

RESEARCH ARTICLE

Biotinylated peptides substituted with D-amino acids with high stability as anti-anaphylactic agents targeting platelet-activating factor

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Platelet-activating factor (PAF) is an important lipid mediator of anaphylaxis and therefore can be an anti-anaphylactic agent target. Recently, we reported that several synthetic biotinylated peptides containing a Tyr-Lys-Asp-Gly sequence markedly inhibited the bioactivities of PAF in vitro and in vivo; it also inhibited anaphylactic reactions such as hypothermia, hypotension, and vascular permeability in vivo. Here, we report the anti-anaphylactic effects of three biotinylated heptapeptides (peptide 1: H-Lys(biotinyl)-Trp-Tyr-Lys-Asp-Gly-Asp-OH, peptide 2: H-D-Lys(biotinyl)-Trp-Tyr-Lys-Asp-Gly-Asp-OH, and peptide 3: H-D-Lys(biotinyl)-Trp-Tyr-Lys-Asp-Gly-D-Asp-OH). The experiment using tryptophan fluorescence spectroscopy showed that the interaction of peptides 2 and 3 with PAF was larger than that of peptide 1. Experiments using a rat model of hind paw edema showed that peptides 1, 3, and 2 inhibited PAF-induced edema by 67.9%, 69.3%, and 79.3%, respectively. In a mouse model of anaphylaxis, both peptides 2 and 3 showed inhibitory effects on anaphylactic hypothermia, whereas peptide 1 did not. Furthermore, experiments involving in vitro rat plasma stability of peptides showed that both peptides 3 and 2 were more stable in plasma compared to peptide 1 (84.0%, 51.8%, and 0%, remained after 6 h, respectively). Our results suggest that both peptides 2 and 3 may show systemic and local inhibitory effects as anti-anaphylactic agents targeting PAF.

KEYWORDS

anaphylaxis, anti-anaphylactic agent, biotinylated peptide, D-amino acid, hypothermia, interaction, plasma stability, platelet-activating factor

1 | INTRODUCTION

Anaphylaxis is a sudden, severe, and life-threatening allergic reaction that is characterized by hypotension, hypothermia, vascular leakage, bronchial constriction, and cardiac arrhythmia in the early stage.^{1–4} Platelet-activating factor (PAF: 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is an important lipid mediator that is synthesized from several types of cells, such as endothelial cells, mast cells, neutrophils, and macrophages.^{5–8} PAF is known to be involved in the pathogenesis of anaphylaxis, depending on the stimuli.^{2,3} PAF acts by binding to its specific PAF receptor and is rapidly inactivated and eliminated by PAF

acetylhydrolase (PAF-AH) in the circulation (the half-life time of PAF: 3–13 min).^{7,9,10} Moreover, serum PAF levels are significantly higher in anaphylactic patients than in control patients and inversely correlate with PAF-AH activity.¹¹ However, there are currently no anti-anaphylactic agents that target PAF in clinical use.

Asp-hemolysin is a hemolytic and toxic protein derived from *Aspergillus fumigatus*.^{12,13} Recently, we revealed that several Asp-hemolysin-related synthetic biotinylated peptides composed of 4 to 29 amino acids (BP4-BP29), all of which have a Tyr-Lys-Asp-Gly sequence that inhibited the bioactivities of PAF in vitro and in vivo by directly binding to PAF, even at doses 150- to 300-fold lower than

that of the PAF antagonist, CV-3988.^{14,15} Moreover, we reported that one of the peptides, BP21, inhibits anaphylactic hypothermia, hypotension, and vascular permeability in a mouse model of anaphylaxis.^{16,17} Furthermore, we previously reported that a synthetic biotinylated heptapeptide (peptide 2: H-D-Lys(biotinyl)-Trp-Tyr-Lys-Asp-Gly-Asp-OH) shows more significant inhibitory effects on PAF-induced edema than a same heptapeptide (peptide 1), H-Lys(biotinyl)-Trp-Tyr-Lys-Asp-Gly-Asp-OH, which consists of all L-amino acids, using a rat hind paw edema model.¹⁸ In this study, we found that peptide 3, which is substituted with D-Asp of peptide 2 at the C-terminus, as well as peptide 2 can have more marked inhibitory effects on both the bioactivities of PAF and anaphylactic hypothermia in vivo with high stability in the plasma than peptide 1 (Figure 1).

