

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



The Anti-Allergic Effects of the His-Ala-Gln Tripeptide and Constituent Amino Acids

Mamoru Tanaka and Takeaki Okamoto

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.68642>

Abstract

The type-1 allergy, as typified by allergic rhinitis, pollen, and food allergies, is defined as a hypersensitivity reaction, and its frequency is increasing worldwide. There is a need to develop therapeutic agents that either prevent sensitization to allergens or suppress the allergic response after initiation. It has been reported that various peptides show anti-allergic effects, but there have been few reports concerning peptides derived from food. Previously, we studied the anti-allergic effect of His-Ala-Gln (HAQ), which is present in CE90GMM, a peptide mixture derived from milk casein. In this chapter, special emphasis is placed on the anti-allergic effects of the HAQ peptide *in vitro* and *in vivo*, and the effect of peptide binding, peptide sequence, number of amino acids, and the electron density of the amino acids is investigated.

Keywords: type-1 allergy, milk casein, peptide, amino acid, anti-allergic effect, *in vitro*, *in vivo*

1. Introduction

The prevalence of allergic diseases has been increasing in recent years, especially in Western countries. More than 25% of people living in developed Western countries suffer from allergies [1]. It has been reported that polyphenolic compounds, such as epigallocatechin gallate, show anti-allergic effects, but there have been few reports regarding the anti-allergenic effect of peptides. It has been reported that bioactive peptides from proteins in foods have several functions, such as reduction in blood pressure [2–4], activation of immune cells [5, 6], antiviral actions [7], and improvement of lipid metabolism [8]. Milk casein yields many peptides through hydrolysis with digestive and other enzymes. Hydrolyzed peptides have been indicated to have a variety of bioactive functions [2–8]. However, there have been few reports on the anti-allergic effects of peptides derived from casein. In the present study, we examined

whether peptides derived from casein can inhibit type-1 allergic reactions *in vitro* and *in vivo*. Furthermore, characteristics of peptides with anti-allergic actions are investigated.

2. Mechanism of the type-1 allergic response

Type-1 allergies are hypersensitivity disorders mediated by immunological mechanisms, and type-1 allergic responses are induced by certain types of antigens, such as those from plants, mammals, microbes, foods, drugs, and chemicals. Type-1 allergic responses are known to be evoked by the antigen-induced activation of high-affinity IgE (FcεRI) expressed in mast cells and basophils.

When allergens invade the body, mast cells, basophils, eosinophils, and T cells are activated. Then, various tissues in the body are damaged by the physiologically active substances produced or released. Allergic sensitization involves T cell priming after dendritic cell (DC) activation, and the resultant T-helper (Th) 2 response is characterized by the production of interleukin (IL)-4, IL-5, and IL-13 from CD4⁺ T cells. This Th2 response leads to IgE production from B cells, and this IgE binds to FcεRI on the surface of mast cells and basophils in the skin, gut, and respiratory and cardiovascular systems, arming them for reactivity upon re-exposure to the allergen. The elicitation of classic allergic symptoms occurs within minutes after allergen exposure, when the IgE-bound mast cells and basophils recognize the allergen and become activated [9]. The bond between antigen and IgE is essential, and is the first step in triggering the signaling cascades that lead to degranulation. These signaling pathways are involved in the activation of protein kinase C, induced by 1,2-diacylglycerol, or Ca²⁺ influx into the intracellular matrix induced by deacylglycerol [10, 11]. The activation of mast cells and basophils triggers the production of many chemical mediators, such as histamine, proteolytic enzymes, prostaglandins, leukotrienes, inflammatory cytokines, and arachidonic acid metabolites, including prostaglandins and leukotrienes [12–15]. These mediators cause immediate allergic reactions. Thus, mast cells and basophils are implicated in the development of diseases, such as asthma, allergic rhinitis, and inflammatory arthritis [13].

Th cells also play a central role in type-1 allergic responses. The cells are classified into two types: Th1 and Th2. These cells are responsible for the modulation of cytokine secretion to maintain homeostasis in the host, and disruption of this balance induces various immunological diseases. Allergic diseases are characterized by an excessive Th2-type immune response. It is generally accepted that IL-4 regulates the differentiation of native CD4⁺ T cells into Th2 cells and immunoglobulin class switching to the IgG1 and IgE isotypes. Excessive IL-4 production by Th2 cells has been associated with an elevation of IgE levels and allergic reactions. Thus, modulation of the Th1/Th2-balanced immune response is important to control allergic symptoms. There are several phases in the pathogenesis of type-1 allergic responses as seen above, and allergic symptoms may be arrested by blocking the response at any of these points.

3. The search for peptides derived from casein with anti-allergic actions

It has been reported that bioactive peptides from proteins in foods have several functions [2–8]. Milk casein yields many peptides through hydrolysis with digestive and other enzymes.

Hydrolyzed peptides have been indicated to have a variety of bioactive functions [2–8]. Therefore, we focused our search on peptides derived from casein, and examined degranulation-inhibitory activity related to anti-allergic effects. Degranulation of rat basophilic leukemia (RBL-2H3) cells was monitored by measuring the activity of released β -hexosaminidase. For antigen stimulation, DNP-specific IgE-primed RBL-2H3 cells were preincubated for 10 min with various concentrations of peptides, then stimulated with antigen (mouse anti-DNP IgE). After 30 min, the medium was collected and 0.2% Triton X-100 was added to the cells. Levels of β -hexosaminidase released into the medium, and within cells, were determined by colorimetric assay using *p*-nitrophenyl-2-acetamide-2-deoxy- β -glucopyranoside and expressed as the percentage of activity released into the medium compared with total activity. In the first stage of this study, we investigated casein and CE90GMM (average molecular weight 640 kDa), a peptide mixture derived from milk casein, on the degranulation of RBL-2H3 cells and found that degranulation of the cells was not suppressed in the presence of casein. On the other hand, significant inhibition of β -hexosaminidase release was seen with CE90GMM stimulation at 250 μ g/mL (**Figure 1**) [16]. Thus, this finding suggested that a peptide capable of inhibiting β -hexosaminidase release is present in CE90GMM. Several varieties of peptides have been identified in CE90GMM [17]. We tested the low-molecular-weight peptides: His-Ala-Gln (HAQ), Glu-Gln-Pro-Ile (EQPI), Asp-Met-Glu-Ser (DMES), and Lys-Ile-Lys-Glu (KIKE), which are present in CE90GMM, using RBL-2H3 cells. With the four peptides, at concentrations ranging from 10 to 500 μ g/mL, the inhibition of β -hexosaminidase release was observed, which was dose dependent (**Figure 2**) [16].

