Study on the intestinal absorption of oligopeptides by electrospray ionization-mass spectrometric assay

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Title : Study on the intestinal absorption of oligopeptides by electrospray ionization-mass spectrometric assay（エレクトロスプレーイオン化質量分析法を用いたオリゴペプチドの腸管吸収に関する研究）

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Thesis Summary

Dietary intake of food derived short peptides (2 – 20 amino acid residues) have been demonstrated to be potentially involved in the prevention of various life-style related diseases including cardiovascular disease, diabetes, hyperlipidemia, etc. Upon oral intake, such bioactive oligopeptides have to be absorbed into blood circulation across the small intestinal barrier to reach targeted tissues and organs to elicit their physiological functions. Therefore, elucidation of the intestinal absorption of oligopeptide is of vital importance. Di-/tripeptides have been demonstrated to be absorbed via intestinal peptide transporter PepT1. In contrast, large remains unknown regarding the absorption of longer peptides of more than three amino acid resides, presumably due to a lack of highly sensitive and selective assay. Electrospray ionization (ESI)-mass spectrometry (MS) is considered as an essential tool for the quantitative analysis of oligopeptides in complex biofluids. Therefore, firstly, the ESI-MS detection characteristics of oligopeptides with chemical derivatization was investigated. Then liquid chromatography (LC)-ESI-time-of-flight (TOF)/MS was applied to investigate the intestinal absorption of bioactive oligopeptides.

Chemical derivatization has been used to enhance ESI-MS detection of small amines and dipeptides, but its effect on longer oligopeptides remains unclarified. Therefore, the ESI-MS detection of a series of synthetic di- to pentapeptides consisting of glycine and sarcosine was characterized by four amine derivatization methods. All the standard and chemically modified oligopeptides were detected by LC-ESI-TOF/MS. A chain length-dependent trend was observed since chemical derivatization induced greater MS signal intensity increase to di-/tripeptides than to longer oligopeptides. A moderate correlation was observed between MS signal intensity and hydrophobicity (log *P*) on the whole but increase in hydrophobicity did not necessarily lead to increase in MS signal intensity for individual peptides, especially for tetra-/pentapeptides. Meanwhile, a bell-shaped trend between MS signal intensity and molecular surface area was observed, with an optimum of 250 – 300 $Å^2$. Consequently, it was concluded that the size of a molecule might be related with its ESI-MS detection characteristics and that addition of hydrophobic tags might be less advantageous for longer oligopeptides. Indeed, chemical derivatization did not furtherly enhance the detection of Cblin pentapeptides.

Then intestinal absorption of muscle atrophy-preventive Cbl-b inhibitory (Cblin) pentapeptides DGpYMP and DGYMP. *In vitro* Caco-2 cell monolayer transport experiments demonstrated that both peptides were transportable across Caco-2 cell monolayers in their intact pentapeptide forms. The

P_{app} was 3.5 ± 1.2 cm/s for DGpYMP and 7.0 ± 0.8 cm/s for DGYMP. A screening of peptide metabolites showed that DGpYMP was partly dephosphorylated to generate DGYMP. Meanwhile, no other peptide fragments were detected from the transport of DGpYMP and DGYMP. PepT1 was not involved in the transport of DGpYMP and DGYMP, while passive diffusion via paracellular tight junction could be the major route involved. *In vivo* single oral administration experiments to Sprague-Dawley (SD) rats (100 mg/kg-body weight) revealed that DGYMP, despite its pentapeptide length, could be absorbed in its intact peptide form into SD rat blood circulation (C_{max}: 2.78 ± 0.17 pmol/mL-plasma, t_{max}: 15 min, $AUC_{0-60\ min}$: 100.35 ± 23.40 pmol·min/mL-plasma, $t_{1/2}$: 28 min). Peptide fragments GYMP and MP were also detected in rat plasma, suggesting that part of DGYMP experienced degradation during the absorption process.

In conclusion, using ESI-MS as a quantitative tool, this study demonstrated that Cblin pentapeptides could cross small intestinal barrier and reach blood circulation. These results suggested that longer oligopeptides with more than tripeptide lengths could be absorbed and potentially elicit bioactivity upon oral intake.