2 | MATERIALS AND METHODS

2.1 | Materials

PAF (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine, C16) was purchased from Enzo Life Sciences Inc. (Plymouth Meeting, PA, USA). Unless otherwise stated, all other reagents were purchased from FUJIFILM Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Peptide name	Sequence
peptide 1	H-Lys(Biotinyl)-Trp-Tyr-Lys-Asp-Gly-Asp-OH
peptide 2	H-D-Lys(Biotinyl)-Trp-Tyr-Lys-Asp-Gly-Asp-OH
peptide 3	H-D-Lys(Biotinyl)-Trp-Tyr-Lys-Asp-Gly-D-Asp-OH

FIGURE 1 Amino acid sequences and structures of biotinylated peptides used in this study. These amino acids are represented by conventional three letter codes

2.2 | Synthetic peptides

The three biotinylated peptides (>95% purity) used in this study were synthesized and purified by GL Biochem (Shanghai, China). The amino acid sequences and structures as well as the analytical data are shown in Figure 1 and Table 1, respectively.

2.3 | Animals

The study protocol was approved by the Institutional Animal Care and Use Committee of Iryo Sosei University (no approved number) and the Genetic Recombination Experiment Committee (approval numbers: 62 and 67). Male Wistar rats (6–9 weeks old, 220–260 g body weight) and male ovalbumin (OVA)-specific IgE transgenic BALB/c mice (OVA-IgE mice, 8–14 weeks old, 25–30 g body weight) were obtained from CLEA Japan, Inc. (Tokyo, Japan). All animals were housed under a 12/12 h light/dark cycle and were fed ad libitum.

2.4 | Measurement of rat hind paw edema induced by PAF

Measurements of rat hind paw edema were conducted as previously described.^{14,18,19} The endpoint was the time of maximal edema, that is, 45 min after PAF injection into the hind paw. PAF (20 µmol/L) was dissolved in a sterile solution containing 10 mmol/L tris(hydroxymethyl)aminomethane, 150 mmol/L sodium chloride, and 0.25% bovine serum albumin (fraction V grade, Merck KGaA, Darmstadt, Germany). All injections were performed in rats under ether anesthesia. The subplantar surface of the hind paw of the rat was injected with PAF (1 nmol); after 45 min, injection was quantified by measuring the increase in paw volume (ml). To examine the effects of biotinylated peptides on rat hind paw edema induced by PAF, each peptide (10 nmol) was dissolved in sterile phosphate buffered saline

Biotinylated peptides	RP-HPLC		ESI-MS	
	t_R (min)	Purity (%)	Observed	Calculated
Peptide 1	8.125	95.16	[M+H] ⁺ 1,138.26	1,137.29
Peptide 2	8.647	96.63	[M-H] ⁻ 1,136.05 [M-2H] ²⁻ 567.70	1,137.29
Peptide 3	8.129	96.05	[M+H] ⁺ 1,137.6 [M+2H] ²⁺ 569.5	1,137.26

TABLE 1 Analytical data of biotinylated peptides used in this study

Note: All data were provided by GL Biochem Ltd. (Shanghai, China). RP-HPLC was performed using C18 column (4.6 mm × 250 mm, 5 µm). The analysis was archived using a linear gradient of 20% to 45% of eluent A for peptide 1, 18% to 43% for peptide 2, and 22% to 47% for peptide 3 (eluent A: 0.1% trifluoroacetic acid in 100% acetonitrile; eluent B: 0.1% trifluoroacetic acid in 100% water) for 25 min with a flow rate of 1.0 ml/min.

(PBS) containing 10 mmol/L phosphate and 150 mmol/L sodium chloride (pH 7.4) was injected into the subplantar surface of the hind paw 15 min prior to PAF injection.

2.5 | Measurement of body temperature of mice

Body temperature was obtained rectally using a Weighing Environment Logger (AS-1687) and an endorectal probe for mice (Φ 1.6 mm) (A&D Company Limited, Tokyo, Japan) as previously described.^{16,17} OVA-IgE mice were injected intraperitoneally with OVA (1.5 mg/mouse) in PBS (pH 7.4) in the absence or presence of each biotinylated peptide (100 nmol/mouse). The rectal temperature was measured at 0, 10, 20, 30, 45, 60, and 75 min after injection.