We next investigated the effect of the four peptides on cytokine production [tumor necrosis factor (TNF)- α and IL-4] from RBL-2H3 cells after antigen stimulation. After stimulation with

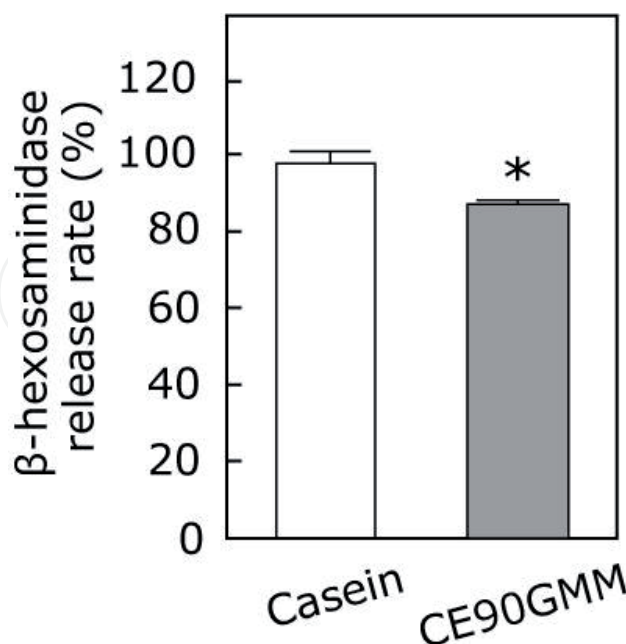


Figure 1. Effects of Casein and CE90GMM on β -hexosaminidase release from RBL-2H3 cells. Data are expressed as the means \pm SD values of triplicate determinations. * $p < 0.05$ vs control. DNP-specific IgE sensitized RBL-2H3 cells were challenged with DNP-HSA for 30 min. Casein and CE90GMM were added 10 min before antigen challenge.

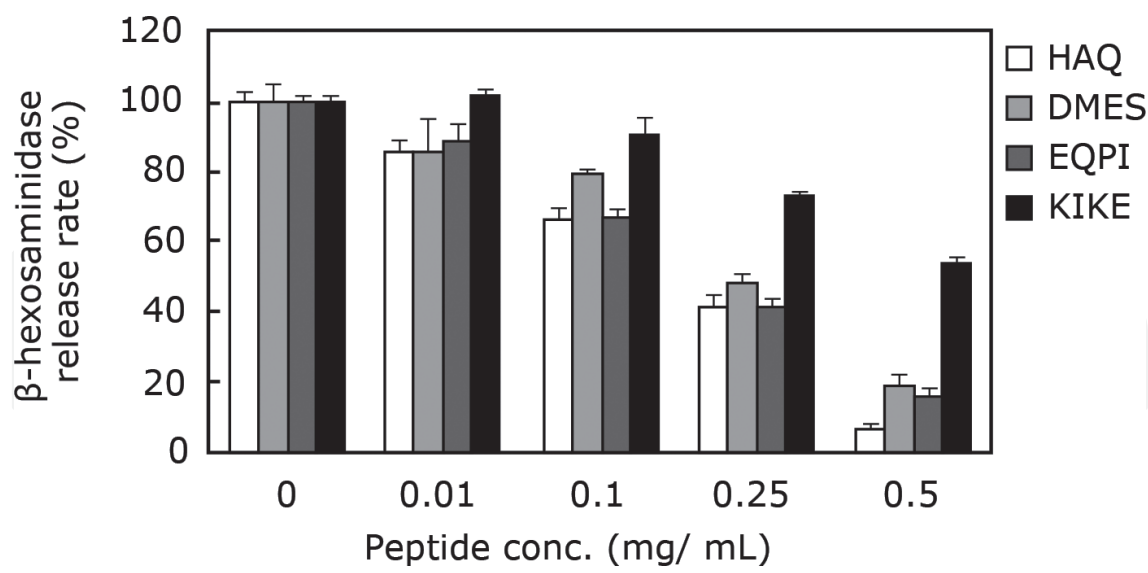


Figure 2. Effects of HAQ, DMES, EQPI and KIKE on β -hexosaminidase release from RBL-2H3 cells. Data are expressed as the means \pm SD values of triplicate determinations. DNP-specific IgE sensitized RBL-2H3 cells were challenged with DNP-HSA for 30 min. Peptides were added 10 min before antigen challenge.

anti-DNP-IgE only, control levels of TNF- α and IL-4 were 9.1 and 90.8 pg/mL, respectively. All peptides inhibited the production of the inflammatory cytokines TNF- α and IL-4. In particular, the HAQ and EQPI peptides showed stronger inhibition of TNF- α and IL-4 than was seen with the DMES and KIKE peptides (**Table 1**). In addition, we also investigated the characteristics of peptides with degranulation depression effects [18]. It is known that concentrations of intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) in mast cells and basophils rise through signaling after the cross-linkage of antigen to Fc ϵ RI through IgE for degranulation. Thus, the degranulation-suppressing effect of the HAQ peptide on $[\text{Ca}^{2+}]_i$ was examined using fluo-3 AM. The $[\text{Ca}^{2+}]_i$ from RBL-2H3 cells was significantly increased by treatment with the HAQ peptide without affecting the proliferation and viability of the cells (**Figure 3**). These results imply that the HAQ peptide suppressed the elevation of $[\text{Ca}^{2+}]_i$ induced by intracellular-signaling pathways caused by the antigen-antibody interaction.

	TNF- α	IL-4
Control	100 \pm 8 (9.1 pg/mL)	100 \pm 5 (90.8 pg/mL)
HAQ	46 \pm 6	52 \pm 1
DMES	48 \pm 3	80 \pm 8
EQPI	33 \pm 9	56 \pm 5
KIKE	61 \pm 10	86 \pm 3

Table 1. Amount of cytokine production.

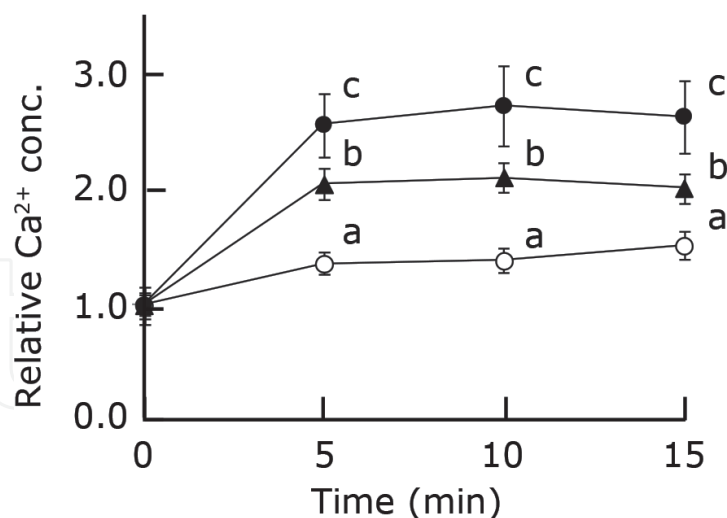


Figure 3. Effects of HAQ peptide on the release of $[Ca^{2+}]_i$ of RBL-2H3 cells. Data are presented as the means \pm SD ($n = 5$). $^*p < 0.05$ between each group. $[Ca^{2+}]_i$ was measured by Calcium Kit-Fluo-3. Anti-DNP IgE-sensitized cells were incubated with Fluo-3 AM for 1 h and then incubated with 500 μ M HAQ or PBS for 10 min. Then, the treated cells were stimulated with DNP-HSA, and the fluorescence intensity was measured. (○), non-HAQ-treated cells not sensitized with anti-DNP IgE; (▲), HAQ-treated cells stimulated with antigen; (●), non-HAQ-t peptide-treated cells stimulated with antigen.

