Labeled amino acids (stable isotope-labeled amino acids)

Various labeled amino acid products are now available from Ajinomotos Amino Acid Department. These products, produced through Ajinomoto's proprietary amino acid fermentation technologies, are primarily used in leading-edge scientific research in the field of protein structural analysis. These labeled amino acids are described below.

What are stable isotopes?

First, the term "stable isotope" will be explained. Do you remember the atomic symbols you learned long ago? [$^{12}_{6}$C] is an atomic symbol representing a carbon atom. The number at the lower left, 6, is the atomic number (number of protons), while the number at the upper left, 12, is the mass number (the sum of the protons and neutrons). The atomic number is often removed, and thus a carbon atom is often represented by [^{12}C].

For example, a hydrogen atom is normally represented by [^{1}H], as it has a mass number of 1 (with no neutron). The vast majority (99.985%) of hydrogen atoms in the world have a mass number of 1. So what are the remaining (0.015%) hydrogen atoms? They are those represented by [^{2}H] with a mass number of 2 (with one neutron). In addition, there is another hydrogen atom with a mass number of 3 (with two neutrons), although it is not found in nature. The ^{1}H hydrogen is commonly represented by [H], ^{2}H hydrogen is called "deuterium" and is expressed by [D], and ^{3}H hydrogen is called "tritium" and is expressed by [T]. (Figure 1)

Figure 1. Isotopes of hydrogen atoms

	Stable isotope		Radioisotope
	^{1}H hydrogen	^{2}H=D Deuterium	^{3}H=T Tritium (Super-heavy hydrogen)
	Electron, Proton	Proton, Neutron	Proton, Neutron
Electron	1	1	1
Proton	1	1	1
Neutron	0	1	2
Mass number	1	2	3

Mass number = Number of protons + Number of neutrons

Atoms with the same atomic number (proton number) but different mass numbers (the sum of the proton and neutron numbers) in this manner are called "isotopes." Among these isotopes, unstable isotopes, which emit radioactive rays and neutrons, are called "radioisotopes," while isotopes that are always stable are called "stable isotopes." In the case of hydrogen, [^{1}H] and [^{2}H=D] atoms are stable isotopes, while [^{3}H=T] atoms are radioisotopes. Likewise, in the case of carbon, [^{12}C] and [^{13}C] atoms are stable isotopes, and there are two stable isotopes of nitrogen, [^{14}N] and [^{15}N]. All isotopes in the products of the Amino Acid Department are stable isotopes.

What are labeled amino acids (stable isotope labeled amino acids)?

Amino acids consist primarily of carbon (C), nitrogen (N), hydrogen (H) and oxygen (O) atoms. As shown in Table 1,

Table 1. Ratios of the stable isotopes present in the major constituent elements of amino acids

Hydrogen		Carbon		Nitrogen		Oxygen	
Isotope	Ratio	Isotope	Ratio	Isotope	Ratio	Isotope	Ratio
^{1}H	99.985	^{12}C	98.892	^{14}N	99.635	^{16}O	99.759
^{2}H	0.015	^{13}C	1.108	^{15}N	0.365	^{17}O	0.037
						^{18}O	0.204

nearly all hydrogen atoms present in nature have a mass number of 1 (^{1}H), nearly all carbon atoms present in nature have a mass number of 12 (^{12}C), nearly all nitrogen atoms have a mass number of 14 (^{14}N), and nearly all oxygen atoms have a mass number of 16 (^{16}O). However, these atoms contain isotopes with different mass numbers, although in trace amounts. Amino acids labeled by special procedures using these stable isotopes, which are present only in small amounts in nature, are called "stable isotope-labeled amino acids" (or "labeled amino acids").

Figure 2. L-glutamine ($C_5H_{10}N_2O_3$)

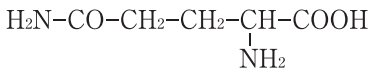


Figure 3. ^{15}N-labeled amino acid

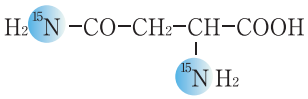
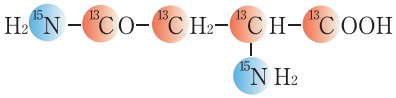


Figure 4. ^{13}C- and ^{15}N-labeled amino acid



For example, in one amino acid, L-glutamine, which is represented by the chemical structure shown in Figure 2, the five carbon atoms are almost always (^{12}C) (98.892%), and the two nitrogen atoms are almost always (^{14}N) (99.635%). On the other hand, amino acids in which some of the normal nitrogen atoms are replaced according to special procedures with ^{15}N atoms having a mass number of 15, are called ^{15}N-labeled amino acids (Figure 3), and amino acids in which the normal carbon atoms are further replaced with ^{13}C, having a mass number of 13, are called ^{13}C- and ^{15}N-labeled amino acids.

There are many labeled amino acids due to the fact that there are many possible combinations, depending on the type of amino acid, the types of labeling atoms, the combination of labeling atoms (^{13}C, ^{15}N and others), and the positioning of labeling atoms.

