Oligopeptide Mixtures Produced from Soy Protein by Enzymatic Modification and Their Nutritional Qualities Evaluated by Feeding Tests with Normal and Malnourished Rats

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Summary Soy protein isolate (SPI) was enzymatically modified to produce oligopeptide mixtures having methionine at approximate levels of 1% and 3%. Each of them had an average molecular weight of slightly lower than 1,000 daltons. They were compared with corresponding amino acid mixtures as well as with SPI for protein efficiency ratio (PER) and several other parameters. Normal and protein-malnourished rats were used as subjects for the comparison tests. When malnourished rats were subjected to a feeding test at a methionine level of 1% in nitrogen source, the oligopeptide mixture, OPM₁, gave a significantly higher PER value than any of SPI and the amino acid mixtures. At a methionine level of 3%, both normal and malnourished rats utilized the oligopeptide mixture, OPM₃, with higher efficiency than the amino acid mixture. These results suggest that the oligopeptide mixtures were utilized similarly to or more efficiently than SPI from which they were derived and the amino acid mixtures with exactly simulated amino acid composition.

Key Words oligopeptide, soy protein, protein hydrolysate, methionine, enzymatic modification

In recent years the view on the absorption of protein digestion products has undergone a radical change. It is now believed that proteins never undergo complete digestion to free amino acids before absorption and that their intralumen and brush border hydrolysis is only partial (1, 2). A number of relevant papers are available dealing with the small-intestinal absorption of synthetic oligopeptides (3–7) and partially hydrolyzed proteins (8–10). An applied study has also been conducted to produce a peptide rather than free amino acid nitrogen source for chemically defined diets (11).
It is likely indeed that oligopeptides, when administered per os, behave differently from corresponding amino acid mixtures in some specific respects in absorptive and postabsorptive stages, but little is known of whether there is any difference with respect to over-all nutritional parameters including protein efficiency ratio (PER).

When an oligopeptide mixture or a partially hydrolyzed protein for use in evaluation of such parameters is intentionally produced from a protein by enzymatic modification or any other available methods, the resulting product must not contain any significant amount of free amino acids which might cause an erroneous interpretation of experimental results. Secondly, the amino acid composition of an oligopeptide mixture should be taken into consideration. When the level of the most limiting amino acid happens to be too low in the oligopeptide mixture, it should be properly supplemented, preferably in a covalent fashion because some untritional differences may exist between an added amino acid and a covalently bound amino acid. Thirdly, it is sometimes preferable to use abnormal animals as subjects because, as far as healthy animals are used, it may be difficult to detect significant differences in any nutritional parameters between an oligopeptide mixture and the corresponding amino acid mixture.

The present paper describes a process developed to produce, from soy protein isolate (SPI), oligopeptide mixtures which have very narrow ranges of molecular weight distribution, containing little if any amounts of free amino acids. For production of such oligopeptide mixtures, we designed a sophisticated enzymatic process with papain [EC 3.4.22.2] in the first place and, secondly, with trypsin [EC 3.4.21.4] or a thiol-independent prolyl endopeptidase [EC 3.4.21.26] of Flavobacterium origin (12), since the use of a simple process would possibly result in formation of a rather heterogeneous protein hydrolysate with a large amount of free amino acids. The use of papain also facilitates covalent attachment of \( \text{L-methionine} \), as earlier studies (13–15) have employed this enzyme for the same purpose. The attachment of methionine is to enhance its content in an oligopeptide mixture up to a level of 3\%\ with which has been reported to be optimal for SPI to give a highest PER value (16). The paper also describes the consequences of feeding tests with normal and protein-malnourished rats to compare nutritional qualities of oligopeptide mixtures with those of SPI and amino acid mixtures.