4. Characteristics of the amino acids in peptides with anti-allergic actions

We hypothesized that the amino acids constituting the peptide may be the reason that the degranulation-inhibitory activity differs depending on the type of peptide. It is known that proteins are broken down into peptides in the stomach, which are then broken down into amino acids, dipeptides, and tripeptides that are absorbed in the bloodstream through the small intestine [19]. Thus, the amino acids making up the HAQ peptide were investigated for degranulation-inhibitory activity, cytokine production, and electron density [16].

We measured β -hexosaminidase levels released during degranulation in the antigen-antibody reaction in the presence of amino acids, and evaluated the anti-allergic effects. For the amino acids making up the HAQ peptide, an inhibitory effect of degranulation was found with L-histidine stimulation, but L-alanine and L-glutamine did not inhibit β -hexosaminidase release (**Figure 4**). L-histidine contains an imidazole group, one of the hetero-aromatic rings in the side chain, whereas L-alanine and L-glutamine do not have hetero-aromatic rings in the side chain. Polyphenols have multiple benzene rings as a construction feature. Therefore, we considered that amino acids with aromatic rings may show anti-allergic effects. To confirm this hypothesis, we investigated the effect of amino acids with aromatic rings, L-histidine, L-tryptophan, L-phenylalanine, and L-tyrosine, on the degranulation of RBL-2H3 cells. A significant inhibitory effect on the release of β -hexosaminidase was found with L-tryptophan, L-phenylalanine, and L-histidine, depending on the dose ($p < 0.05$), but L-tyrosine did not inhibit β -hexosaminidase release (**Figure 5**).

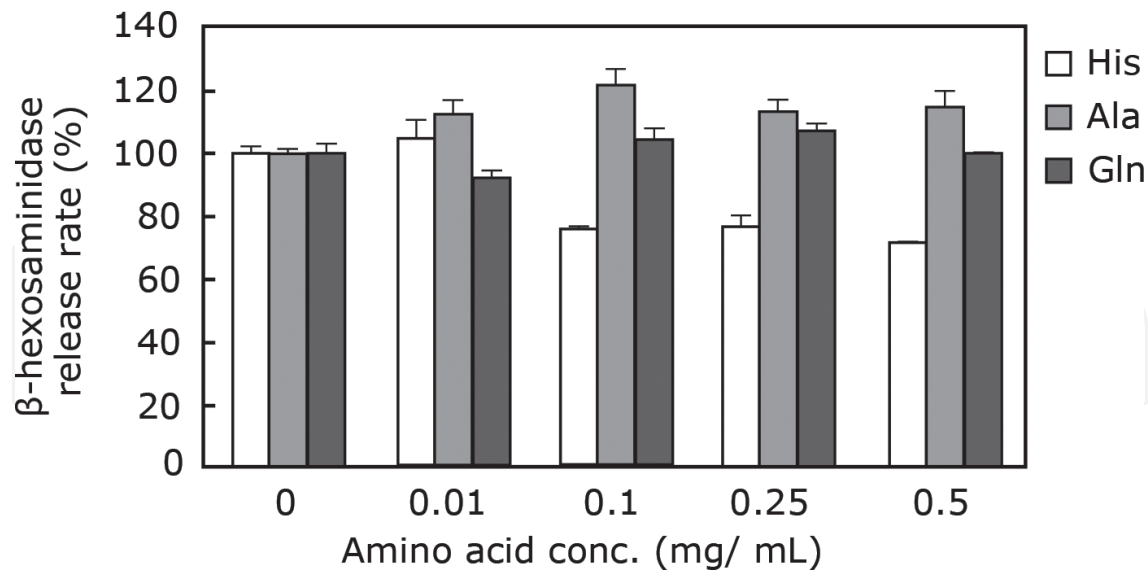


Figure 4. Effects of amino acids (His, Ala, Gln) on β -hexosaminidase release from RBL-2H3. Data are expressed as the means \pm SD values of triplicate determinations. DNP-specific IgE sensitized RBL-2H3 cells were challenged with DNP-HSA for 30 min. Amino acids were added 10 min before antigen challenge.

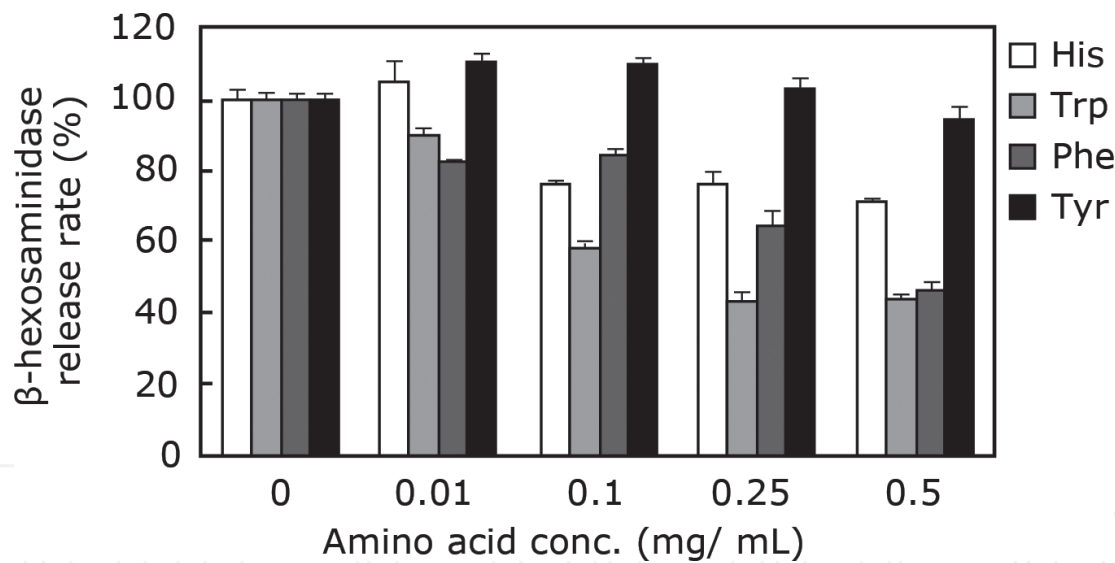


Figure 5. Effects of amino acids (His, Trp, Phe, Tyr) on β -hexosaminidase release from RBL-2H3. Data are expressed as the means \pm SD values of triplicate determinations. DNP-specific IgE sensitized RBL-2H3 cells were challenged with DNP-HSA for 30 min. Amino acids were added 10 min before antigen challenge.

