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Letter to the Editor

Development of a liquid chromatography-tandem mass spectrometry method for quantification of the egg allergen ovalbumin in rat plasma

Dear Editor,

Monitoring of serum concentrations of food allergens during challenge tests provides important information when diagnosing food allergies.[1] Sandwich ELISA has been used to measure the concentrations of food allergens in foodstuffs[2] and in biological samples.[1] However, the antibodies used in ELISA sometimes show cross-reactivity and/or interference with multiple components in samples, resulting in overestimation and/or underestimation. Thus, the widespread use of ELISA is limited because of poor specificity. Recently, liquid chromatography tandem mass spectrometry (LC-MS/MS) has been used for the analysis of food allergens in processed foods.[2,3] These reports suggest that detection of food allergens in processed foods using mass spectrometry (MS) is helpful for quality control. Furthermore, MS has been used for quantification of biological origin peptides[4] and protein-based biopharmaceuticals[5] in biological samples. However, there are no studies that the plasma (or serum) concentrations of food allergens were measured using MS. Compared to the detection of food allergens in processed foods using MS, detection of allergen in plasma is complicated since concentrations of food allergen in plasma are generally much lower than those in processed foods. To detect a target protein at low concentrations in biological samples, it is necessary to remove any abundant impurities from the samples by appropriate methods such as immunocapture methods. We applied immunoprecipitation of egg allergen OVA and nanoLC combined with quadrupole time of flight tandem mass spectrometer to measure the concentration of OVA in rat plasma after oral ingestion.

Figure 1 shows our schematic strategy to determine the concentrations of OVA in plasma. In this study, we used Ekspert™ nanoLC 425 (AB sciex) combined with a TripleTOF 5600+ (AB Sciex) as a LC-MS/MS system. Experiments involving animals were approved by the animal committee of Hiroshima University (Approval No. A16-138). All details are shown in Supplementary Methods.

To determine the signature peptide ions for OVA quantification, we first confirmed the correlation between the OVA concentrations in water and peak areas of the product ions generated from tryptic digested OVA. When the peptides from tryptic digested OVA were analyzed using nanoLC-MS/MS and Skyline software, 11 precursor ion peaks were obtained (Supplementary Table 1). Among these precursor ions, five OVA-specific precursor ions exhibited correlations between the OVA concentrations and peak areas of the product ions (Supplementary Table 2). In particular, GGLEPINFQTAADQAR $[M+2H]^{2+}$ showed the highest intensity and reproducibility for measuring the OVA concentration in the range of 50–500 μg/mL. GGLEPINFQTAADQAR peptide has been identified as a marker that can be used to quantify the OVA contents in processed foods.[3] In addition, this peptide contains a partial IgE-binding epitope sequence of OVA (RGGLEPINFQ) for patients with type I hypersensitivity to eggs.[6] Thus, we determined GGLEPINFQTAADQAR as a signature peptide and synthesized its stable isotope-labeled (SIL) peptide. SIL-peptide was used as internal standard in OVA quantification.

Next, we tried to detect OVA in rat plasma and confirmed correlation between the standard OVA concentration in rat plasma and peak area ratios of product ions from OVA to SIL-peptide. To eliminate the quantitative interferences by large amounts of biological proteins, we concentrated OVA from plasma samples by the immunoprecipitation method using polyclonal anti-OVA antibody-coupled magnetic beads. After immunoprecipitation, we could also obtain the signals of GGLEPINFQTAADQAR $[M+2H]^{2+}$ (Supplementary Fig. 1). When the peak area ratios of the integrated product ions generated from OVA to those generated from an internal standard were plotted against the OVA concentrations (0–150 ng/mL), a high correlation coefficient was observed ($r = 0.9956$, Supplementary Fig. 2). The calculated lower limit of detection (signal to noise ratio = 3) and lower limit of quantitation (signal to noise ratio = 10) of OVA were 6.6 ng/mL and 29.5 ng/mL, respectively.

Finally, we measured OVA concentration in plasma after oral administration of OVA in rats. When OVA (50 mg) was administered orally 30 min after the vehicle (PBS), the plasma concentration of OVA was low (approximately 74 ng/mL). By contrast, after pretreatment with aspirin, the plasma concentration of OVA was approximately 149 ng/mL following ingestion by rats (Table 1). These results agree with our previous finding that aspirin disrupted the intestinal barrier, which was followed by OVA absorption through the paracellular pathway.[7] In this study, 50 mg of OVA was administered to each rat with a body weight of approximately 250 g, which gave an OVA administration rate of 200 mg/kg. Husby *et al.* reported a serum concentration of OVA of 4–178 ng/mL measured by ELISA after 2 mL/kg of raw egg white was administered to children through a gastric tube, which corresponded to a dosage rate of approximately 100 mg of OVA per kilogram.[8] According to Food and Drug Administration guidelines, 100 mg/kg of OVA in human corresponds to 620 mg/kg in rats.[9] Hence, the dosage of OVA (200 mg/kg) in this study is considered to be reasonable.