The following codes denote types of samples with two different methionine levels: SPI\(_1\), soy protein isolate which per se contains methionine at a level of ca. 1\%; SPI\(_3\), soy protein isolate to which free L-methionine is added to enhance its methionine level up to ca. 3\%; OPM\(_1\), oligopeptide mixture with a methionine level of ca. 1\%; OPM\(_1\)-T, OPM\(_1\) produced by using trypsin; OPM\(_1\)-P, OPM\(_1\) produced by using prolyl endopeptidase; OPM\(_3\), oligopeptide mixture with a methionine level of ca. 3\%; OPM\(_3\)-T, OPM\(_3\) produced by using trypsin; OPM\(_3\)-P, OPM\(_3\) produced by using prolyl endopeptidase; AAM\(_1\), amino acid mixture formulated by simulating OPM\(_1\)-T; and AAM\(_3\), amino acid mixture formulated by simulating OPM\(_3\)-T.
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EXPERIMENTAL

Protein and amino acids. SPI, an acid-precipitate product with a protein content of 92%, was obtained from Fuji Oil Mill Co., Japan; its amino acid composition is shown in Table 1. Glycine and other L-amino acids, all being crystallized preparations, were purchased from Ajinomoto Co., Japan.

Methionine ethyl ester. Crystallized L-methionine was esterified with absolute ethanol by the method of Boissonas et al. (17). The resulting product was crystallized from ethanol-ether to obtain methionine ethyl ester hydrochloride in needle.

Enzymes. The three enzymes were obtained from commercial sources: papain (25 BAEE units/min/mg; lot: 14F-8100) from Sigma Chemical Co., trypsin (9200 BAEE units/min/mg; lot: T-8003) from Sigma Chemical Co., and prolyl endopeptidase (20 units/min/bial) from Seikagaku Kogyo Co. Tokyo, Japan.

Amino acid analysis. SPI and OPM products were each hydrolyzed with 6 N HCl at 110°C for 24 h and the resulting amino acids quantified with an amino acid analyzer (Hitachi KLA 4-A). Cysteine was analyzed by the performic acid oxidation (18) followed by the amino acid analysis. Tryptophan was determined by the method using 3 N mercaptoethane sulfonic acid (19). For analysis of free amino acids remaining, OPM products were dissolved in a citric acid buffer (pH 2.2) and the solution injected directly to the amino acid analyzer.

Molecular weight estimation. OPM products were chromatographed on Bio-gel P-2 (Biorad) by using 0.2 M phosphate buffer (pH 8.0) as eluent. For calibration of a molecular weight-elution volume relationship, glycine (Ajinomoto Co., Japan), L-phenylalanine (Ajinomoto Co., Japan), triglutamic acid (Protein Research Foundation, Japan), antipain (Sigma Chemical Co.), human angiotensin III (Protein Research Foundation, Japan) and human angiotensin I (Protein Research Foundation, Japan) were used as markers, which have molecular weights of 75, 165, 402, 604, 931, and 1,296, respectively.

Feeding tests. Male rats (Wistar strain) were fed experimental diets ad libitum at 22 ± 1°C with a 12-h cycle of light (6 a.m.—6 p.m.) and dark (6 p.m.—6 a.m.). The feeding room was maintained at a relative humidity of 65%. The diet composition and the feeding schedule are described in detail later.

Evaluation of nutritional parameters. Daily body weight gains of rats, their food intake, and fecal nitrogen were measured to obtain PER. Systemic blood samples were taken from rats at the tail to measure their hemoglobin (Hb) concentrations. With these blood samples, serum glutamine-oxoacid transaminase (GOT) and glutamine-pyruvate aminotransferase (GPT) activities were measured by the usual methods (20).

