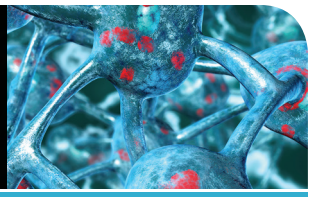



# Utility of Stable Isotopes in Mass Spectrometry

The use of stable isotopes to define metabolic pathways and turnover of body constituents occurred very quickly after the discovery of deuterium and a method for isolating it, both by Harold Urey. Urey was awarded the Nobel Prize in 1934 for discovery of deuterium, but by the mid-1930s Rudolf Schoenheimer had already begun synthesizing deuterated molecules that he administered to rodents. With his young student, David Rittenberg, Schoenheimer defined synthesis and degradation pathways of many compounds, including fatty acids and cholesterol, that we take for granted today. When enriched nitrogen-15 ($^{15}N$) became available, Schoenheimer and Rittenberg demonstrated that proteins were dynamic in that they were both continually being synthesized and degraded.[1] All of this work was performed in only a few years using crude methods of preparation of labeled compounds and tedious measurement by isotope ratio mass spectrometry (IRMS) that requires all compounds be reduced to simple gases ($CO_2$, $H_2$, $N_2$) for measurement of isotopic enrichments.

After World War II, use of stable isotopes in biochemistry was mostly displaced by the availability of tritium and carbon-14 ($^{14}C$) radioisotopes. Although use of $^{15}N$ continued to study the nitrogen side of amino acid and protein metabolism and turnover (as there is no long-lived radioisotope of nitrogen), even this work was limited. It was not until the late 1960s that attention began to be paid to measurement of protein turnover using glycine ($^{15}N$, 98%) (NLM-202) as the tracer and measurement of $^{15}N$ in urea, the end product of protein metabolism by Sir John Waterlow and colleagues.[2] There was a flurry of work in the 1970s, but again the primary reasons for the work were that glycine ($^{15}N$, 98%) was easy to synthesize (the only amino acid without an optically active center), and the urea ($^{15}N_2$, 98%+) (NLM-233) end product was easy to isolate from urine and prepare for measurement by IRMS. Use of $^{15}N$ and measurement directly in proteins was done, but that work was dwarfed by use of $^{14}C$ to make the same measurements.[2] Although both enriched carbon-13 ($^{13}C$) and deuterium were both available in the 1960s, their use as tracers in metabolism was extremely limited compared to $^{14}C$ and tritium.

The first major turning point for use of stable isotopes ($^{2}H$, $^{13}C$, and $^{15}N$) came with the development of gas chromatography/mass spectrometry (GC/MS). GC/MS provided separation of complicated mixtures of components and the ability to measure mass differences in those compounds. However, early GC/MS instruments were magnetic sector mass spectrometers. Nonetheless, Sweeley developed a system to perform limited selected ion monitoring (SIM) on an early instrument and demonstrated measurement of D-glucose (1,2,3,4,5,6,6-$D_7$, 98%) (DLM-2062).[3] As quadrupole GC/MS instruments arose, measurement of stable isotopically labeled enrichments became common place over a wide range of compounds.[4] Stable isotopically labeled compounds (often deuterated) were used as internal standards for quantification and were proposed as "gold standard" methods for clinical chemistry measurement of even simple compounds, such as glucose in plasma. The other more important use of GC/MS was for measurement of stable isotopically labeled compounds as *in vivo* tracers for studies of metabolite kinetics in mammals.[4]

Two notable tracers arose in the 1970s that have become standard tracers reported in hundreds of studies and administered to thousands of people: D-glucose (6,6-$D_2$, 99%) (DLM-349) for measuring the rate of glucose production and L-leucine (1-$^{13}C$, 99%) (CLM-468) for measuring the rate of protein turnover and oxidation.[4] These compounds became popular only because companies, such as CIL, were able to develop cost-effective syntheses of the optically active compounds in large quantities. Because a gram of D-glucose (6,6-$D_2$, 99%) may be administered intravenously, orders for the material are often 100 grams or more. Use of both of these compounds relied on GC/MS, but GC/MS is limited in terms of how low an enrichment can be measured. The development of GC-combustion-MS (GC-C-MS), also in the 1970s,[5] added a method for measuring very low enrichments of stable isotopically labeled tracers. This technique allowed relatively straightforward direct measurement of protein synthetic rates and could be defined as a very early proteomic method.

GC/MS remained the method of choice for the greatest range of stable isotope tracer measurements until the invention of electrospray ionization (ESI) by Fenn in the 1980s and its commercialization as the interface for liquid chromatography/mass spectrometry (LC/MS) in the 1990s. ESI-LC/MS allowed measurement of much larger and much more polar molecules, such as peptides, that could never be measured by GC/MS. ESI-LC/MS along with MALDI-TOF opened the door for mass spectrometry into proteomics. Just as GC/MS was initially used primarily to identify compounds, so was LC/MS initially used in proteomics to identify peptides. However, the next logical step was quantification with identification, and we again return to the use of stable isotopically labeled compounds as internal standards.

*(continued)*

The simplest and most cost-effective isotopically labeled internal standards were not labeled peptides, but labeled derivatization reagents. Peptides derived from one protein sample were chemically modified with an unlabeled derivatization reagent and combined with peptides from another protein sample that were chemically derivatized with a stable isotopically labeled reagent, and the ratio of the unlabeled-to-labeled peptides measured.[6]

For cell culture experiments, cells could be directly grown using stable isotopically labeled substrates to incorporate stable isotope labels directly into cellular proteins.[7] For simple cells such as yeast,[8] the media could be D-glucose ($^{13}C$) and/or ammonium salts ($^{15}N$). For mammalian cells, the labels had to be introduced directly as labeled amino acids, typically labeled lysine and arginine. Stable isotope companies, such as CIL, developed products to make growing labeled cells in culture no more difficult than growing normal cells. However, the ultimate stable isotopically labeled internal standards in proteomics has been the growth of whole labeled animals, initially a $^{15}N$-labeled rat,[9] but later as a range of small animals from mice[10] to worms.[11] Labeled protein, even as prepared animal feed, can be purchased directly from companies, such as CIL, for growing your own stable isotope-labeled animal to encompass all stable isotope possibilities.

The whole process of use of stable isotopes has evolved around both technology development for innovative mass spectrometric techniques to measure molecules in complicated matrices and technology development of cost-effective preparation of stable isotopes at high isotopic enrichments and strategies for incorporation of the isotopes into organic molecules. It is interesting to note that initially all incorporation was done via classical organic chemical synthesis, but more recently methods have been developed to incorporate isotopes into molecules using biochemical and cell biological techniques. One point is true: as biological science evolves, use of stable isotopes will evolve with it and remain a key tool in research.

Dwight E. Matthews, PhD
Professor of Chemistry and Medicine
The University of Vermont, Burlington, VT

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