We also performed *ab initio* molecular orbital calculations for the aromatic amino acids, to elucidate the mechanism of the anti-allergic action. Geometrical optimization was carried out and the molecular electrostatic potential was calculated for each amino acid. The electrostatic potential maps enabled visualization of the charge distributions of the molecules (**Figure 6**). The aromatic ring of L-tyrosine, which does not show an anti-allergic effect, is positively charged. L-tyrosine has a hydroxyl group on the side chain of the aromatic ring, which is not present in L-histidine, L-tryptophan, or L-phenylalanine. The charge of this hydroxyl group

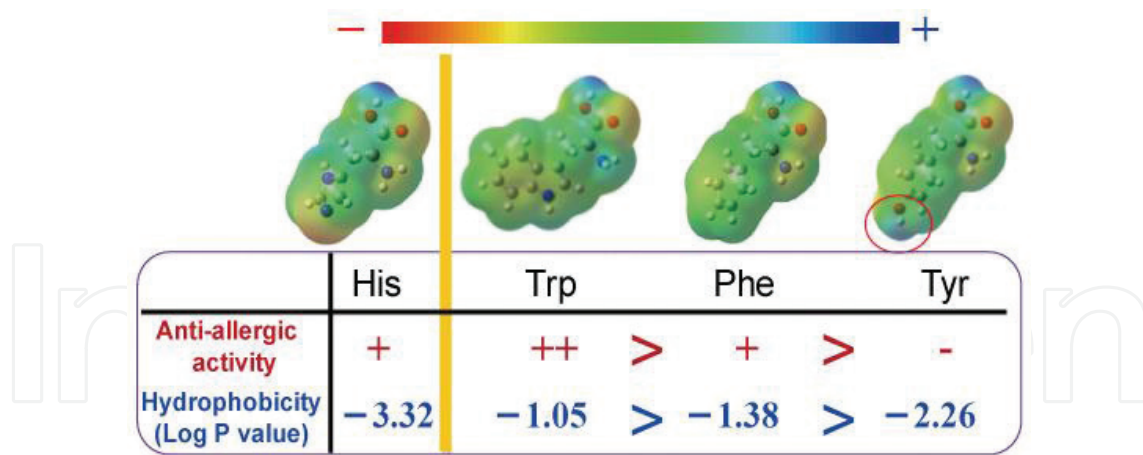


Figure 6. Charge distribution on four kinds of aromatic amino acids (His, Trp, Phe, Tyr).

is positive. Furthermore, according to the octanol/water partition coefficient (LogP), L-tyrosine is less hydrophobic than the other amino acids containing aromatic rings. One reason why degranulation is not observed with L-tyrosine may be that it is difficult for L-tyrosine to pass through the membrane lipid bilayer and incorporate into the cell. On the other hand, L-histidine is a hydrophilic amino acid and shows degranulation-inhibitory activity. In addition to this, the charge distribution on the aromatic ring of L-histidine is largely negative. L-tryptophan and L-phenylalanine are hydrophobic amino acid and the charge distribution on the aromatic ring of L-tryptophan and L-phenylalanine is largely neutral. Consequently, the mechanism of the degranulation-suppressing effect of L-histidine is likely to be different from that of L-tryptophan and L-phenylalanine. These results indicate that amino acids with an anti-allergic action have aromatic rings and neutral or negatively charged side chains.

5. Importance of peptide binding, number of amino acid residues, and peptide sequence on the anti-allergic effect

The degranulation-inhibitory activity differs depending on the type of amino acids. Thus, we examined the effect of peptide binding, number of amino acid residues, and peptide sequence on degranulation inhibition [20, 21].

Initially, we revealed that the level of degranulation-inhibitory activity depends on the peptide binding. In other words, a significant inhibitory effect on the release of β -hexosaminidase was found with the HAQ peptide, depending on the dose, but mixtures of the amino acids in the HAQ peptide did not show an inhibitory effect (Figure 7). It has been reported that bioactive peptides from proteins in foods have several functions [2–8]. Our results, indicating that the HAQ peptide can suppress the antigen-induced degranulation of RBL-2H3 cells, are consistent with those observed for other bioactive peptides.

We further found that the level of degranulation-inhibitory activity depended on the number of amino acid residues in the peptide. Our group has previously reported that peptides

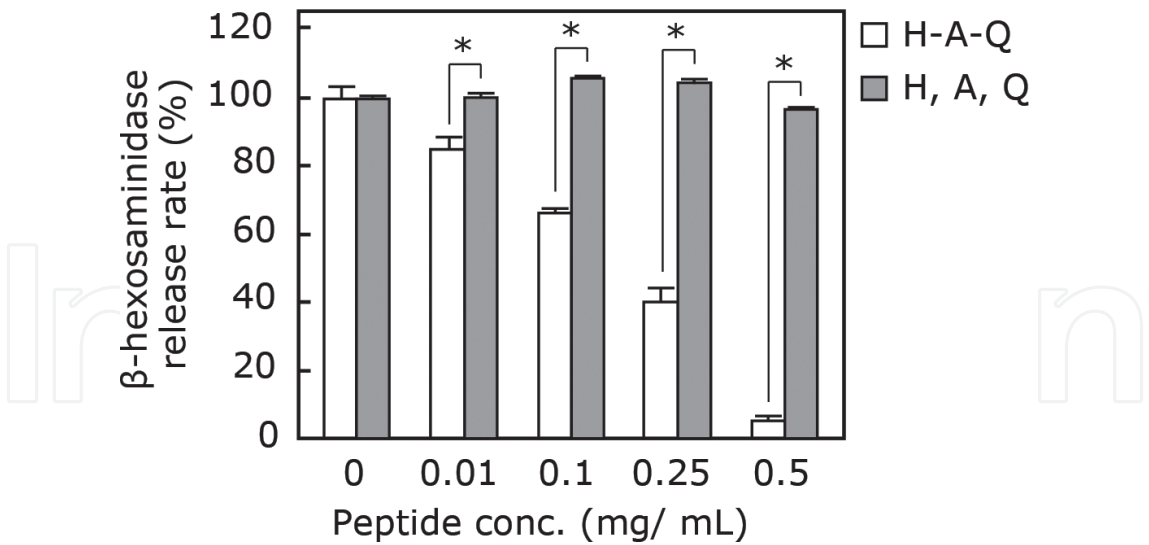


Figure 7. Effects of HAQ and amino acid mixtures made up of HAQ on β -hexosaminidase release from RBL-2H3 cells. Data are expressed as the means \pm SD values of triplicate determinations. * $p < 0.05$. DNP-specific IgE sensitized RBL-2H3 cells were challenged with DNP-HSA for 30 min. HAQ and amino acid mixtures made up of HAQ were added 10 min before antigen challenge.

with an anti-allergic action contain amino acids with aromatic rings and neutral or hydrophobic side chains [16]. Thus, we used peptides containing L-histidine, with an imidazole functional group, to assess the inhibitory effect of amino acid residues on the IgE-induced allergic response in IgE-sensitized RBL-2H3 cells. Imidazole peptides containing only L-histidine decreased the release of β -hexosaminidase. In particular, the tri- and tetra-peptides demonstrated degranulation-inhibitory activity (**Figure 8**).