Statistical analysis. Comparisons of two means were made by Student’s t-test (21).
RESULTS AND DISCUSSION

Production and chemical properties of oligopeptide mixtures

SPI (100 g) was mixed with NaHCO₃ (15 g) and Na₂CO₃ (4.5 g) and then kneaded homogeneously with added water (300 ml). Thus, the mixture reached a pH value of 9.0 which had been reported to be optimal for papain to catalyze covalent attachment of methionine ethyl ester (13). To the mixture were added L-methionine ethyl ester hydrochloride (3.56 g) and an enzyme solution (5 ml) which comprised papain (1 g) and L-cysteine (70 mg) as solutes. The mixture was incubated at 37°C for 2 h and then treated with 0.5 N NaOH (2.5 liters) for 2 h at room temperature, to terminate the enzymatic reaction and, at the same time, to saponify ethyl ester linkages that might remain intact. The alkali-treated solution was placed in an ultrafiltration membrane tube (spectrapore) and dialyzed in running water at 5°C for 2 days until the inner solution reached neutrality. The lyophilization of the non-diffusible fraction afforded a papain-modified SPI product in a yield of 85 g on a dry-matter basis. The same process was repeated to accumulate a similar product when needed. This product (100 g) was dissolved in water (5 liters) and adjusted to pH 7.8 by adding 1 N NaOH. The solution was incubated with trypsin (100 mg) at 37°C for 15 min. Absolute ethanol (10 liters) was added to terminate the enzymatic reaction and the resulting 66% ethanol solution allowed to stand for 6 h at room temperature. The resultant insoluble fraction was precipitated by centrifugation at 4,000 rpm for 10 min to obtain a supernatant. The precipitate (42 g on a dry-matter basis) was submitted to a repetition of the tryptic hydrolysis followed by the ethanol treatment to obtain the second supernatant. The first and second supernatants were pooled prior to evaporation of the ethanol in vacuo. The lyophilization of the residue gave OPM₃-T in a yield of 87 g. For production of OPM₃-P, the papain-modified SPI product (10 g) was dissolved in water (500 ml) and adjusted to pH 7.8 by adding 1 N NaOH. This was mixed with prolyl endopeptidase (1 bial) and the mixture incubated at 37°C for 30 min. Absolute ethanol (1 liter) was added to terminate the enzymatic reaction and the resulting 66% ethanol solution allowed to stand for 6 h at room temperature. The resultant insoluble fraction was precipitated by centrifugation to obtain a supernatant. The precipitate (4 g on a dry-matter basis) was hydrolyzed again with prolyl endopeptidase similarly to the above. The ethanol treatment was followed to obtain the second supernatant. Both were pooled and lyophilized with production of OPM₃-P in a yield of 6.3 g.

To produce OPM₁-T and OPM₁-P, the modification of SPI was conducted without L-methionine ethyl ester. SPI (100 g) was mixed with NaHCO₃ (15 g) and Na₂CO₃ (4.5 g) in water (300 ml) and treated with papain (1 g) in the presence of L-cysteine (70 mg). The conditions used for incubation of the mixture, termination of the enzymatic reaction, and dialysis to obtain a non-diffusible fraction, were the same as those described in connection with the production of a papain-modified SPI product for OPM₃-T and OPM₃-P. Thus, the lyophilization of the non-diffusible fraction afforded another papain-modified SPI product in a yield of ca. 80 g. With
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this product (100 g), repeated tryptic hydrolysis was conducted as described above, to obtain the first and second supernatants. The lyophilization of a pooled supernatant yielded 87 g of OPM$_1$-T. Similar treatments on the papain-modified SPI product (10 g) were carried out by using prolyl endopeptidase (1 bial) instead of trypsin, to produce OPM$_1$-P in a yield of 6.7 g.

Amino acid analysis showed that OPM$_3$-T and OPM$_3$-P, as their subscript numbering indicates, had methionine at an approximate level of 3% each (Table 1). Total amounts of free amino acids remaining were as small as 4.44% in OPM$_3$-T and 0.18% in OPM$_3$-P. Methionine levels observed for OPM$_1$-T and OPM$_1$-P were approximately 1%, reflecting the original methionine content of the SPI used. Also in these cases, total amounts of free amino acids remaining were at small levels, approximating 4.15% in OPM$_1$-T and 0.14% in OPM$_1$-P. Though the OPM$_1$ products differed from the OPM$_3$ products in terms of the level of methionine, there were no appreciable differences in the levels of other amino acids including cystine. Any of the OPM products also resembled SPI with respect to the levels of amino acids other than methionine.