2.6 | Tryptophan fluorescence spectroscopy

Interactions of biotinylated peptides with PAF can be revealed by measuring the fluorescence of tryptophan residues, as previously described.^{14,15,20,21} The spectra were recorded using a Hitachi F2500 fluorescence spectrophotometer (Tokyo, Japan) at room temperature (25°C). The excitation wavelength was set to 295 nm; emission was monitored from 320 to 356 nm. Moreover, tryptophan fluorescence intensity was measured at an excitation wavelength of 295 nm and an emission wavelength of 348 nm to minimize interference from tyrosine fluorescence. Each biotinylated peptide (1 μ mol/L) was incubated with different concentrations of PAF (0–30 μ mol/L) for 30 min at 37°C in PBS (pH 7.4); the changes in intensity between the lipid and lipid-free (peptide alone) samples were calculated.

2.7 | In vitro stability of biotinylated peptides in rat plasma

The in vitro stability of biotinylated peptides was determined using rat plasma. Blood obtained from diethyl ether anaesthetized rats was collected in a plastic tube containing 0.38% sodium citrate; the plasma was isolated by centrifuging the blood sample at 1,450 $\times$ g for 20 min. Each biotinylated peptide (0.135 mmol/L, corresponding to approximately 0.15 mg/ml) was incubated with rat plasma (25 μ l), which was pre-incubated at 37°C for 20 min, at 37°C for four different time points (0, 0.5, 2, and 6 h). The reaction was terminated by the addition of an equal volume of ice-cold acetonitrile; the mixtures were vortexed for 1 min and then centrifuged at 4°C for 15 min at 4,500 $\times$ g. The supernatants containing the remaining unchanged peptide were collected, dried under a nitrogen flow, and re-dissolved in distilled water. The samples were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis using precast 16.5% Tris/Tricine gels (Bio-Rad Laboratories, Hercules, CA, USA). After electrophoresis, the peptide was electroblotted onto a polyvinylidene difluoride membrane (Bio-Rad Laboratories) in a buffer (pH 8.3) containing 25 mmol/L Tris, 192 mmol/L glycine, 15% methanol, and 0.1%

SDS for 70 min at 70 V. Biotinylated peptides were detected using horseradish peroxidase (HRP)-conjugated streptavidin (GeneTex, Inc., Los Angeles, CA, USA) to detect biotinylated peptides. Enhanced chemiluminescence detection reagents (Cytiva, Ltd., Buckinghamshire, UK) were used as the substrate for HRP; the chemiluminescence emitted was analyzed using an LAS-3000 luminescent image analyzer (FUJIFILM, Tokyo, Japan).

2.8 | Statistical analysis

All results are expressed as the mean $\pm$ standard deviation (SD). Significant differences were analyzed using the Student's *t* test. Differences were considered statistically significant at *p* < 0.05.

3 | RESULTS

3.1 | Interaction of biotinylated peptides with PAF

All biotinylated peptides used in this study contain a tryptophan residue (Figure 1). To assess the interaction of each peptide with PAF, intrinsic tryptophan fluorescence experiments were performed. The interaction of each peptide (1 μ mol/L) with PAF was analyzed by monitoring the tryptophan fluorescence spectra of the peptide in the presence of different concentrations of PAF (0–30 μ mol/L) at an excitation wavelength of 295 nm (Figure 2A–C). The fluorescence intensities of all peptides used in this study increased in a PAF-concentration-dependent manner; however, there was no shift in the maximal wavelength of peptides with different concentrations of PAF (maximum emission peak of all peptides: λ_{max} = 350 nm). Therefore, a tryptophan residue in these peptides is located at the PAF-binding site; that is, another residue in the peptides is important for the binding of the peptide to PAF.

As shown in Figure 2D, the change in the fluorescence intensity of peptide 1, which consists of all L-amino acids, was the lowest. This finding indicated that both peptides 2 and 3, both of which contain D-amino acid(s), can bind more strongly to PAF than peptide 1. Furthermore, the tryptophan fluorescence intensity of each peptide increased as the concentration of PAF increased to approximately 3 μ mol/L, with the intensity almost reaching a plateau above 3 μ mol/L. In fact, PAF exists as a monomer at concentrations below the critical micellar concentration (CMC) and forms micelles at concentrations exceeding the CMC. Previously, we reported that the CMC of PAF (C16) is approximately 2.6 μ mol/L.¹⁶ Therefore, these results suggest that all biotinylated peptides selectively recognize monomeric PAF.