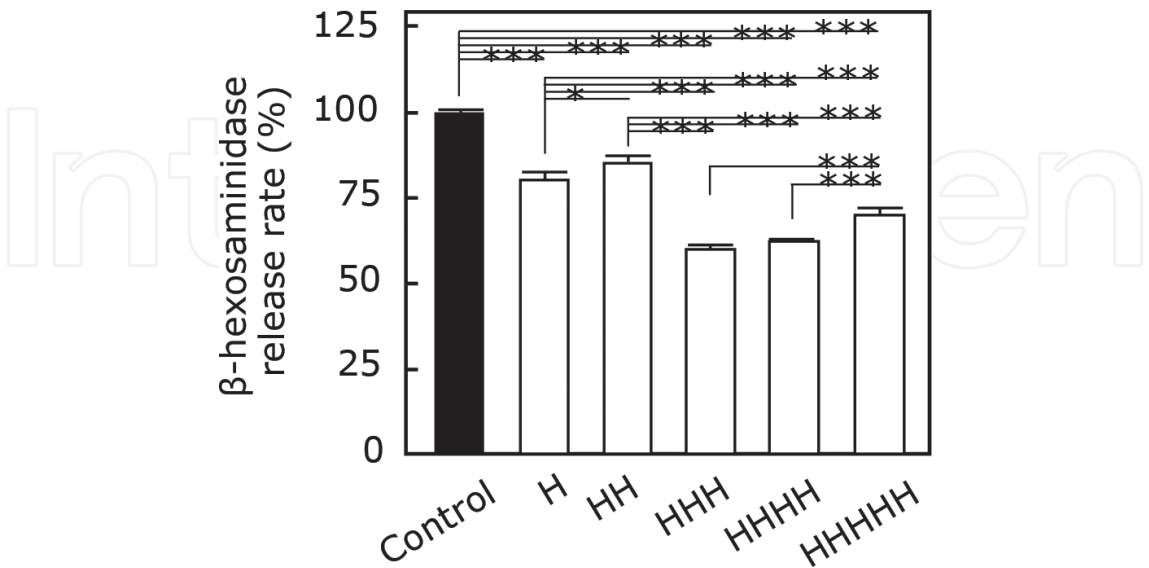


Figure 8. Effects of His and imidazole peptides of HAQ on β -hexosaminidase release from RBL-2H3 cells. Data are expressed as the means \pm SD values of triplicate determinations. * $p < 0.05$. DNP-specific IgE sensitized RBL-2H3 cells were challenged with DNP-HSA for 30 min. His and imidazole peptides were added 10 min before antigen challenge.

Finally, we investigated the amino acid sequence of the HAQ peptide. The activity of released β -hexosaminidase was evaluated for three peptides, with the L-histidine residue at different positions, HAQ, Ala-Gln-His (AQH), and Gln-His-Ala (QHA). The level of degranulation-inhibitory activity was found to depend on the peptide sequence (**Figure 9**). Nishida et al. reported that metallic ion concentration was important for the degranulation-inhibitory reaction and cytokine production from mast cells and basophils [22]. Therefore, we hypothesized that the degranulation from mast cells and basophils is related to an exaggerated effect of chelation. It has been reported that the HAQ peptide has a high affinity for metal ions, and peptides with histidine at the *N*-terminus of the sequence show a strong chelation effect for copper ions compared with peptides with L-histidine at the *C*-terminus [23]. Therefore, the suppression of degranulation may be related to an increase in ability to chelate metal ions, because the HAQ peptide having an L-histidine residue at the *N*-terminus exhibited a stronger inhibitory action against β -hexosaminidase than either AQH or QHA. In conclusion, we propose that the level of degranulation-inhibitory activity depends on the peptide binding, the number of amino acid residues, and the peptide sequence.

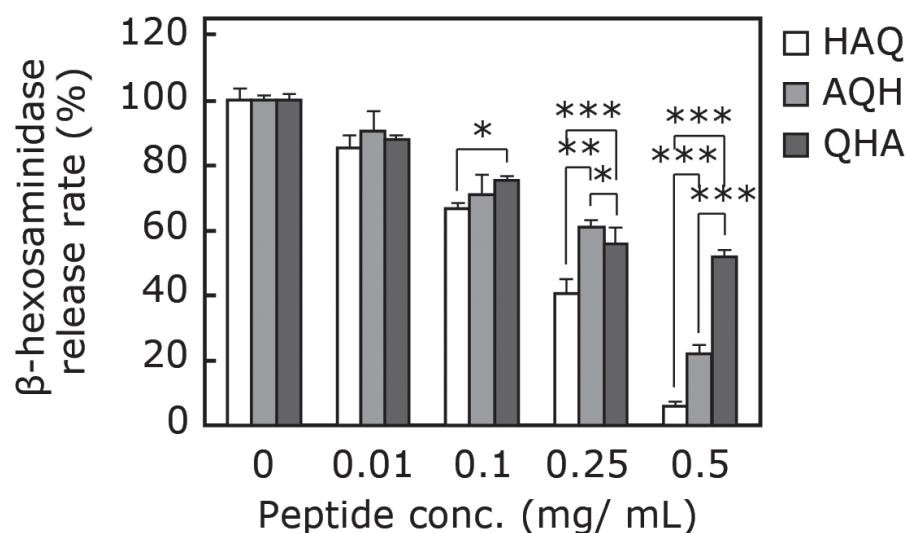


Figure 9. Effects of HAQ, AQH and QHA on β -hexosaminidase release from RBL-2H3 cells. Data are expressed as the means \pm SD values of triplicate determinations. * $p < 0.05$. DNP-specific IgE sensitized RBL-2H3 cells were challenged with DNP-HSA for 30 min. HAQ, AQH and QHA were added 10 min before antigen challenge.