Table 1. Total and free amino acid composition of soy protein isolate (SPI) and four kinds of oligopeptide mixtures.

| Amino acid       | SPI     | OPM$_1$-T | OPM$_1$-P | OPM$_3$-T | OPM$_3$-P |
|------------------|---------|-----------|-----------|-----------|-----------|
|                  | Total   | Total Free (g amino acid/16 g nitrogen) | Total Free | Total Free | Total Free |
| Aspartic acid    | 11.13   | 11.08 0.40 | 12.26 n.d. | 12.12 0.39 | 12.12 n.d. |
| Threonine        | 3.65    | 3.84 0.07  | 3.08 n.d.  | 3.69 0.06  | 3.02 n.d.  |
| Serine           | 4.81    | 4.02 0.01  | 4.40 n.d.  | 4.91 0.02  | 4.35 n.d.  |
| Glutamic acid    | 20.42   | 18.20 1.52 | 21.64 0.07 | 21.44 1.61 | 20.94 0.07 |
| Proline          | 5.66    | 3.65 n.d.  | 3.03 0.04  | 2.43 n.d.  | 3.14 0.03  |
| Glycine          | 3.97    | 4.09 0.08  | 4.02 n.d.  | 4.45 0.05  | 4.12 n.d.  |
| Alanine          | 4.33    | 4.62 0.36  | 3.64 n.d.  | 4.62 0.18  | 3.60 n.d.  |
| Cystine          | 1.04    | 1.10 0.04  | 1.14 n.d.  | 1.10 0.04  | 1.10 n.d.  |
| Valine           | 5.04    | 5.08 n.d.  | 3.86 n.d.  | 5.20 0.03  | 4.10 n.d.  |
| Methionine       | 1.07    | 1.08 0.06  | 1.09 n.d.  | 3.11 0.22  | 3.07 0.04  |
| Isoleucine       | 4.78    | 5.20 0.21  | 4.88 n.d.  | 5.52 0.20  | 4.80 n.d.  |
| Leucine          | 7.05    | 7.69 0.41  | 6.73 n.d.  | 7.59 0.41  | 6.67 n.d.  |
| Tyrosine         | 3.79    | 4.05 0.10  | 2.95 n.d.  | 3.57 0.21  | 3.15 n.d.  |
| Phenylalanine    | 5.25    | 5.95 0.25  | 4.61 0.03  | 5.92 0.36  | 4.97 0.04  |
| Tryptophan       | 0.98    | 1.00 n.d.  | 0.98 n.d.  | 0.99 n.d.  | 0.98 n.d.  |
| Lysine           | 5.72    | 5.95 0.14  | 6.16 n.d.  | 5.42 0.13  | 6.06 n.d.  |
| Histidine        | 2.65    | 2.74 0.02  | 3.07 n.d.  | 2.02 0.02  | 3.01 n.d.  |
| Arginine         | 7.22    | 7.63 0.48  | 8.86 n.d.  | 5.52 0.51  | 8.72 n.d.  |
| Sum              | 98.56   | 96.97 4.15 | 96.40 0.14 | 99.62 4.44 | 97.92 0.18 |

n.d., not detected.

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Fig. 1. Bio-gel P-2 chromatography of four kinds of oligopeptide mixtures. Each OPM product (50 mg) dissolved in water (1 ml) was loaded onto a Bio-gel P-2 column (2 × 100 cm) which had been equilibrated with 0.2 M phosphate buffer (pH 8.0) at 20°C. Elution was made by using the same buffer at the rate of 1 ml/min. Every 5 ml was collected to determine its absorbance at 280 nm. The six markers with known molecular weights (see the text) were eluted out at the positions denoted by the arrows.