3.2 | Effects of biotinylated peptides on rat paw edema induced by PAF

We examined the inhibitory effects of biotinylated peptides on the bioactivities of PAF in a rat hind paw edema model. As shown in

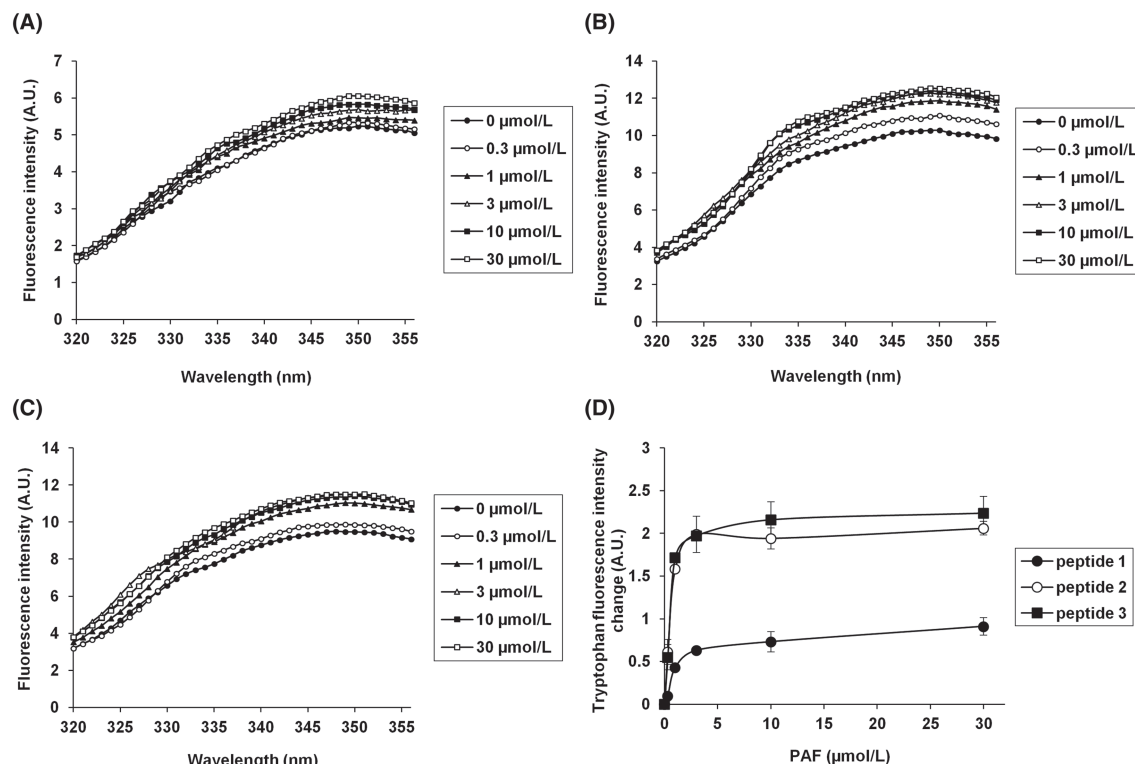


FIGURE 2 Effects of PAF on the intrinsic tryptophan fluorescence of biotinylated peptides. Tryptophan fluorescence spectrum of peptide 1 (A), peptide 2 (B), and peptide 3 (C) (each 1 μmol/L) in the absence or presence of PAF (0–30 μmol/L). Excitation wavelength was set to 295 nm and emission wavelength to 320–356 nm. Panel D shows changes in fluorescence intensity of biotinylated peptides in the absence or presence of PAF at 348 nm by exciting at 295 nm. Each value represents the change compared to the lipid-free sample (peptide only) and is presented as the mean ± SD of three individual experiments

Figure 3A, the intraplantar injection of all peptides (each 10 nmol per paw), especially peptides 2 and 3, led to significant and marked inhibition of paw edema induced by PAF (1 nmol per paw). In contrast, none of the peptides used in this study induced paw edema (data not shown). Moreover, the change in tryptophan fluorescence intensity of biotinylated peptides was inversely proportional to the increase in PAF-induced paw edema in the presence of the peptides (Figure 3B), indicating that all biotinylated peptides used in this study inhibited PAF-induced inflammation by binding to PAF in vivo.

