

## Study on the intestinal absorption of small and oligopeptides in rats

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<https://doi.org/10.15017/1866364>

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出版情報 : 九州大学, 2017, 博士 (農学), 課程博士  
バージョン :  
権利関係 :



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Title : Study on the intestinal absorption of small and oligopeptides in rats

(ラットにおける低分子およびオリゴペプチドの腸管吸収に関する研究)

Category : Kou

### Thesis Summary

It has been known that peptides derived from protein hydrolysate may exert preventive effects against lifestyle-related diseases such as hypertension, diabetes, and inflammation. However, the lack of accurate and reliable analytical assay systems for bioactive peptides limited researches on physiological role and bioavailability of peptides. In this study, thus, high sensitive liquid chromatography-mass spectrometry (LC-MS) assay was extensively applied for reliable peptide detection without any suppressed ionization efficiency of target peptides by matrix effect. The study also attempted to clarify *in vivo* intestinal absorption behavior of di- to pentapeptides in spontaneously hypertensive rats (SHRs) in terms of aging.

In order to overwhelm the issue on poor MS detection of analytes in a mixture, a standard addition method was applied for dipeptide assay in soybean hydrolysate by LC-time-of flight (TOF)-MS in combination to a 2,4,6-trinitrobenzene sulfonate (TNBS) derivatization technique. LC conditions were optimized to separate trinitrophenyl (TNP)-dipeptides at a linear gradient elution of methanol (60-100% over 40 min) containing 0.1 % formic acid at flow rate of 0.25 mL/min at 40 °C on Biosuite C<sub>18</sub> column. To compensate matrix suppression, the standard addition method using target peptide standards was applied for this study. Namely, soybean hydrolysate samples (10 mg/mL) spiked with peptide standard solutions (4.0, 8.0, and 16.0 µg/mL) were reacted with TNBS at 30 °C for 30 min. At the optimal LC-MS conditions, there was a good relationship (correlation coefficient of  $r^2 > 0.979$ ) between spiked concentrations and MS signal intensities of five target dipeptides. However, a 7- to 24-fold decrease in the slope of the standard addition curves of hydrolysate was obtained compared to the absolute calibration curves of dipeptide standard solution. This indicates that ionization of peptide was suppressed by contaminants in the hydrolysate. By using the curve by standard addition method, five target peptides (Gly-Tyr, Tyr-Gly, Ser-Tyr, Tyr-Ser, and Ile-Tyr) were successfully quantified ( $424 \pm 12$ ,  $184 \pm 5$ ,  $2188 \pm 114$ ,  $327 \pm 9$ , and  $2211 \pm 77$  µg/g of hydrolysate, respectively). Therefore, the proposed TNBS derivatization-aided LC-MS assay using the standard addition method would provide the convenient evaluation of peptide profile in hydrolysate without the use of an isotope labeled internal standard.

Secondly, this study aimed to investigate whether oligopeptides (tri- to pentapeptides) can be absorbed in their intact form after single oral administration to SHRs by using the TNBS-LC-MS technique. Peptides (Gly-Sar-Sar, Gly-Sar-Sar-Sar, and Gly-Sar-Sar-Sar-Sar) were administered at each dose of 10 mg/kg to 8-wk SHRs. It was demonstrated for the first time that these oligopeptides were absorbed in intact form into SHR blood system. It was also clear that their absorption behavior was in a peptide length-dependent manner: Gly-Sar-Sar (AUC,  $267.4 \pm 34.3$  nmol·min/mL-plasma) > Gly-Sar-Sar-Sar (AUC,  $88.2 \pm 17.6$

nmol·min/mL-plasma) > Gly-Sar-Sar-Sar-Sar (AUC,  $71.7 \pm 2.8$  nmol·min/mL-plasma).

Finally, effect of aging on the absorption of peptides was investigated in 8-wk and 40-wk SHR. Herein, peptides including Gly-Sar and Trp-His as dipeptide, Gly-Sar-Sar as tripeptide, Gly-Sar-Sar-Sar as tetrapeptide, Gly-Sar-Sar-Sar-Sar as pentapeptide, and captopril as dipeptidomimetic anti-hypertensive drug were administered at each dose of 10 mg/kg. It was demonstrated that the absorption of di-/tripeptides used in this study was significantly enhanced by aging of rats. In contrast, the intestinal absorption of tetrapeptide (Gly-Sar-Sar-Sar) and pentapeptide (Gly-Sar-Sar-Sar-Sar) was not affected by aging. Western blotting analyses of intestinal PepT1 and claudin-1 at the up-jejunum, mid-jejunum, and ileum segments from 8- and 40-wk SHR revealed that PepT1 expression in the mid-jejunum was significantly increased in 40-wk SHR compared to 8-wk SHR, whereas aging did not affect the expression of claudin-1, a tight junction-related protein. Therefore, SHR aging may increase the absorption of di-/tripeptides through the enhanced PepT1 transport route, whereas the intestinal absorption of oligopeptides was not affected by aging of SHR.

In conclusion, the present study proposed reliable peptide quantitative assays in food hydrolysate by a standard addition method with TNBS-aided LC-MS technique. This study also demonstrated that oligopeptides are a possible *in vivo* penetrant across intestinal membrane in a peptide length-dependent manner. It should be noted that aging of rats may result in enhanced absorption of small (di-/tripeptides), but not oligopeptides.