Chromatography on Bio-gel P-2 demonstrated that any of the four OPM products had a narrow range of molecular weight distribution, the elution peaks centering at the position of 980 daltons in OPM1-T, 850 daltons in OPM1-P, 850 daltons in OPM3-T, and 650 daltons in OPM3-P (Fig. 1). In each case, no great amounts of higher-molecular-weight and lower-molecular-weight species occurred, according to the chromatograms. It seemed that OPM1-T and OPM3-T were chromatographically more homogeneous than OPM1-P and OPM3-P, although free amino acid contents of OPM1-P and OPM3-P were much smaller than those of OPM1-T and OPM3-T (Table 1). However, it was too expensive to use prolyl endopeptidase for large-scale production of OPM1-P and OPM3-P. In comparison, OPM1-T and OPM3-T could more easily be produced in large amounts and we therefore used them as nitrogen sources for the experimental diets in the following feeding tests.

Feeding tests and their consequences

Six kinds of experimental diets were prepared based on SPI1, SPI3, OPM1-T, OPM3-T, AAM1, and AAM3 as nitrogen sources. SPI per se containing 1.07% methionine (Table 1) was used as SPI1 without any prior treatment, whereas the addition of free L-methionine to SPI produced SPI3 having finally a total me-
Table 2. Composition of diets.

| Ingredient   | SPI₁ diet | OPM₁₋T diet | AAM₁ diet | SPI₃ diet | OPM₃₋T diet | AAM₃ diet | Protein-free diet (g/100 g diet) |
|--------------|-----------|-------------|-----------|-----------|-------------|-----------|--------------------------------|
| SPI₁         | 10.9ᵃ     | —           | —         | —         | —           | —         | —                              |
| OPM₁₋T      | —         | 10.5ᵇ      | —         | —         | —           | —         | —                              |
| AAM₁        | —         | —           | 9.7ᵃ      | —         | —           | —         | —                              |
| SPI₃         | —         | —           | —         | 10.9ᵇ     | —           | —         | —                              |
| OPM₃₋T      | —         | —           | —         | —         | 10.5ᵃ      | —         | —                              |
| AAM₃         | —         | —           | —         | —         | —           | 9.7ᵃ      | —                              |
| Corn starchᵇ | 68.75     | 69.15       | 69.95     | 68.75     | 69.15       | 69.95     | 79.65                          |
| Sucrose      | 10.0      | 10.0        | 10.0      | 10.0      | 10.0       | 10.0      | 10.0                          |
| Soybean oilᶜ | 5.0       | 5.0         | 5.0       | 5.0       | 5.0        | 5.0       | 5.0                          |
| Celluloseᵈ   | 1.0       | 1.0         | 1.0       | 1.0       | 1.0        | 1.0       | 1.0                          |
| Mineral mixtureᵉ | 4.0   | 4.0         | 4.0       | 4.0       | 4.0        | 4.0       | 4.0                          |
| Vitamin mixtureᵉ | 0.20  | 0.20        | 0.20      | 0.20      | 0.20       | 0.20      | 0.20                          |
| Choline chlorideᶠ | 0.15  | 0.15        | 0.15      | 0.15      | 0.15       | 0.15      | 0.15                          |

ᵃ Equivalent to 1.5 g nitrogen. ᵇ Produced by Oriental Yeast Industry, Ltd. (Japan). ᵈ Produced by Fuji Oil Co. (Japan). ᵙ Produced by Sanyo Pulp Industry, Ltd. (Japan). ᵙ Formulated according to the reference (22). ᵇ Obtained from Wako Pharmaceutical Co. (Japan).

The thionine level of 3.11% AAM₁ and AAM₃ were formulated by exactly simulating the composition of OPM₁₋T and that of OPM₃₋T, respectively (Table 1). With these sources used at a nitrogen level of 1.5% each, experimental diets of the Harper composition (22) were prepared (Table 2). Besides those, a protein-free diet was used which comprised starch in replacement of the nitrogen source.