3.3 | Effects of biotinylated peptides on hypothermia during anaphylaxis

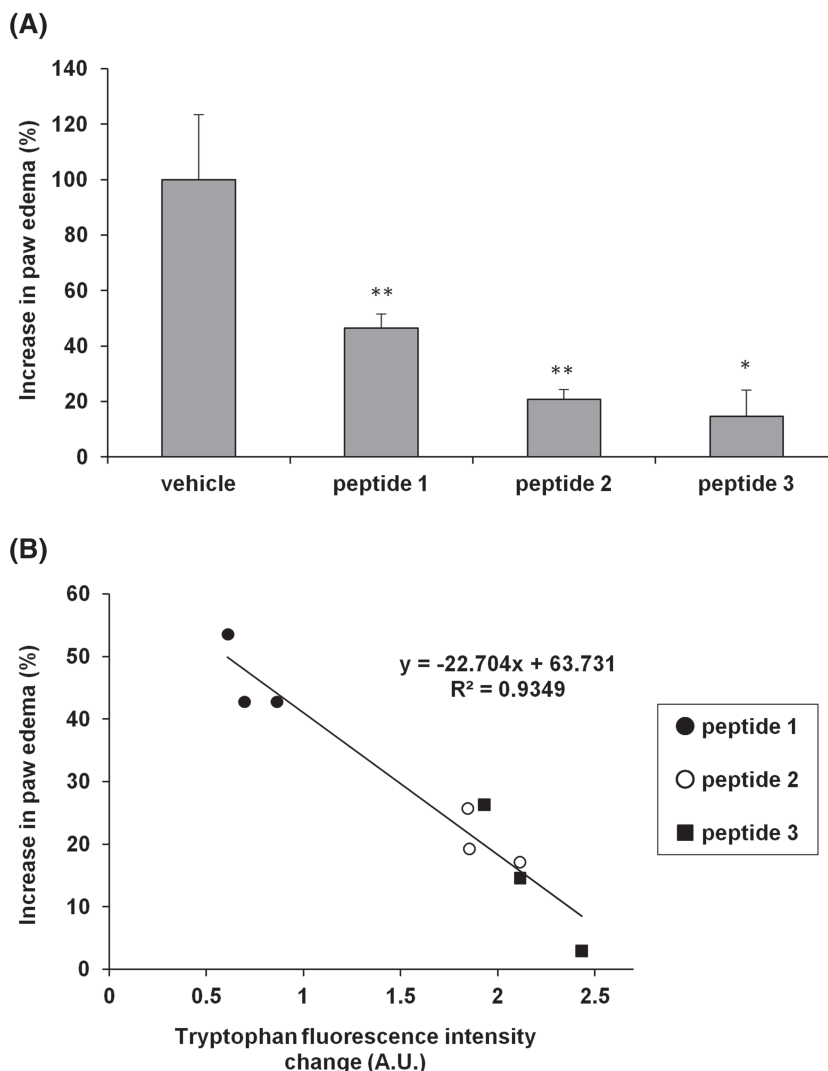
PAF is known to induce hypothermia during anaphylaxis. Here, we examined the inhibitory effects of biotinylated peptides on hypothermia in a mouse model of anaphylaxis (OVA-IgE mice). As shown in Figure 4, intraperitoneal administration of OVA (1.5 mg/mouse) significantly decreased rectal temperature in OVA-IgE mice. Intraperitoneal injection of peptide 2 or 3, both of which contain D-amino acid(s) (100 nmol/mouse), especially peptide 2, showed both marked and significant inhibition of hypothermia after administration. On the other hand, peptide 1, which consists of all L-amino acids (100 nmol/mouse), did not inhibit hypothermia. In contrast, none of

the peptides induced hypothermia. These results suggest that marked and sustainable inhibitory effects of peptides 2 and 3 on the anaphylactic hypothermia than peptide 1 may probably depend on high stability in vivo.

3.4 | In vitro plasma stability of biotinylated peptides

Generally, peptides, which consist of L-amino acids, cannot circulate in the blood for more than a few minutes due to enzymatic degradation. Here, the in vitro stability of biotinylated peptides in rat plasma was examined using Western blotting. As shown in Figure 5, peptide 3 has the highest stability in plasma; the percentage of unchanged peptide remaining after 6 h was $84.0 \pm 3.8\%$. Peptide 2 also showed high stability in plasma; the percentage of unchanged peptide remaining after 6 h was $51.8 \pm 20.4\%$. On the other hand, peptide 1 was rapidly degraded; the percentage of unchanged peptide remaining after 0.5 h was $16.0 \pm 6.2\%$. It did not completely remain after 6 h in the plasma. These results indicate that peptides 2 and 3, both partially made of D-amino acid(s), can have prolonged and marked inhibitory effects on anaphylactic hypothermia in vivo, owing to their higher stability in the plasma as compared to peptide 1, which is made of L-amino acids.

