Molecular Specificity of CN^- Secondary Ion Formation using Multi-Isotope Imaging Mass Spectrometry

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The fundamental discovery that proteins in biological tissues are continuously renewed was made using a custom-made mass spectrometer to measure the incorporation into proteins of the amino acid leucine labelled with the stable nitrogen isotope ^{15}N [1]. These seminal studies could not be pursued at the intra-cellular level because there was no methodology to simultaneously image and quantitate a stable isotope, and because there is no useful radioactive isotope of nitrogen. Imaging of stable-isotope distribution has been possible, however, since the development of secondary ion mass spectrometry (SIMS). Yet until now, SIMS technology has presented irreconcilable tradeoffs [2] that have severely limited its use as a major discovery tool in biology. In order to use secondary ion mass spectrometry to locate and measure isotope tags in subcellular volumes, we needed solutions to three major problems. First, in order to obtain quantitative ultrastructural images, the analysis must have sufficiently high lateral resolution, and the quantitation and imaging must be associated. Second, because the quantitation of label is based upon the measurement of isotope ratios, we ought to be able to simultaneously record data from several isotopes, which must be exactly in register. Third, because in practice ^{15}N ions do not form, nitrogen is detected as a cyanide ion. Thus, the mass resolution needs to be high enough to separate the isobars $^{12}C^{15}N^-$ and $^{13}C^{14}N^-$. A new generation of secondary ion mass spectrometer meets these requirements. It can measure several ion masses in parallel, it has high mass resolution at high transmission, and it has a spatial resolution of ~35 nm [3, 4]. These extraordinary capabilities opened the possibility of using stable isotopes as molecular markers, in particular ^{15}N. We developed the methodology of multi-imaging mass spectrometry (MIMS) for locating and measuring stable-isotope labelled molecules in intra-cellular compartments.

An essential question related to CN^- quantitative imaging is whether the secondary CN^- ion signal arises predominantly from: 1) the primary production of C-N molecular fragments, 2) the recombination of C and N atoms produced separately, or 3) the combination of both mechanisms. To answer this question we synthesized a series of ^{13}C- and ^{15}N- labelled polyglycines (Figure 1),

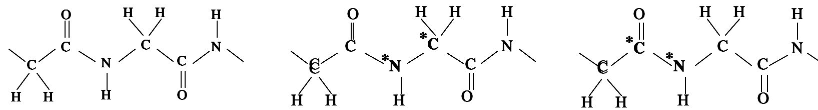

Figure 1. A portion of the unlabeled polyglycine (OH(COCH$_2$ NH)$_5$ H) is shown at the top left. The relative positions of the ^{13}C- and ^{15}N- labels in the isotopically labeled derivative polyglycines used in this study are indicated (*) in the adjacent figures.

varying two parameters: the distance between the labels, and the labels' neighbouring environment (i.e., ^{13}C present or not as part of a carbonyl group). Powdered polyglycines were solubilized in deionised water to a concentration of 73mM to 130mM and were applied as microdroplets to cleaned Si chips. The polyglycines were analyzed by MIMS by applying a primary ion current of 0.64pA onto each sample. Five series of four masses were simultaneously measured, always including ^{12}C and ^{13}C but varying the combination of recorded CN$^-$ ions paired as follows: ($^{12}C^{14}N^-$, $^{12}C^{15}N^-$), ($^{12}C^{14}N^-$, $^{13}C^{14}N^-$), ($^{12}C^{14}N^-$, $^{13}C^{15}N^-$), ($^{12}C^{15}N^-$, $^{13}C^{15}N^-$), and ($^{13}C^{14}N^-$, $^{13}C^{15}N^-$).

Based upon the magnitude of the observed $^{13}C^{15}N/^{12}C^{14}N$ ratios relative to the position of the ^{13}C- and ^{15}N- labels within the various polyglycines a number of conclusions can be drawn:

1. Recombination of the C and N atoms is a significant source of secondary CN$^-$ ions.

2. This recombination is higher the closer the two labels are to each other.

3. When the two labels are immediately adjacent there is an increase of 3-orders of magnitude in the $^{13}C^{15}N/^{12}C^{14}N$ ratio relative to the unlabeled polyglycine.

4. The amount of recombination is reduced 40-50% when the ^{13}C label is part of a carbonyl group, irrespective if the ^{15}N label is immediately adjacent to ^{13}C or separated by a glycine unit.

In addition to increasing our understanding of basic processes, this study should enable the development of astute labelling schemes for biological experiments, paving the way for even more sensitive measurements of isotope ratios in subcellular domains.

[1] Schoenheimer, R., *The dynamic state of body constituents*. The Edward K. Dunham Lectures for the Promotion of the Medical Sciences. 1942, Cambridge, MA: Harvard UP. 78.
[2] Ireland, T., *New Tools for Isotopic Analysis.* Science, 1999. **286**: p. 2289-2290.
[3] Slodzian, G., et al. *A high resolution scanning ion microscope with parallel detection of secondary ions*. in *8th International Conference on Secondary Ion Mass Spectrometry*. 1991. Amsterdam, The Netherlands.
[4] Slodzian, G., et al., *Scanning secondary ion analytical microscopy with parallel detection.* Biol Cell, 1992. **74**(1): p. 43-50.

¥National Resource for Imaging Mass Spectrometry