Structure and biological activities of hydrophobic short chain pyroglutamyl peptides in fermented foods and food protein hydrolysates

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（発酵食品及び食品タンパク質加水分解物中に存在する疎水性短鎖ピログルタミルペプチドの構造とその生理機能）

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Abstract（要約）

Food peptides are usually produced by protease digestion of food proteins. Some peptides in foods have been demonstrated to exert beneficial activities beyond amino acid source. Pyroglutamyl (5-oxoprolyl) (pyroGlu) peptide is spontaneously generated from peptides with a glutaminyl residue at the amino terminal during processing and storage. There are several reports demonstrating *in vivo* and *in vitro* biological activities of short chain pyroglutamyl peptides. The presences of pyroglutamyl peptides in food protein hydrolysates and some Japanese traditional fermented foods have been demonstrated.

Japanese diet (washoku) has been believed to contribute to longevity of Japanese. Some studies have demonstrated the health benefits of washoku. However, the study on the active compounds in washoku is limited. One of characteristics of washoku is using traditional fermented seasonings, such as salted soy paste (miso), soy sauce (shoyu), and Japanese rice wine (sake). There is a possibility that pyroglutamyl peptides in Japanese traditional fermented seasoning might exert beneficial activities as demonstrated by epidemiological studies.

The present study shows the structure, content, and biological activity of pyroglutamyl peptides in miso in Chapter 1. Chapter 2 provides new mechanism for attenuation of high fat diet-induced dysbiosis by pyroGlu-Leu. In chapter 3, the tissue distribution of short chain hydrophobic pyroglutamyl peptides after the oral administration is shown. In addition, generation of a novel peptide by hydrophobic pyroglutamyl peptides is reported.

Chapter 1

Identification of hydrophobic short chain pyroglutamyl peptides in Japanese traditional fermented food, miso, and their potential anti-obesity activity

Japanese salted soy paste (miso) is one of Japanese traditional fermented foods. Some compounds in miso have been suggested to be responsible for the beneficial activity of miso, while, it has not been confirmed even by animal experiment. In the present study, newly developed LC-MS/MS method based on precursor scan targeting immonium ion of pyroglutamyl residue revealed the presence of 13 pyroglutamyl peptides in miso without pre-fractionation. In animal experiment, the hydrophobic compounds without amino group from soybean miso (0.6 g/kg of body weight of rat) significantly suppressed high fat diet-induced body weight gain. The main pyroglutamyl peptides in this fraction (pyroGlu-Pro, pyroGlu-Val, pyroGlu-Ile, and pyroGlu-Leu) also suppressed body weight gain, which suggests that hydrophobic pyroglutamyl peptide are responsible for health benefits by intake of miso.

Chapter 2

Pyroglutamyl leucine, a peptide in fermented foods and food protein hydrolysates, attenuates dysbiosis by increasing host antimicrobial peptide

The relationship between obesity and dysbiosis has been demonstrated. Previous study has also demonstrated that the oral administration of pyroGlu-Leu (0.1 mg/kg body weight) attenuates dysbiosis in mice with DSS-induced colitis. In the present study, high fat diet extensively increased the ratio of *Firmicutes*/*Bacteroidetes* in feces of rats compared to that of control diet groups. However, administration of pyroGlu-Leu (1 mg/kg body weight) significantly attenuated dysbiosis. By using newly developed direct detection of antimicrobial peptide by LC-MS based on the multivalent ions, it was found that pyroGlu-Leu significantly increased the level of 4962 Da peptides, which was identified as the propeptide of rattusin or defensin alpha 9. Tryptic fragment peptides from rattusin in the lumen were also observed. These findings indicate that orally administered pyroGlu-Leu attenuates dysbiosis by increasing host antimicrobial peptide, rattusin,

which are novel concept for modulation of gut microbiota by food component.

Chapter 3

Generation of a novel leucine-derivative, propionyl leucine, in ileum after oral administration of pyroglutamyl peptides

To evaluate tissue distribution and metabolic fate, rats were orally administered hydrophobic pyroglutamyl peptides (pyroGlu-Val, pyroGlu-Ile, and pyroGlu-Leu). All peptides were absorbed into small intestine, especially into ileum, 1 h after the administration. However, no significant amounts of these peptides were absorbed into blood circulation system and peripheral tissues, suggesting that these peptides are metabolized in ileum. To detect the potential metabolite in ileum, precursor ion scan mode of LC-MS/MS, targeting immonium ion of Val, Ile, and Leu, was used. As a result, the increase of a novel peptide; propionyl-Leu was detected. Finding of propionyl-Leu might provide breakthrough for elucidating the mechanism for biological activities of hydrophobic short chain pyroglutamyl peptides.

In conclusion, the present study suggests that hydrophobic short chain pyroglutamyl peptides may be key players in health benefits by consumption of washoku and Japanese traditional fermented foods. Effects of the administration of pyroglutamyl peptides should be further confirmed by well-designed human clinical study.