FIGURE 3 Effects of biotinylated peptides on PAF-induced edema. (A) Rats were treated by intraplantar injection of biotinylated peptides (10 nmol per paw) 15 min prior to intraplantar injection of PAF (1 nmol per paw). After 45 min, paw edema was quantified by measuring the increase in volume (ml). Each value represents the mean $\pm$ SD (%) of three to four rats, as compared to the increase by vehicle (peptide none). * $P < 0.05$ and ** $P < 0.01$, as compared to rats treated with vehicle (peptide none) (Student's *t* test). (B) The linear relationship between the inhibitory effects of biotinylated peptides on paw edema and the change in intrinsic tryptophan fluorescence intensity of biotinylated peptides in the presence of PAF



4 | DISCUSSION

We previously found the inhibitory effects of several Asp-hemolysin-related synthetic biotinylated peptides, all of which have a Tyr-Lys-Asp-Gly sequence that is essential for specific binding to PAF, on the bioactivities and anaphylactic hypothermia in vivo.^{14–17} However, these peptides synthesized from L-amino acids are rapidly degraded by peptidases in the circulation, whereas peptides synthesized from D-amino acids remain stable, as naturally occurring peptidases can degrade L-form peptide bonds but cannot degrade D-form peptide bonds.²² In the present study, a synthetic biotinylated heptapeptide, peptide 1, which consists of L-amino acids only and has a Tyr-Lys-Asp-Gly sequence, was rapidly degraded in rat plasma in vitro (Figure 5). Two biotinylated peptides, peptides 2 and 3, both having a Tyr-Lys-Asp-Gly sequence and partially D-amino acid(s), can show inhibitory effects on both the bioactivities of PAF and anaphylactic hypothermia with high in vivo stability in the plasma. This suggests that both peptides 2 and 3 may be useful as potent anti-anaphylactic agents targeting PAF (Figure 6). Furthermore, we previously reported that the inhibitory effect on the in vivo bioactivities of PAF of a

Tyr-Lys-Asp-Gly tetrapeptide synthesized from D-amino acids was weaker than that from L-amino acids.¹⁸ This indicates that a Tyr-Lys-Asp-Gly sequence consisting of L-amino acids, in peptides 2 and 3, is essential for the inhibition of PAF bioactivities.

PAF is a potent proinflammatory mediator involved in the pathophysiology of several allergic diseases, including asthma and anaphylaxis.¹⁰ PAF is biosynthesized by two distinct pathways: a remodeling or de novo pathway.^{23–26} Under inflammatory conditions, PAF is biosynthesized through a remodeling pathway in inflammatory cells, such as macrophages and neutrophils, in response to extracellular stimuli.²⁷ PAF acts by binding to its specific PAF receptor.^{7,9} PAF, histamine, and serotonin are known to be released during anaphylaxis and induce early phase anaphylaxis.^{4,28} In anaphylactic patients, serum PAF levels are significantly higher than in the control patients and inversely correlate with the activity of PAF-AH, which converts PAF to inactive lyso-PAF.¹¹ PAF and histamine increase vascular hyperpermeability; PAF, and, to a lesser extent, histamine, mainly mediates anaphylactic hypotension in mice.^{2,28,29} Previously, we found several synthetic biotinylated peptides having a Tyr-Lys-Asp-Gly sequence, such as BP4 and BP21, as PAF inhibitors.¹⁵ In the present study, we demonstrated

