

Study on the analytical evaluation on intestinal absorption of small peptides by mass spectrometries

洪, 性珉

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氏 名 : 洪 性珉

論文題名 : Study on the analytical evaluation on intestinal absorption of small peptides by mass spectrometries
(質量分析法による低分子ペプチドの腸管吸収性の分析化学的評価に関する研究)

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論 文 内 容 の 要 旨

Clinical evidences revealed that the intake of peptides improved promoting blood pressure through the suppression of pressor systems. Irrespective to the health-benefit of peptides, however, few studies on their absorption behavior or bioavailability have been reported due to the lack of appropriate analytical assay systems. Thus, the aim of this study is to develop analytical methods for intestinal absorption and distribution behavior of bioactive peptides by the aid of mass spectrometries (MS).

Firstly, to get insights of absorption process and distribution of peptides in intestinal membrane, an attempt for their visualization in Sprague-Dawley (SD) rat intestines was performed by matrix assisted laser desorption/ionization (MALDI)-MS imaging method. In order to solve the issues on poor ionization efficiency of localized targets by unexpected matrix cluster formation with alkali metals, a matrix additive method using chelators was conducted. As a result, the addition of phytic acid bearing six phosphate groups into 2',4',6'-trihydroxyacetophenone (THAP) matrix solution significantly improved the poor MS detection of di-peptides. For the MS detection of Gly-Sar as a peptide transport model *via* intestinal peptide transporter 1 (PepT1), a 2.4-fold higher MS detection by phytic acid was obtained compared to THAP alone, by the suppression of matrix cluster formation and by the formation of homogenous matrix crystals on tissue section.

Secondly, the phytic acid-aided MALDI-MS imaging method was applied for the visualization of anti-atherosclerotic di-peptide, Trp-His, in SD rat intestinal tissues, together with the absorption behavior into rat blood by amine-derivatization liquid chromatography (LC)-MS/MS method. By the LC-MS/MS analysis, Trp-His was found to be a penetrant with maximal concentration of 28.7 ± 8.9 pmol/mL-plasma at a dose of 10 mg/kg. The phytic acid-aided MALDI-MS imaging method revealed the PepT1 transport pathway of absorbable Trp-His, in which the visualized distribution of Trp-His by the imaging method was completely abolished by Gly-Sar. The combination study of LC-MS/MS with MALDI-MS imaging method also provided the evidence that His-Trp, a reversed sequence of Trp-His, was far from a penetrant due to rapid protease hydrolysis at the brush border.

Finally, an attempt to design a model peptide for the evaluation of oligo-peptide absorption behavior was performed. Among the synthesized tri- to penta-peptides, peptides with Gly-Sar skeleton were candidates for intact transport of oligo-peptides across rat intestinal membrane. The apparent permeability of designed oligo-peptides decreased with the descending order of tri-> tetra-> penta-peptide

length. In addition, it was revealed that di- and tri-peptides were involved in PepT1 transport pathway, while tetra-and penta-peptides were transported through paracellular tight-junction pathway.

In conclusion, the present study demonstrated that the question on low MS detection ability of bioactive peptides could be solved by the phytic acid-aided matrix additive method. The combinatorial MS methods by derivatization LC-MS/MS and phytic acid-aided MALDI-MS imaging would provide a new aspect on absorption study of bioactive peptides.