6. Effect of the HAQ peptide on antibody production in mice

Th1 and Th2 polarization occurs according to cytokine patterns, which begin when antigen-presenting cells interact with naïve T cells and polarize into type 1 and type 2 cells in response to the type of antigen encountered [12–15]. Th1 cells produce IFN- γ and TNF- α which induce T-cell-mediated immunity and IgG2a production and downregulate Th2 cells. On the other hand, IgE production in mice is induced by IL-4 and IL-5 secreted by Th2 cells. IgE response is accompanied by IgG1 production, which is also induced by IL-4, resulting in allergic diseases.

IgA antibodies play an essential role in mucosal protection. Several properties of IgA antibodies, including an ability to be secreted when linked to secretory components, resistance to proteolysis, and an inability to trigger the complement cascade, allow the antibodies to clear antigens from mucosal surfaces in a process called immune exclusion [24]. Antigen delivery on surfaces may induce either immunization or unresponsiveness. Oral tolerance is usually defined as the suppression of humoral and cellular immune responses after mucosal presentation of a putative protein antigen [25]. It has been reported that an increase in specific IgA antibodies occurs concomitantly with the systemic suppression induced by oral tolerance [26]. One proposed explanation is that the induction of regulatory T cells (Treg) causes not only enhancement of the Th1 response and suppression of the Th2 response but also results in an allergy-suppressive mechanism. Thus, we examined immune responses (antibody and cytokine production) to continuous ingestion of the HAQ peptide in a mouse model of type-1 allergy to ovalbumin (OVA) [27]. BALB/c mice were randomly divided into phosphate-buffered saline (PBS)-PBS, PBS-OVA, and HAQ-OVA groups. An HAQ peptide-added diet was orally administered to BALB/c mice for 35 days. The mice were immunized intraperitoneally with OVA on days 8, 18, and 25. Blood, feces, and spleen lymphocytes were obtained from the mice on day 35 or 36. The levels of total IgA, IFN- γ , IL-4, and OVA-specific IgE, IgG1, IgG2a, and IgA were analyzed by enzyme-linked immunosorbent assay (ELISA) (Table 2 and Figure 10). BALB/c mice that were orally administered the HAQ peptide exhibited lower OVA-specific IgE and IgG1 secretion, whereas OVA-specific IgG2a levels remained unchanged in the serum. An increase in OVA-specific IgA was observed in feces, whereas total IgA levels remained unchanged. An increase in IgG1, IgG2a, and IFN- γ levels was observed, while the IL-4 level was decreased, in spleen lymphocytes, in the

	PBS-PBS	PBS-OVA	HAQ-OVA
Serum			
Specific IgE (A490)	0.05 \pm 0.02 ^a	1.15 \pm 0.57 ^b	0.82 \pm 0.18 ^c
Specific IgG1 (A490)	0.05 \pm 0.01 ^a	1.33 \pm 0.10 ^b	1.25 \pm 0.07 ^b
Specific IgG2a (A490)	0.00 \pm 0.00 ^a	0.57 \pm 0.10 ^b	0.54 \pm 0.06 ^b
Feces			
Specific IgA (A490)	0.05 \pm 0.03 ^a	0.93 \pm 0.13 ^b	1.66 \pm 0.18 ^c
Total IgA (8g/g)	3.25 \pm 0.74 ^a	3.48 \pm 0.74 ^b	3.07 \pm 0.74 ^c
Spleen lymphocytes			
Specific IgE (A490)	0.00 \pm 0.01	0.02 \pm 0.00	0.05 \pm 0.1
Specific IgG1 (A490)	0.02 \pm 0.00 ^a	0.28 \pm 0.05 ^b	0.72 \pm 0.07 ^c
Specific IgG2a (A490)	0.01 \pm 0.01 ^a	0.06 \pm 0.02 ^b	0.13 \pm 0.02 ^c

Data are expressed as the mean \pm SEM. a-b, a-c, b-c: $p < 0.05$, b-d: $p = 0.09$. Groups of that had received orally administered HAQ or PBS during sensitization to OVA or PBS were challenged intraperitoneally. Unsensitized animals received the vehicle alone. Blood was obtained from the orbital veins of mice at 35 or 36 days after injection and subjected to ELISA specific for IgE or IgG1 against OVA.

Table 2. Effect of continuous ingestion of HAQ on antibodies production from OVA-sensitized mice [27].

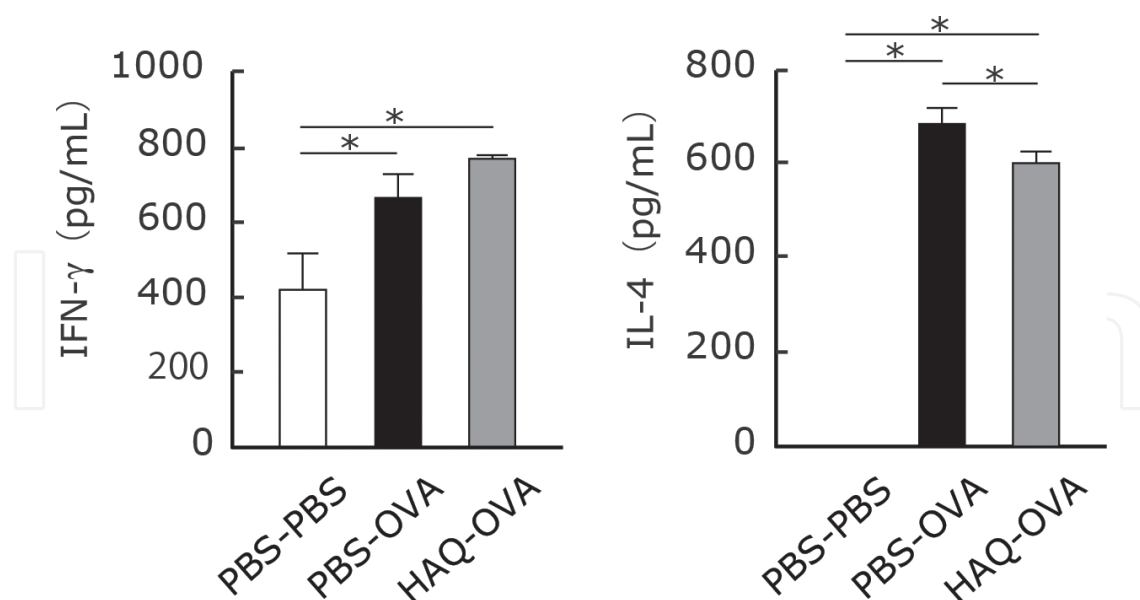


Figure 10. Effect of continuous ingestion of HAQ on IFN- γ and IL-4 production by mouse spleen lymphocytes (72 h). Data are expressed as the mean \pm SD values of triplicate determinations. * $p < 0.05$.

presence of the HAQ peptide. These findings suggest that the HAQ peptide may cause a shift from a Th2-type immune response toward a Th1-type response. Thus, the HAQ peptide has a potential regulatory effect on antibody production in a type-1 allergic response.

