

ISOTOPE RATIO IMAGING OF BIOLOGICAL TISSUES WITH MULTI-ISOTOPE IMAGING MASS SPECTROMETRY (MIMS)

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The fundamental discovery that proteins in biological tissues were turning over was made using a custom-built mass spectrometer to detect and measure the stable nitrogen isotope, ^{15}N , which was used as a marker of amino acids introduced to mice through diet (Schoenheimer and Rittenberg, 1939; Schoenheimer, 1942). Since then, these seminal studies could not be pursued at the cellular and/or intra-cellular level due to the lack of suitable methodology and the lack of a usable radioactive isotope of nitrogen. The recent development of a secondary ion mass spectrometer with the capability of simultaneously measuring several ion masses and of providing high resolution imaging capability (Slodzian et al. 1992) opened the possibility of using ^{15}N as a molecular marker for identifying and measuring the turnover of nitrogen-labelled molecules in intra-cellular compartments.

Intestines and kidneys from mice were fixed in glutaraldehyde, embedded in epon, and sectioned at 0.5 μm ; sections were deposited on silicon pieces for MIMS analysis. The mice were on either a control diet or an experimental diet supplemented with ^{15}N -L-leucine for 25 days. The $^{12}\text{C}^{15}\text{N}/^{12}\text{C}^{14}\text{N}$ ratios were measured in the control and experimental food by combustion mass spectrometry.

Control tissue isotope ratios of $^{12}\text{C}^{15}\text{N}/^{12}\text{C}^{14}\text{N}$ were first measured with a stationary primary cesium ion beam ($\sim 4\text{-}\mu\text{m}$ diameter; 2-pA primary current). They were equivalent to that of the $^{15}\text{N}/^{14}\text{N}$ terrestrial ratio (0.367%). Similar results were observed when the isotope ratios were calculated from the pair of $^{12}\text{C}^{15}\text{N}$ and $^{12}\text{C}^{14}\text{N}$ images obtained simultaneously by scanning the primary beam over the same samples.

Experimental intestinal and kidney tissue isotope ratios of $^{12}\text{C}^{15}\text{N}/^{12}\text{C}^{14}\text{N}$, however, were equivalent to that of the experimental diet. This means that, in 25 days, all the tissue nitrogen (i.e. proteins) had been renewed in the proximal tubule of the kidney and in the microvillae and apical areas of the intestine.

In the embedding medium, but very close to tissue that had excess ^{15}N , the $^{12}\text{C}^{15}\text{N}/^{12}\text{C}^{14}\text{N}$ isotope ratio was equivalent to its terrestrial value. This demonstrates the lack of gross leaching of the marker from the tissue.

Multi-isotope Imaging Mass Spectrometry allows -- for the first time -- both the visualization (at high resolution) and the measurement (with high precision) of molecules or elements labelled with the stable isotope ^{15}N in subcellular compartments of individual cells.

References

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