Correlation between Molecular Weight Distribution of Oligo-L-methionine Prepared by Papain-catalyzed Polymerization and Its Supplementary Effect in a Low Protein Diet

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Oligo-L-methionine (OM) prepared by papain-catalyzed polymerization of MetOEt is a mixture of oligomers with different degrees of polymerization and shows interesting characteristics when it is added to low protein diets to feed rats. OM has nearly the same supplementary effect as free Met for growth of rats when it is added to a low casein diet, but has little supplementary effect on a low soybean protein diet. In a few cases, however, OM has good supplementary effects for both low casein and soybean protein diets. The varied supplementary effect of OM on low soybean protein diets is considered to be due to the difference in composition of OM. The degree of polymerization of OM has been reported to be about 5, 7.2-8.7 (8 as major), and 6-8 with traces of higher oligomers (T. Kasai, by FDMS analysis, unpublished results), but the precise composition of OM has not been clarified. This paper deals with analysis of OM sulfone with HPLC and the correlation between polymerization degree of OM and its supplementary effects on low soybean protein diet for the growth of rats.

Preparation of OM and OM sulfone. OM was prepared by papain catalyzed polymerization of MetOEt as described by Arai et al. and Jost et al. with a little modification. MetOEt was prepared in anhyd. ethanol with conc. H2SO4 instead of HCl gas as an acid catalyst, because a small amount of ethionine ethylester was formed by bubbling HCl gas into suspension of Met in anhyd. ethanol and incorporated in OM by papain-catalyzed polymerization of the ester. OM was scarcely soluble in H2O and precipitated out of the reaction mixture. Jost et al. oxidized OM to H2O soluble OM sulfoxide to analyze by paper electrophoresis and NMR spectrum, but we derived OM to H2O soluble OM sulfone by exhaustive oxidation with performic acid, because it was difficult to stop the oxidation reaction at sulfoxide and many diastereoisomers of OM sulfoxide which might cause a complex profile on HPLC could be formed.

HPLC analysis of OM sulfone. The reversed phase column method for analytical separation of OM sulfone by HPLC was done with a Waters 600 Multisolvant Delivery System. Separation was done on a WakoLi-518 (4.6 x 250 mm) at 50°C. The gradient was linear from 100% A (M/45 phosphate buffer, pH 6.5)/0% B (20% acetonitrile) to 60% A/40% B at 20 min with the flow rate of 1 ml/min. The OD210 nm was monitored. Identification of OM with polymerization degrees 5 to 9 was done by comparison of elution time with that of chemically synthesized authentic specimens. Synthesis of each OM will be described in the next report together with their nutritional evaluation. Figure 1(a) shows a typical HPLC pattern of sulfone of OM which has a different supplementary effect on low casein and soybean protein diets, that is, improved the growth of rats when added to the low casein diet, but had little effect when added to the low soybean protein diet. As shown in Fig. 1(a), OM was a mixture of several oligomers with polymerization degrees of 5 to 12. Although the heptamer and octamer were the major constituents, the content of nonamer and decamer was also significant, taking into consideration the number of Met residues constituting those oligomers, while the major constituents of OM which had supplementary

Fig. 1. HPLC patterns of Oligo-L-methionine Sulfone, (MetSO)₇, Prepared by Papain-catalyzed Polymerization of L-Methionine Ethylester and Oxidation with Performic Acid.

(a): Sulfone of oligo-L-methionine which has little effect on the growth of rats when added to a low soybean protein diet.

(b): Sulfone of oligo-L-methionine which improves the growth of rats when added to a low soybean protein diet.

See the text for the analytical conditions.

Abbreviations: Met, L-methionine; MetOEt, L-methionine ethylester; OM, oligo-L-methionine.
Molecular Weight Distribution of Oligo-L-methionine

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[(n=5-7)/(n=5-12)] \times 100 \text{ in } (\text{Met})_n
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Fig. 2. Correlation between Molar Ratio (%) of Oligomers with Polymerization Degree of 5, 6, and 7 to Total Oligomers (Polymerization Degree of 5 to 12) in an Oligo-L-methionine, and Weight Gain of Rats Fed 10% Soybean Protein Diet Supplemented with 0.3% of the Oligo-L-methionine.

- It was shown in this study that the major component of OM prepared by papain-catalyzed polymerization of MetOEt was the octamer in many cases, but the components with lower polymerization degree such as pentamer, hexamer, and heptamer were the main constituents in some preparations. The condition(s) that decide the molecular weight distribution of OM are still obscure.

- Supplementary effects of various OM preparations on soybean protein diet for the growth of rats. Male weanling rats of the Sprague-Dawley strain (about 50 g, Japan SLC Inc.) were fed a 25% casein diet for 3 days, followed by the experimental diet for 2 weeks. The composition of experimental diet was as follows (in 1 kg): soybean protein isolate (Fujipro, Fuji Oil Co., Ltd.), 100 g; each of various OM preparations, 3 g; retinyl palmitate (Chocola A, Eisai), 4 mg; ergocalciferol (Chocola D, Eisai), 20 μg; all-rac-α-tocopheryl acetate (Juvela, Eisai), 200 mg; vitamin mixture other than A, D, and E prepared in accordance with the AIN-76 mixture \(^7\) (except L-ascorbic acid and menadione which were added to make 50 mg/kg\(^8\) and 1 mg/kg\(^9\) of diet, respectively), 10 g; choline chloride (50% in 50% ethanol solution) 2 g; mineral mixture, 2 g and 38 g for mixture micro and macro, \(^10\) respectively; Sucrose, up to 1 kg. Highly significant correlation was observed between the molar ratio of oligomers with polymerization degree of 5, 6, and 7 to total oligomers (polymerization degree of 5 to 12) in an OM and weight gain of rats fed the OM added to the low soybean protein diet (Fig. 2). It is suggested that digestibility of OM with polymerization degree below 8 in the digestive tract of rats is significantly higher than those with polymerization degrees of 8 and above. Chemical synthesis of OM with polymerization degrees of 6, 7, 8, and 9, their in vitro digestion with pepsin and bile pancreatic juice, and supplementary effects on 8% casein and 10% soybean protein diets for the growth of young rats will be described in our next report. The study to elucidate the cause of different effects for the growth of rats between casein and soybean protein supplemented with OM has also been done in this laboratory.\(^11\)

- Analysis of OM sulfone with an amino acid analyzer was unsuccessful (Hitachi 835 high-speed amino acid analyzer equipped with Hitachi custom ion-exchange resin #2619 (2.6 x 250 mm) under the conditions for physiological fluid analysis). A peak of (Met), sulfone was eluted faster on the analyzer with increasing polymerization degrees \(n\) from 2 and the OM sulfones with polymerization degrees of 7 and above were eluted together at the elution front.

**References**