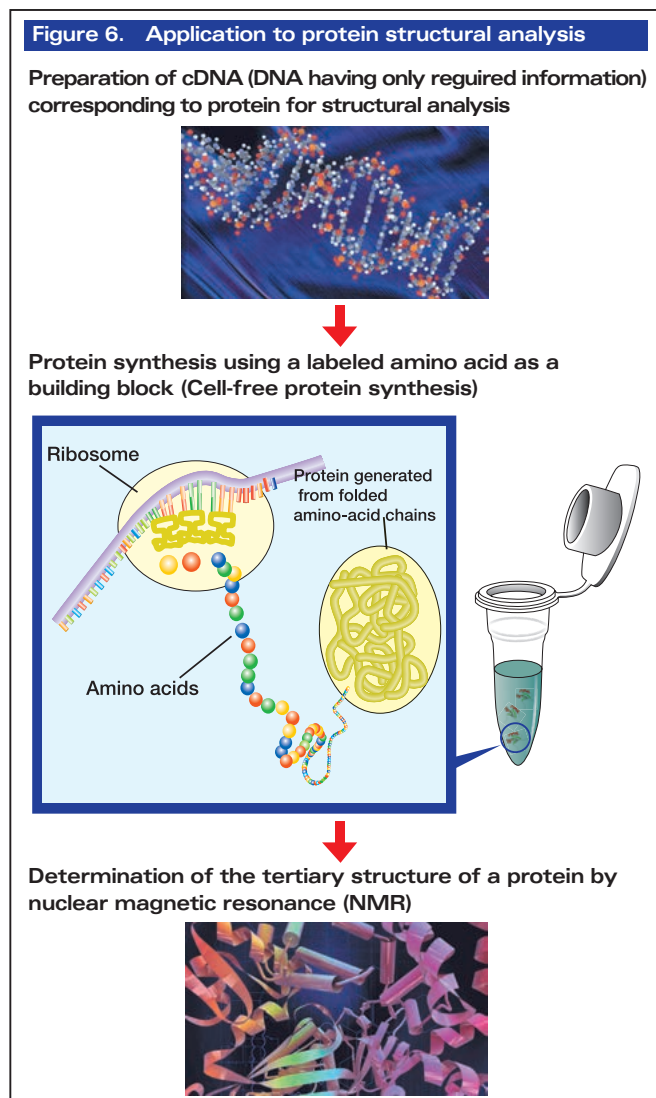
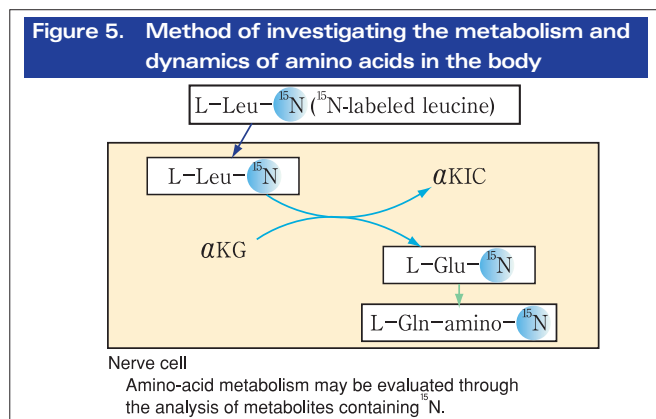




Three types of labeling elements are currently available,  $^{15}\text{N}$ ,  $^{13}\text{C}$ , and  $^2\text{H}$  (D), the combinations of which include four types: (1)  $^{15}\text{N}$ -label, (2)  $^{13}\text{C}$ - and  $^{15}\text{N}$ -double label, (3)  $^{15}\text{N}$ - and  $^2\text{H}$ (D)-double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



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

In 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  $^{13}\text{C}$  and  $^{15}\text{N}$  are separated from gases such as carbon monoxide (CO), methane ( $\text{CH}_4$ ), 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 ( $^{13}\text{CO}_2$  or  $^{15}\text{N}_2$ ), 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.