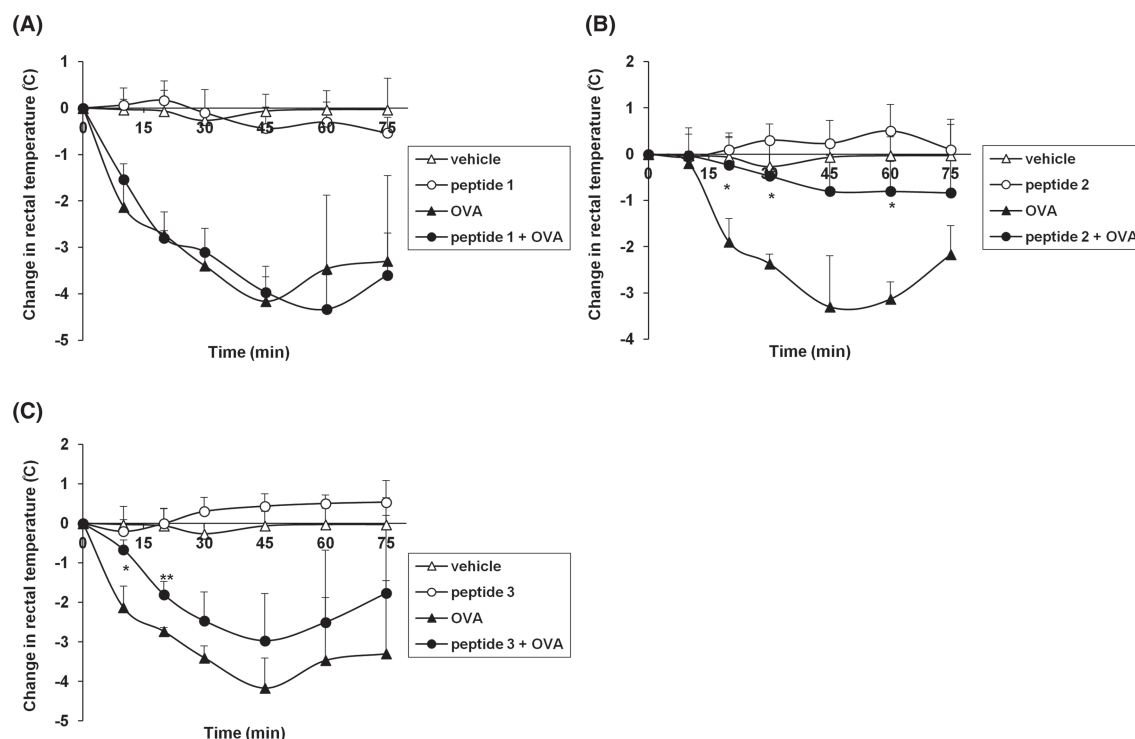


FIGURE 4 Effects of biotinylated peptides on hypothermia in a mouse model of anaphylaxis. OVA-IgE mice were injected intraperitoneally with OVA (1.5 mg/mouse) in the absence or presence of (A) peptide 1, (B) peptide 2, or (C) peptide 3 (each 100 nmol/mouse). All figures show the changes in rectal temperatures at 0–75 min after the injection. Each value represents the mean $\pm$ SD of changes in the temperatures of three to four mice. * P < 0.05 and ** P < 0.01 compared to mice treated with OVA alone (peptide none) (Student's t test)

the inhibitory effects of two biotinylated peptides consisting of partial D-amino acid(s), peptides 2 and 3, with high stability in plasma on PAF bioactivities and anaphylactic hypothermia in vivo. Moreover, we recently reported that one of the Asp-hemolysin-related biotinylated peptides, BP21, efficiently inhibits PAF-induced paw edema even at doses 150–300 times lower than doses of a known PAF antagonist, CV-3988,^{14,15} and also inhibits anaphylactic hypothermia in mice, even at doses 20 times lower than those of CV-3988.¹⁶ Therefore, both peptides 2 and 3 can be most useful as anti-anaphylactic agents targeting PAF among Asp-hemolysin-related biotinylated peptides (including BP21) that we previously found.

Recent studies have shown that 234 peptidases in the plasma have been identified and focused on exopeptidases: 34 aminopeptidases that hydrolyze the amino-terminal of peptides and 25 carboxypeptidases that hydrolyze the carboxy-terminal of peptides.³⁰ In addition, peptides made of partial D-amino acids are known to show strong resistance to proteolytic degradation.²² In the present study, peptide 1 was rapidly degraded by both aminopeptidases and carboxypeptidases. Peptide 2 can be degraded by carboxypeptidases, while peptide 3 is hardly degraded by peptidases; both amino-terminal and carboxy-terminal are composed of D-amino acids. Therefore, peptide 3 was more stable than peptide 2 in rat plasma (Figure 5). However, the inhibitory effect of peptide 3 on anaphylactic hypothermia in mice was lower than that of peptide 2 (Figure 4); therefore, further studies are needed to investigate these inhibitory effects in mice.

Moreover, peptides 2 and 3 markedly inhibited PAF-induced paw edema and anaphylactic hypothermia, whereas peptide 1 markedly inhibited PAF-induced edema in vivo (Figures 3 and 4). These findings indicate that both peptides 2 and 3 show systemic and local inhibitory effects, as compared to peptide 1, which shows only local inhibitory effects, on PAF-induced diseases such as anaphylaxis in vivo.

