and MIMS measurement were performed simultaneously in a single experiment.

Results

Two approaches were investigated: the post-MIMS labeling antibody, to study cesium ion beam sputtering effects on tissue antigenicity, and the pre-MIMS labeling antibody using gold nanoparticles as a reporter group. Antigenicity was lost after cesium bombardment.

Gold nanoparticles (1.4 nm) conjugated to antibodies were visualized using MIMS on the brush border (microvilli) of the intestinal villus (Figure 1) and on the synaptic ribbon of retina (Figures 2 and 3) without Au amplification. These results correlate well with traditional immunofluorescence (Figure 1) and demonstrate the feasibility of using MIMS to quantify gold nanoparticle-conjugated antibodies.

Conclusion

An immunoassay method, e.g. fluorescence microscopy or immunogold and TEM, gives qualitative information on the presence and location of a specific protein at medium to high resolution. MIMS gives quantitative information on metabolic turnover. MIMS can be combined with gold nanoparticle immuno-reporter tags so that in a single analysis in one instrument one can localize and quantitate the turnover of a specific protein.

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Figure 1.

Figure 2.

Figure 3.