Selective Amino Acid-Type Labeling

Selective amino acid-type labeling is used to simplify crowded spectra in order to aid in spectral interpretation or to provide specific probes for dynamic and structural studies. This technique requires the addition of free labeled amino acids (~50->250 mg/L) to either minimal or rich growth media prior to protein induction. The cells will generally utilize the supplemented amino acids for protein synthesis prior to undergoing the *de novo* synthesis of the target amino acids. Please see page 25 for a complete listing of amino acids that may be used with either *in vivo* growth systems or cell-free protein expression methods.

Selective Isotope-Labeling Methods for Protein Structural Studies

Hanudatta S. Atreya, Ph.D.
NMR Research Centre, Indian Institute of Science, Bangalore, India

One of the major contributing factors to the rapid advance of biomolecular NMR spectroscopy is the emergence of different isotope labeling methods. Recent developments in biotechnology have made it easier and economical to introduce ^{13}C,^{15}N and ^{2}H into proteins and nucleic acids. At the same time, there has been an explosion in the number of NMR experiments that utilize such isotope-labeled samples. Thus, a combination of isotopic labeling and multidimensional, multinuclear experiments has significantly expanded the range of problems in structural biology amenable to NMR.

Isotope labeling in proteins can be broadly classified into four categories: Uniform, amino acid-type selective, site-specific and random/fractional labeling. The beginning of systematized isotope labeling in proteins can be traced back to late 60's in the group of Jardetsky and Katz and coworkers.[1,2] Theirs was also one of the first amino acid-type selective labeling methods involving incorporation of specific protonated amino acids against a deuterated background. In the 80's uniform (^{13}C/^{15}N) and selective incorporation of ^{15}N-labeled amino acids against an unlabeled (^{12}C/^{14}N) background was developed.[3] Subsequently, a variety of labeling methods have emerged (reviewed in [4] and [5] and illustrated in Figure 1).

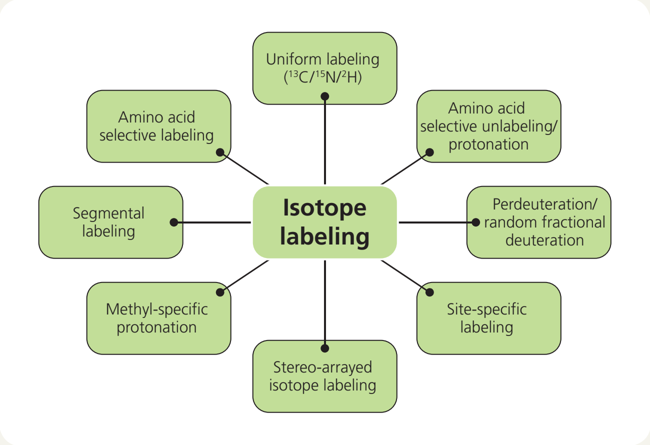

Figure 1. Different isotope-labeling methods.

In addition to uniform (^{13}C/^{15}N/^{2}H) labeling, amino acid-type or site-selective labeling is often pursued as it helps in spectral simplification and provides specific probes for structural and dynamic studies. Selective amino acid-type labeling also aids in sequence-specific resonance assignments by helping to identify resonances which are otherwise buried in the crowded regions of 2D and 3D NMR spectra. However, a disadvantage of this method is the possible mis-incorporation of ^{15}N label in undesired amino acids (also called as "isotope scrambling").[3] This happens due to metabolic conversion of one amino acid to another in the bio-synthetic pathway of the cell. The problem becomes more severe for amino acids higher up or intermediates in the metabolic pathway such as Asp, Glu and Gln (See Figure 2 showing the biosynthetic pathway in *E. coli*). For those which are end-products in the production pipeline (Ala, Arg, Asn, Cys, His, Ile, Lys, Met, Pro and Trp) isotope scrambling is minimal and the remaining (Gly, Phe, Leu, Ser, Thr, Tyr and Val) have medium to weak interconversion. Isotope scrambling in *E. coli* can be minimized by reducing the activity of the enzyme(s) catalyzing the inter-conversion or amino transfer using either specific (auxotrophic) strains[3] or using enzyme inhibitors.[6] Another alternative is to use cell-free or *in vitro* expression systems which lack these enzymes.[4]

One drawback of amino acid selective labeling is the expense associated with the use of ^{13}C/^{15}N labeled amino acids. A relatively inexpensive method is that of amino acid selective "unlabeling" or reverse labeling. In this method, the host organism is grown on a medium containing the desired unlabeled (*i.e.*, ^{1}H/^{12}C/^{14}N) amino acid against a labeled (^{13}C/^{15}N) background. This is somewhat akin to the selective protonation experiment by Jardetsky[1] and Katz.[2] Reverse labeling was first used by Bax and coworkers[7] and developed further by other groups for different applications.[8,9,10] The problem of isotope scrambling (in this case being the mis-incorporation of ^{14}N) remains largely the same as in the selective-labeling approach mentioned above (for a detailed table of possible scrambling of ^{14}N see reference 10).

(continued)

Selective Amino Acid-Type Labeling

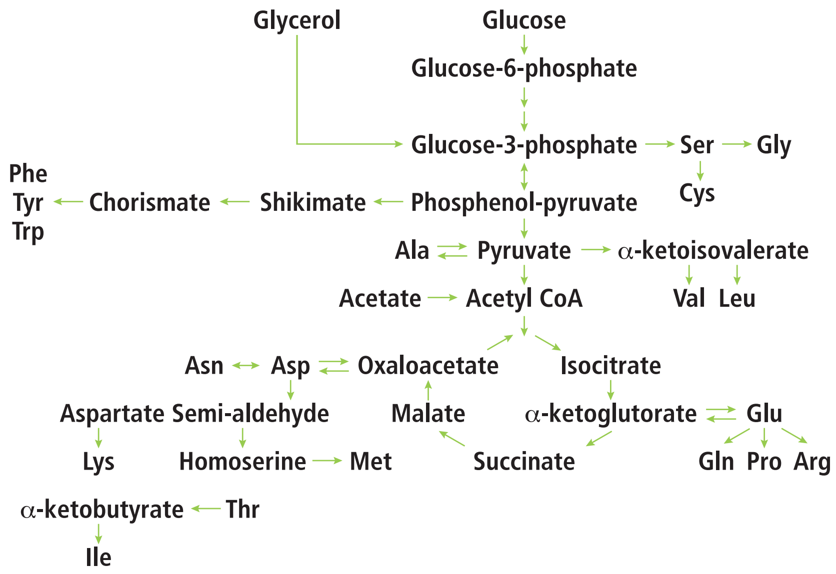

Figure 2. Amino acid biosynthesis in *E. coli.*

In addition to the above, new isotope-labeling methods continue to be developed. More recent methods of segmental labeling[11] and stereo-arrayed isotope labeling (SAIL)[12] open up new avenues in protein structural studies. The future points towards a combination of different isotope-labeling methods to address challenging and complex problems in structural biology.

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