In a feeding test with normal subjects, male rats aged 4 weeks, weighing about 80 g each, were fed the experimental diets for 3 weeks. On the other hand, male rats aged 6 weeks, weighing about 170 g, were depleted of nitrogen source by feeding with the protein-free diet for 2 weeks until their average body weight decreased down to approximately 140 g with an accompanying anemic symptom. The protein-malnourished rats were then fed the experimental diets for the following 3 weeks.

Table 3 summarizes the resulting data on various items including food intake, digestibility (obtained from fecal nitrogen and dietary nitrogen) and PER calculated therefrom. It is shown that rats accepted diets almost equally, without distinct degrees of aversion against and preference for any particular one of the diets, and also that the diets were similarly digested and absorbed. The data in this table suggest that, at a methionine level of ca. 1% in nitrogen source, i.e., ca. 0.1% in diet, no significant difference exists between diets in terms of PER and the other parameters, Hb concentration, and serum GOT and GPT activities. Significant differences resulted when malnourished rats were used, which indicates that OPM₁-
Table 3. Performance of three-week feeding of normal and protein-malnourished rats on the SPI-, OPM-, and AAM-based diets at methionine levels of 0.1% and 0.3%.

| State of rats   | Number of rats | Nitrogen source | Methionine level in diet (%) | Diet intakea (g/day) | Digestibilityb (%) | PERa (g/100 ml) | Hba (g/100 ml) | GOTa (Karmen units) | GPTa (Karmen units) |
|----------------|----------------|-----------------|------------------------------|----------------------|-------------------|----------------|---------------|------------------|-------------------|
| Normal         | 5              | SPI1            | 0.1                          | 19.7 ± 1.3           | 84.0              | 2.05 ± 0.38    | 12.0 ± 0.46    | 88 ± 0.5         | 15.0 ± 1.3        |
|                | 5              | OPM1-T         | 0.1                          | 19.9 ± 1.6           | 87.6              | 2.11 ± 0.27    | 12.3 ± 0.17    | 87 ± 5.4         | 13.7 ± 1.5        |
|                | 5              | AAM1           | 0.1                          | 20.0 ± 1.4           | 90.0              | 2.14 ± 0.34    | 12.6 ± 0.45    | 78 ± 6.8         | 18.3 ± 1.2        |
| Malnourished   | 5              | SPI1           | 0.1                          | 23.7 ± 2.7           | 89.1              | 2.54 ± 0.19    | 10.9 ± 0.45    | 95 ± 9.2         | 16.9 ± 0.7        |
|                | 5              | OPM1-T         | 0.1                          | 24.0 ± 1.3           | 89.6              | 3.13 ± 0.13    | *10.9 ± 0.28    | 74 ± 4.0         | 10.7 ± 0.7        |
|                | 5              | AAM1           | 0.1                          | 24.0 ± 2.3           | 90.2              | 2.13 ± 0.51    | *10.3 ± 0.42    | 74 ± 4.2         | 12.8 ± 0.7        |
| Normal         | 5              | SPI1           | 0.3                          | 22.7 ± 1.3           | 87.0              | 2.96 ± 0.24    | 12.6 ± 0.46    | 66 ± 4.0         | 8.3 ± 1.8         |
|                | 5              | OPM3-T         | 0.3                          | 22.3 ± 1.6           | 92.3              | 3.12 ± 0.02    | 12.2 ± 0.44    | 55 ± 1.2         | 11.0 ± 2.0        |
|                | 5              | AAM3           | 0.3                          | 23.2 ± 1.4           | 92.0              | 2.72 ± 0.14    | 12.9 ± 0.28    | 69 ± 3.6         | 14.0 ± 1.5        |
| Malnourished   | 5              | SPI1           | 0.3                          | 24.0 ± 1.5           | 87.0              | 3.88 ± 0.27    | 11.6 ± 0.46    | 68 ± 8.0         | 12.0 ± 0.3        |
|                | 5              | OPM3-T         | 0.3                          | 24.0 ± 1.0           | 91.1              | 3.97 ± 0.15    | 12.2 ± 0.21    | 55 ± 4.5         | 11.0 ± 0.1        |
|                | 5              | AAM3           | 0.3                          | 24.2 ± 1.8           | 92.6              | 3.49 ± 0.13    | 12.2 ± 0.35    | 65 ± 3.9         | 13.5 ± 0.3        |