7. Effect of the HAQ peptide on allergic symptoms in mice

To investigate the effects of the HAQ peptide on the allergic reaction, we performed animal experiments using a murine type-1 allergy model. C3H/HeJ mice were randomly divided into HAQ-LHE, PBS-PBS, and PBS-LHE groups. The PBS-PBS and PBS-LHE groups were orally administered PBS alone. The HAQ-LHE and PBS-LHE groups were initially injected intraperitoneally with 100 μ g lysozyme from hen egg white (LHE) and 4 mg aluminum hydroxide in 0.2-mL PBS on day 1. LHE was then reduced to 50 μ g and injected intraperitoneally with 4 mg aluminum hydroxide in 0.2 mL PBS on day 8. To assess the sensitization, blood was obtained from the orbital veins of mice under light anesthesia, 11 days after the initial injection, and subjected to ELISA. The HAQ-LHE group was orally administered HAQ peptide (1 mg/day) in 0.2 mL PBS by gavage throughout the experimental period of 14 days.

We evaluated the effect of orally administered LHE on the suppression of allergic reactions in a murine model. The score assessment commonly increases and whole-body temperature is commonly reduced during systemic anaphylaxis [28, 29]. Score assessment and body temperature measurements were therefore conducted to assess anti-allergic effects in mice. The HAQ-LHE and PBS-LHE groups were orally administered 10 mg LHE in 0.5 mL PBS, and then score assessment and rectal temperature measurements were performed to evaluate allergic symptoms (**Figure 11**). The PBS-PBS group was orally administered 0.5 mL PBS alone. Score assessments

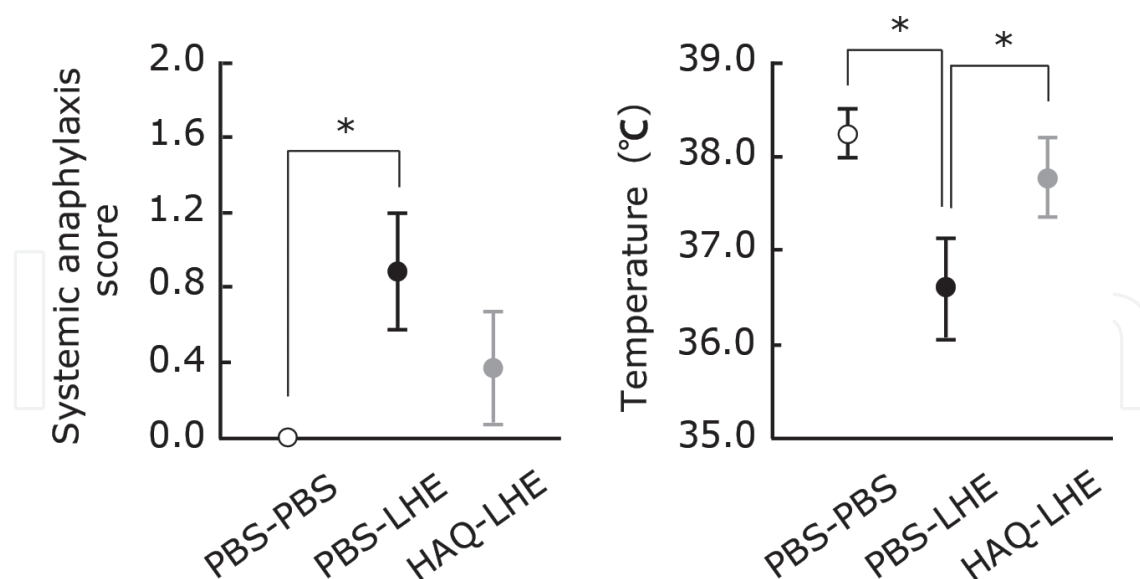


Figure 11. Allergic symptoms after oral challenge in mice presensitized in the presence of HAQ peptide. ○, PBS-PBS PBS orally administered group ($n = 5$); ●, PBS-LHE, PBS orally administered group ($n = 9$); ●, HAQ-LHE, HAQ orally administered group ($n = 8$). Data are expressed as the mean \pm SEM. * $p < 0.05$ between each group. Groups of mice that had received orally administered HAQ peptide or PBS during sensitization to LHE or PBS were challenged intraperitoneally. (A) Symptom scores of systemic anaphylaxis. Anaphylaxis-like symptoms were scored at 20 min after oral challenge on a scale from 0 (no symptoms) to 5 (death) as described in the Materials and Methods. (B) Rectal temperature. The rectal temperature was measured at 30 min after oral challenge.

were performed at 20 min after challenge with LHE using the scoring system as described by Li et al. [28]. To evaluate body temperature, the rectal temperature was measured at 30 min after oral administration of LHE. In the score assessment, the PBS-LHE group score (0.9 ± 0.3) was significantly higher than the PBS-PBS group (0.0 ± 0.0) ($p < 0.05$), but not the HAQ-LHE group (0.4 ± 0.3). In terms of rectal temperature, the PBS-LHE group ($36.6 \pm 0.5^\circ\text{C}$) had a significant decrease in body temperature compared with the PBS-PBS ($38.2 \pm 0.3^\circ\text{C}$) and HAQ-LHE ($37.8 \pm 0.4^\circ\text{C}$) groups ($p < 0.05$). These data demonstrate that continuous administration of the HAQ peptide to sensitized mice suppresses the consistent allergic symptoms induced by antigen stimulation. These results indicate that the HAQ peptide has anti-allergic effects *in vivo* as well as *in vitro* [16].

8. Conclusion

The frequency of allergic disorders is increasing worldwide, and this causes serious issues, including escalation of medical costs, reduction in health levels, and decline in labor productivity. We studied the anti-allergic effects of the HAQ peptide, which is present in CE90GMM, a peptide mixture derived from milk casein. In these studies, we observed five major findings *in vitro* and *in vivo* as follows:

1. CE90GMM and four peptides (HAQ, EQPI, DMES, and KIKE) inhibited the degranulation of RBL-2H3 and the effect varied with the dose.

2. The level of degranulation-inhibitory activity depended on peptide binding, peptide sequence, and the number of amino acids.
3. Peptides with anti-allergic actions possess aromatic rings and neutral or hydrophobic side chains.
4. The HAQ peptide has a potential regulatory effect on antibody and cytokine production in type-1 allergic responses.
5. Continuous administration of HAQ peptide suppressed the mild allergic symptoms in a murine model of type-1 allergy.

Our studies suggest a possible use of the HAQ peptide in preventing type-1 allergic responses. To use HAQ clinically for the prevention of allergic disease, the optimal dosage, efficacy, and adverse effects in humans should be determined.