In conclusion, we developed a new technique to measure the plasma OVA concentrations using LC-MS/MS followed by immunoprecipitation. Our new method enables the measurement of low plasma concentrations of OVA even in clinical challenge tests. This

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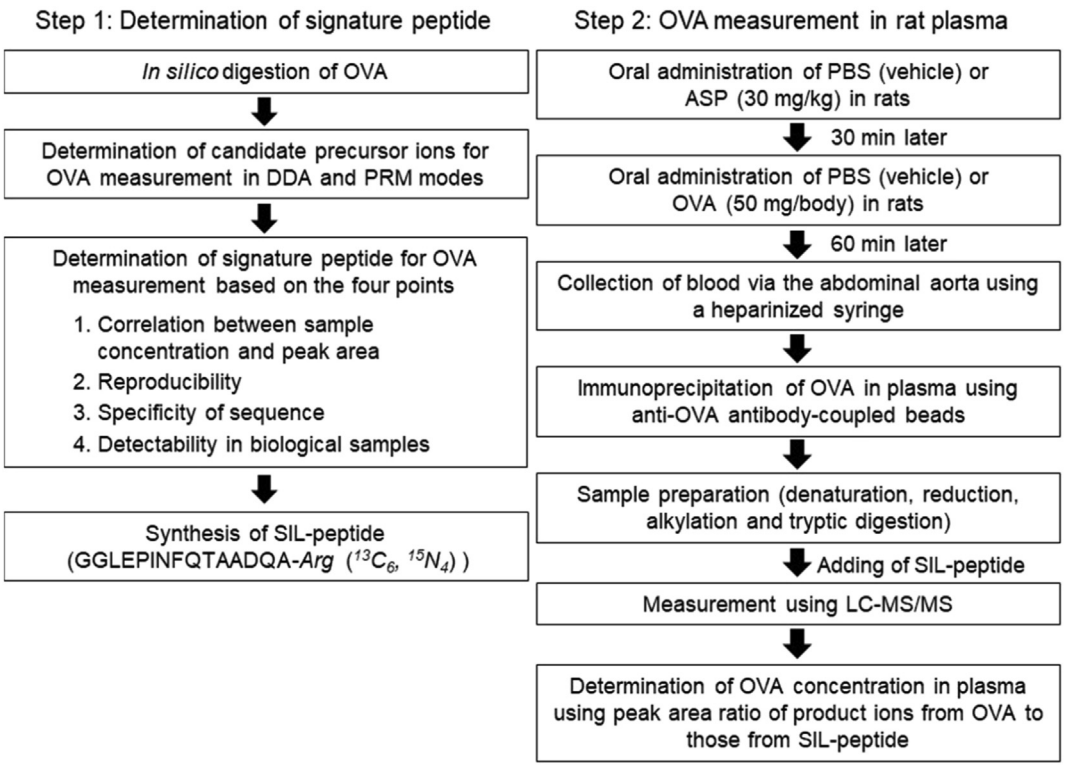

Fig. 1. Strategy for measurement of plasma OVA concentration using liquid chromatography tandem mass spectrometry (LC-MS/MS) in this study (schematic). *In silico* digestion and peak area analysis were conducted using Skyline software (Version 3.5, MacCoss Lab Software). Stable isotope-labeled (SIL)- GGLEPINFQTAADQAR peptide were synthesized by Sigma–Aldrich, which has six carbon (^{12}C) and four nitrogen (^{14}N) atoms of the C-terminal arginine substituted with ^{13}C and ^{15}N, respectively. ASP, aspirin; DDA, data-dependent acquisition; PRM, parallel reaction monitoring.

Table 1
Effect of aspirin on plasma concentrations of OVA after oral administration in rats.

	PBS		OVA	
Aspirin treatment	−	+	−	+
OVA concentration (ng/mL)	n.d.	n.d.	73.5 ± 16.3	148.5 ± 9.2*

Aspirin (30 mg/kg) was administered orally 30 min before oral administration of OVA (50 mg). Each value is the mean ± standard error for three rats. *$P < 0.01$, significantly different from OVA ingestion without aspirin treatment. n.d., not detected.

method could be used to elucidate the relationship between serum allergen concentrations and development of symptoms in food allergy patients after ingestion of an allergen. However, immunoprecipitation is not suitable for measuring large numbers of samples in clinical situations because it is complicated and expensive. Furthermore, it may be difficult to concentratate allergen in plasma from patients with food allergy by immunoprecipitation because immunoglobulins specific to allergen may be increased. A method that is simpler and quicker than immunoprecipitation is required to remove proteins such as albumin and globulins from the plasma samples. Additionally, further studies are also required to confirm the relationship between the plasma concentration of OVA determined by LC-MS/MS and allergic symptoms. The limitation in our assay is method validation. Various validation factors can affect the accuracy quantification by LC-MS/MS, SIL-protein was reported more useful than SIL-peptide to improve method validation.[10] If these problems are resolved, LC-MS/MS may be useful for precise quantification of serum allergen concentrations following ingestion of allergenic foods.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2018.12.005.

Conflict of interest
The authors have no conflict of interest to declare.

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