* Means ± SEM (n = 5). b Means. *p < 0.05.
T was utilized with significantly higher efficiency than SPI$_1$. It is also indicated that the malnourished rats utilized OPM$_1$-T with much higher efficiency than AAM$_1$. When the methionine level was enhanced up to ca. 3% in nitrogen source, partly different performance resulted. Both normal and malnourished rats utilized SPI$_3$ and OPM$_3$-T with similar efficiency. Compared to OPM$_3$-T, however, AAM$_3$ showed a significantly lower PER value. Throughout the feeding tests, no significant difference was found in Hb concentration, while in some cases there were significant differences in GOT activity.

A comment should be made on the observed superiority of OPM$_3$-T to OPM$_1$-T. The methionine existing in OPM$_3$-T is estimated, from the data in Table 1, to be totally in a covalent state. This estimation is supported by a recent paper (23).

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**Figure 2.** Stepwise changes in PER values during three-week feeding of normal rats (A) and protein-malnourished rats (B) on SPI-, OPM-, and AAM-based diets. Each column represents a mean ± SEM (n = 5).

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presented by our group as well as by a preceding paper (15) presented by an independent group. The data obtained from the present study (Table 3) is also consistent with the knowledge (16) that such a state of methionine at a 3% level is as bioavailable as the free state of methionine at the same level.

The RER data (Table 3) were rearranged in order to show their stepwise changes during the feeding period divided into the three sub-periods—the first, second, and third week. Figure 2 shows that when normal rats were fed at a methionine level of ca. 1% in nitrogen source, OPM1-T and AAM1 gave almost constant PER values through the 3 weeks, whereas the PER value for SPI1 tended to increase gradually. In the feeding test with malnourished rats, however, SPI1 showed a constant PER value and OPM1 gave a constant but significantly higher PER value. AAM1 was utilized as efficiently as SPI1 in the first week only, the efficiency decreasing distinctly thereafter. At a methionine level of 3%, on the other hand, no significant difference was observed between any particular two of the three diets, as far as normal rats were used. When malnourished rats were used, it was found that, as the feeding time elapsed, AAM3 gradually decreased its nutritional efficiency down to a significantly lower level than OPM3-T did.

Based on all the data obtained from the present tests, it is concluded that the superior nutritional effect found in the OPM products are probably due to a function intrinsic to these oligopeptides, since, in respect to the composition of amino acids including sulfur-containing amino acids, OPM1-T and OPM3-T are almost similar to SPI1 and SPI3, respectively, and are exactly simulated by AAM1 and AAM3, respectively. A better nutritional effect found in each of OPM1-T and OPM3-T may be ascribed to their function of supplying a better balance of amino acids to the body tissues in the postabsorptive stage, as Hara et al. (10) have estimated from their work on portal absorption of a protein hydrolysate in rats. In relevance to this, Meister (24) has suggested that in general the superioritility of a protein to the corresponding amino acid mixture could be related to temporal factors in the absorption of amino acids, emphasizing the potential importance of such factors in the presentation of amino acids to the tissues for protein synthesis.

We produced four kinds of oligopeptide mixtures for use in evaluation of their overall nutritional qualities. The present study demonstrated the superior qualities of OPM1-T and OPM3-T whose molecular weights are exclusively at an approximate level of slightly lower than 1,000 daltons. It is, however, still unknown whether this level is the most preferable. For more precise information, further basic experimentation is required. Applied studies should also be extended since social demands are increasing for development of dietetic oligopeptide products for medical and therapeutic use.

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