Note

Enzymatic Synthesis of [^{15}N]-Methionine-incorporated Peptides by Papain

Shin-ya TANIMOTO, Yumiko SHOJI,*
Hiroko KIMURA* and Soichi ARAI**

Department of Home Economics,
Aoyama Gakuin Women's Junior College,
Shibuya-ku, Tokyo 150, Japan

* Department of Nutrition, Kagawa Nutrition College,
Toshima-ku, Tokyo 170, Japan

** Department of Agricultural Chemistry,
The University of Tokyo, Bunkyo-ku,
Tokyo 113, Japan

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In the nutritional evaluation of food proteins, the concept of essential amino acids has been deduced from nutritional experiments with free amino acid mixtures. There has long been the conception that, in the digestive tract intake, proteins are digested to amino acids prior to being absorbed.¹⁾ However, recent research has elucidated a digestion and absorption mechanism involving oligopeptides.^{2–4)} It is now believed that these peptides formed by intralumen hydrolysis of the intake proteins are mostly absorbed prior to their complete hydrolysis to free amino acids.⁵⁾

There is the problem to be solved in these absorption experiments that peptide digestives are indistinguishable from endogenous amino acids. One way of solving this is to use isotope-labeled materials.

We fortified soy protein by [^{15}N]methionine by using the plastein reaction⁶⁾ to investigate the absorption and metabolism of the resulting peptide-bound methionine. The plastein reaction was carried out with an enzyme, is recrystallized papain (EC 3.4.22.2) from Sigma being used without further purification. This was in a suspension form containing 25 mg of protein/ml.

Enzymes are not only chemically specific, being also stereospecific and able to resolve racemates. Actually, papain has an affinity specific to L-amino acid and incorporates L-methionine to form a peptide bond.^{7,8)} Firstly, the reactivity of racemates and the L-form of [^{15}N]methionine was respectively examined with *N*-benzoylglycine ethyl ester as a substrate and methionine ethyl ester as a nucleophile. The racemates of the [^{15}N]methionine ethyl ester in the concentration of 10 mM showed an incorporation rate of 0.17 $\mu\text{mol}/\text{min}$, ml the same as that of the L-form [^{15}N]methionine ethyl ester at a concentration of 5 mM. Therefore, DL-[^{15}N]methionine

(95 atom %; Molecules Marquee, France) was used to synthesize the ^{15}N -labeled peptides, instead of using L-[^{15}N]methionine at a cost 2.5 times that of the racemates. Fujipro-R (Fuji Oil Mill Co.), a commercial acid-precipitated soy protein isolate (SPI), was dissolved at a final concentration of 25% (w/w) in a 19.5% carbonate solution. To this solution, DL-[^{15}N]methionine ethyl ester and papain were respectively added at 21% (w/w) and 1% (w/w) of SPI. Furthermore, L-cysteine was mixed in at a final concentration of 7% (w/w) as an activator of the enzyme. The resulting mixture was homogenized for the most efficient hydrolysis of SPI. Incorporation of methionine into the hydrolysate was carried out at 37°C for 3.5 hr, before the reaction mixture was dialyzed in a cellulose tube (Spectrapor, MW 1,000 cut-off, Spectrum Medical Industries Inc., U.S.A.) against water. The non-diffusible fraction was lyophilized, and the average yield calculated by the micro-Kjeldahl method was 86%. This figure signified that the hydrolytic reaction had not proceeded very far. Its amino acid composition determined with an amino acid analyzer showed that the concentration of methionine had increased from 0.7% to 3.8%, while the variance of other amino acids compared to the soy protein amino acid composition was slight. The average molecular weight was considered to be 5,000 from the results of SDS polyacrylamide gel electrophoresis. The ^{15}N content was determined after gasifying the sample by the Dumas method, according to an improved emission spectrum analysis with a ^{15}N analyzer (Heavy Nitrogen Analyzer Model N-150, Japan Spectroscopic Co. Ltd).⁹⁾ The ^{15}N content was found to be 1.7% (Table I), suggesting that [^{15}N]methionine had been incorporated. If the peptide thus obtained is mixed with a protein or hydrolysate, a ^{15}N -labeled peptide mixture containing the desired level of methionine can be produced.

It has been reported that the quantity of incorporated amino acid varies according on the amount of amino acid ester added to the reaction system, as well as to the reaction time employed.¹⁰⁾ Thus, ^{15}N amino acid incorporated peptides or proteins may be prepared as desired, if the quantity of ^{15}N amino acid to be incorporated is controlled by changing these conditions. Precise information and

Table I. ABUNDANCE RATIO OF ^{15}N IN PEPTIDES

Sample no.	Abundance ratio of ^{15}N
1	1.69
2	1.69
3	1.69
4	1.73
Mean value	1.70
Standard deviation	0.02

The experimental conditions are described in the text.

knowledge can be obtained about protein digestion and absorption by using new materials prepared according to the enzymatic technique developed in the present work.

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