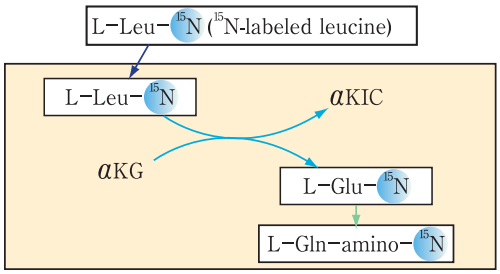
Currently, Ajinomoto's labeled amino acids are those in which all constituent elements are uniformly labeled. For example, ^{13}C-labeled glutamine has five carbon atoms, all of which are labeled with ^{13}C atoms, and ^{15}N-label glutamine has two nitrogen atoms, both of which are labeled with ^{15}N atoms.

Three types of labeling elements are currently available, ^{15}N, ^{13}C, and ^{2}H (D), the combinations of which include four types: (1) ^{15}N-label, (2) ^{13}C- and ^{15}N-double label, (3) ^{15}N- and ^{2}H(D)-double label, and (4) ^{13}C-, ^{15}N- and ^{2}H(D)-triple label.

What are the applications of labeled amino acids?

There are two major applications of labeled amino acids. One is as a material aiding investigation of the metabolism/dynamics of amino acids in microorganisms and animals (Figure 5), and another is as a reagent for analysis of the tertiary structure of proteins (Figure 6).

Figure 5. Method of investigating the metabolism and dynamics of amino acids in the body

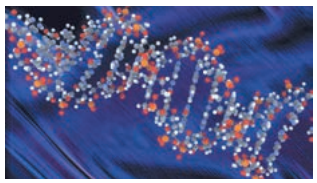


Nerve cell

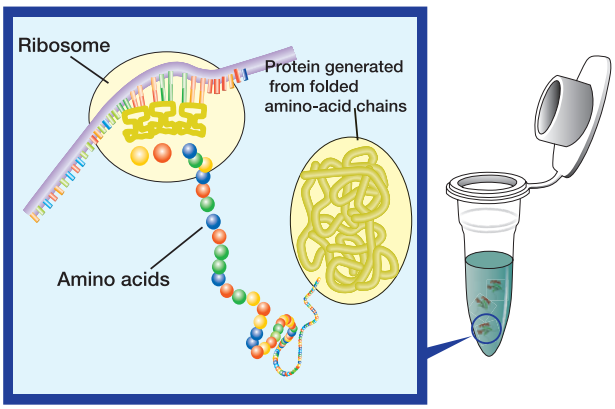
Amino-acid metabolism may be evaluated through the analysis of metabolites containing ^{15}N.

Figure 6. Application to protein structural analysis

Preparation of cDNA (DNA having only reguired information) corresponding to protein for structural analysis



Protein synthesis using a labeled amino acid as a building block (Cell-free protein synthesis)



Determination of the tertiary structure of a protein by nuclear magnetic resonance (NMR)

Labeled amino acids differ in weight from normal amino acids, as they are labeled with stable isotopes. The intended amino acid actions may be investigated based on this weight difference

IIn addition, the structure of proteins (polymers of amino acids) containing labeled amino acids may be analyzed by nuclear magnetic resonance (NMR). The structure of proteins relatively lower in molecular weight, as well as particular portions of protein structures may be determined by this method in a shorter period of time than by X-ray crystallography.

Currently, the applications to protein structural analysis are expanding rapidly. This is due to the fact that many large-scale projects are planned or underway throughout the world for the genomic development of new drugs based on the results of the Human Genome Project. These projects are endeavoring to explain biological phenomena and develop new drugs through analysis of the structure and functions of proteins. Here in Japan, a new project, the "Protein 3000 Project (2002-2006)," was established in 2002 by the Ministry of Education, Culture, Sports, Science and Technology. Other similar projects are also underway, and further protein structural analysis studies are now being conducted in many laboratories in universities and pharmaceutical companies.

How are the labeled amino acids produced?

First, gases containing ^{13}C and ^{15}N are separated from gases such as carbon monoxide (CO), methane (CH_4), and nitrogen monoxide (NO) through distillation based on the weight differences among such gases.

A commonly used method for the production of labeled amino acids involves the culturing of an alga (photosynthetic microorganism) in a container under a mixed stable isotope gas atmosphere ($^{13}CO_2$ or $^{15}N_2$), and the labeled amino acids are extracted from the hydrolysates of the labeled proteins produced. Using this procedure, labeled amino acids are produced in an amount proportional to the amino acid composition of cultured proteins, and hence, the procedure involves the disadvantage that it is difficult to produce labeled amino acids that are contained in smaller amounts in cultured proteins, although the amino acids more abundantly contained are well supplied.

In contrast, labeled amino acids are produced here at Ajinomoto through our proprietary fermentation technique, which uses glucose labeled with a stable isotope (labeled glucose) and ammonia labeled with a stable isotope (labeled ammonia) as raw materials. Although the production volumes are extremely small, in the order of grams and at approximately one-millionth of the volumes of amino acids produced for medicines and foods (several tons), these labeled amino acids are produced based on Ajinomoto's amino acid production technology. Any particular desired amino acid can be produced by the fermentation method. In addition, large-scale production is also possible simply by changing the capacity of the cultivation tank and the quantities of raw materials used.