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学位論文題目	Improvement of anti-viral and anti-allergenic activities of milk casein phosphopeptide by chemical modifications（化学修飾による牛乳カゼインホスホペプチドの抗ウィルス性及び抗アレルギー性の改善）
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論　文　内　容　の　要　旨

The effects of anti-viral and anti-allergenic activities of milk casein phosphopeptides on phosphorylation and dephosphorylation were investigated in this doctoral dissertation.

Chapter 1 describes the introduction, background literature, and objectives. Studies on functional foods in Japan has been fast-tracked and already given birth to Food for Specified Health Uses (FOSHU). Among the FOSHU, Casein phosphopeptide (CPP) has been reported to be one of the potent bioactive agents from milk. CPP is a phosphoserine-rich enzymatic hydrolysis having the basic structure of three phosphoseryl residues followed by two glutamic acid, SerP-SerP-SerP-Glu-Glu. Since the bioactivity of CPP has been related to negative electrostatic charge of phosphate groups attached to its amino acid chain, we hypothesized that modification of the peptide by addition of extra phosphates through phosphorylation can enhance its antiviral and antiallergic activities.

In Chapter 2, the effects of additional phosphorylation and dephosphorylation on antiviral activity of the milk casein-derived peptides rich in phosphate groups called casein phosphopeptide III (CPP III) in vitro. Feline calicivirus (FCV) strain F9, a typical norovirus surrogate, and Crandell Rees feline kidney (CRFK) cells were used as the target virus and host cells, respectively. Higher cell viability was observed in the host cells treated with phosphorylated CPP-III (P-CPP). The expression of anti-viral cytokines such as IFN-α and IFN-β was significantly induced by the treatment with P-CPP, compared to that with native CPP-III (N-CPP). In contrast, dephosphorylation of CPP-III resulted in a decrease in the anti-FCV effect. These results suggest that the anti-viral activity of CPP was enhanced by the introduction of additional phosphates and conversely weakened by their elimination.

Chapter 3 deals with the anti-allergenic effects of P-CPP and dephosphorylated CPP III (D-CPP) in OVA-sensitized mice. Female BALB/c mice were intraperitoneally sensitized with OVA twice at intervals of 14 days and then orally fed N-CPP, P-CPP, and D-CPP for 6 weeks. Next, the mice were orally challenged with 50 mg of OVA. Oral administration of P-CPP significantly suppressed total and OVA-specific IgE levels in the serum. The treatment with P-CPP exhibited low levels of OVA-specific IgG1 and increased OVA-specific IgG2a. The treatment with P-CPP also suppressed

IL-4 production, while D-CPP showed similar a level compared to that of the control. N-CPP and P-CPP increased the regulatory T cells population in spleen compared with D-CPP. Furthermore, the population of the T follicular helper (Tfh) cells in the spleen was increased by P-CPP treatment. These results suggest that additional phosphorylation of CPP can enhance the attenuation of OVA-specific IgE-modulated allergic reactions in OVA-sensitized mice.

Finally, Chapter 4 provides a summary and conclusion of the studies. In this study, it was demonstrated that P-CPP showed anti-viral activity against FCV infection and anti-allergenic activity in OVA-sensitized mice, and these activities were dependent on the phosphate groups. These findings suggest that highly phosphorylated CPP III holds potential as an accessible and cheaper alternative to the food-based nutraceutical ingredients and supplements used to boost the immune system against viral infections and to attenuate the allergenic response. This information will contribute to the development of safe and effective anti-viral and anti-allergenic agents using natural dietary compounds in conjunction with modification techniques to enhance their functionality.