We expect that the findings obtained in these studies will contribute to the prevention, and improve the treatment, of type-1 allergies.

Acknowledgements

This study was supported by JSPS KAKENHI (grant number 25750051). The authors thank Kenji Yamagishi, Takuya Sugahara, Junko Hirano, and Rina Hirata for assistance with experiments.

Author details

Mamoru Tanaka^{1*} and Takeaki Okamoto²

*Address all correspondence to: m-tanaka@cc.u-kochi.ac.jp

1 Department of Nutrition, University of Kochi, Kochi, Japan

2 Faculty of Education, Ehime University, Matsuyama, Ehime, Japan

References

- [1] Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature*. 2008;**454**:445-454
- [2] Nakamura Y, Yamamoto N, Sakai K, et al. Purification and characterization of angiotensinI-converting enzyme inhibitors from sour milk. *Journal of Dairy Science*. 1995;**78**:777-783

- [3] Sato M, Hosokawa T, Yamaguchi T, et al. AngiotensinI-converting enzyme inhibitory peptides derived from Wakame (*Undaria pinnatifida*) and their antihypertensive effect in spontaneously hypertensive rats. *Journal of Agriculture and Food Chemistry*. 2002;**80**:6245-6252
- [4] Tokunaga KH, Yoshida C, Suzuki KM, et al. Antihypertensive effect of peptides from royal jelly in spontaneously hypertensive rats. *Biological and Pharmaceutical Bulletin*. 2004;**27**:189-192
- [5] Hata I, Higashiyama S, Otani H. Identification of a phosphopeptide in bovine α s1-casein digest as a factor influencing proliferation and immunoglobulin production in lymphocyte cultures. *Journal of Dairy Research*. 1998;**65**:569-578
- [6] Otani H, Watanabe T, Tashiro Y. Effects of bovine β -casein (1-28) and its chemically synthesized partial fragments on proliferative responses and immunoglobulin production in mouse spleen cell culture. *Bioscience, Biotechnology, and Biochemistry*. 2001;**65**:2489-2495
- [7] Civra A, Giuffrida MG, Donalisio M, et al. Identification of Equine Lactadherin-derived peptides that inhibit rotavirus infection via integrin receptor competition. *Journal of Biological Chemistry*. 2015;**290**:12403-12414
- [8] Yoshikawa M, Fujita H, Matoba N, et al. Bioactive peptides derived from food proteins preventing lifestyle-related diseases. *Biofactors*. 2000;**12**:143-146
- [9] Vickery BP, Chin S, Burks AW. Pathophysiology of food allergy. *Pediatric Clinics of North America*. 2011;**58**:363-376
- [10] Siraganian RP. Mast cell signal transduction from the high-affinity IgE receptor. *Current Opinion in Immunology*. 2003;**15**:639-646
- [11] Blank U, Rivera J. The ins and outs of IgE-dependent mast-cell exocytosis. *Trends in Immunology*. 2004;**25**:266-273
- [12] Church MK, Levi-Schaffer F. The human mast cell. *Journal of Allergy and Clinical Immunology*. 1997;**99**:155-160
- [13] Wedemeyer J, Tsai M, Galli SJ. Role of mast cells and basophils in innate and acquired immunity. *Current Opinion in Immunology*. 2000;**12**:624-631
- [14] Kidd P. Th1/Th2 balance: The hypothesis, its limitations, and implications for health and disease. *Alternative Medicine Review*. 2003;**8**:223-246
- [15] Mosmann TR, Cherwinski H, Bond MW, et al. Two types of murine helper T cell clone. I. definition according to profiles of lymphokine activities and secreted proteins. *Journal of Immunology*. 1986;**175**:5-14
- [16] Tanaka M, Yamagishi K, Sugahara T, et al. Impact of peptides from casein and peptide-related amino acids on degranulation in rat basophilic leukemia cell line RBL-2H3. *Nippon Shokuhin Kagaku Kaishi (in Japanese)*. 2012;**59**:556-561

- [17] Hashimoto K, Sato K, Nakamura Y, et al. Development of a large-scale (50L) apparatus for ampholyte-free isoelectric focusing (autofocusing) of peptides in enzymatic hydrolysates of food proteins. *Journal of Agricultural Food Chemistry*. 2005;**53**:3801-3806
- [18] Tanaka M, Watanabe H, Yoshimoto Y, et al. Anti-allergic effects of His-Ala-Gln tripeptide *in vitro* and *in vivo*. *Bioscience, Biotechnology, and Biochemistry*. 2016;**81**:380-383
- [19] Silk DB. Protein, peptides and amino acid: Which and what? Nestle Nutrition Workshop Series: Clinical and Performance Programme. 2000;**3**:257-271
- [20] Okamoto T, Hirano J, Tagashira A, et al. Anti-allergic effect of imidazole peptides *in vivo* and *in vitro*. In: *The 6th Asian Congress of Dietetics, Taipei, Taiwan; 2016*. p. 223
- [21] Tanaka M, Watanabe H, Kozai H, et al. Degranulation inhibitory activity may depend on peptide binding and peptide sequence. In: *The 12th Asian Congress of Nutrition, Yokohama, Japan; 2017*. p. 311
- [22] Nishida K, Hirano T. Role of zinc/zinc transporter in allergic response. *Seikagaku* (in Japanese). 2010;**9**:814-824
- [23] Muramoto K, Chen HM, Saito K, et al. Synergistic effect of antioxidative peptides from a soybean protein digest on the antioxidative activity of nonpeptidic antioxidants. *Soy Protein Research*. 1997;**18**:55-61
- [24] Challacombe SJ, Rahman D, Jeffery H, Davis SS, O'Hagan DT. Enhanced secretory IgA and systemic IgG antibody responses after oral immunization with biodegradable microparticles containing antigen. *Immunology*. 1992;**76**:164-168
- [25] Garside P, Mowat AM. Oral tolerance. *Seminars in Immunology*. 2001;**13**:177-185
- [26] Challacombe SJ, Tomasi TB, Jr. Systemic tolerance and secretory immunity after oral immunization. *Journal of Experimental Medicine*. 1980;**152**:1459-1472
- [27] Okamoto T, Hirata R, Tagashira A, et al. Effect of His-Ala-Gln peptide on antibody producibility in mice. *Journal of Shikoku Public Health Society* (in Japanese). 2017;**62**:63-70
- [28] Li XM, Schofield BH, Huang CK, et al. A murine model of IgE-mediated cow's milk hypersensitivity. *Journal of Allergy and Clinical Immunology*. 1999;**103**:206-214
- [29] Tanaka M, Nagano T, Yano H, et al. Impact of ω -5 gliadin on wheat-dependent exercise-induced anaphylaxis in mice. *Bioscience, Biotechnology, and Biochemistry*. 2011;**75**: 313-317