The intrinsic tryptophan fluorescence of peptides/proteins varies when they bind to ligands or undergo conformational changes; therefore, these changes could be representative of ligand binding to the peptides/proteins.^{20,21} In the present study, we analyzed the interaction of biotinylated peptides with PAF using tryptophan fluorescence spectroscopy (Figure 2). We found that both peptides 2 and 3 strongly interacted with PAF, as compared to peptide 1; all peptides used in this study were also found to recognize monomeric PAF selectively at concentrations below 3 μ mol/L because the CMC of PAF(C16) was calculated to be approximately 2.6 μ mol/L.¹⁶ In general, the tryptophan fluorescence intensity increases; the spectrum also causes a blue shift in the emission maximum wavelength (a shift toward shorter wavelengths) when tryptophan is exposed to a hydrophobic environment, such as PAF molecules. However, regardless of the presence of PAF at different concentrations, there was no shift in the maximal wavelength of the emission of the biotinylated peptides used in this study, thereby suggesting that a tryptophan residue in the peptides may not be involved in the peptide-PAF interaction. Therefore, a Tyr-Lys-Asp-Gly sequence in the peptides is essential for the peptide-PAF interaction.

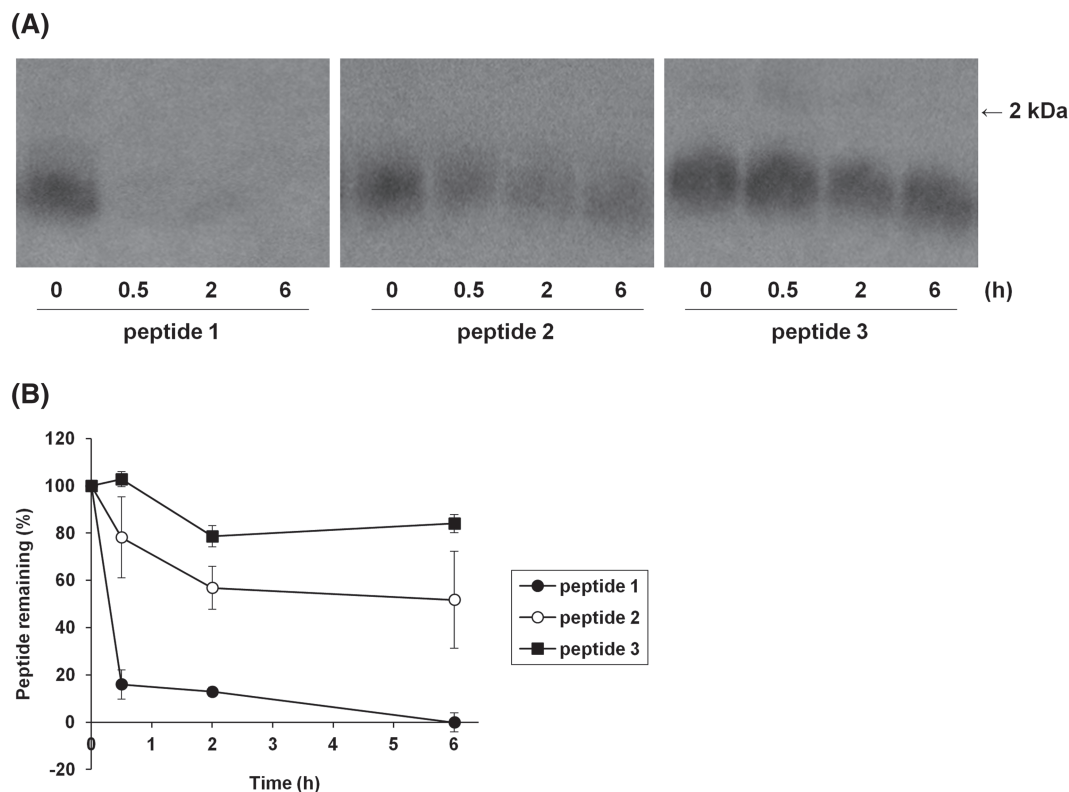


FIGURE 5 In vitro stability of biotinylated peptides in rat plasma. Each peptide (0.135 mmol/L, which corresponds to 0.15 mg/ml) was incubated with rat plasma. (A) The remaining unchanged peptides were analyzed using Western blotting. (B) Each value represents the mean $\pm$ SD (%) of three individual experiments

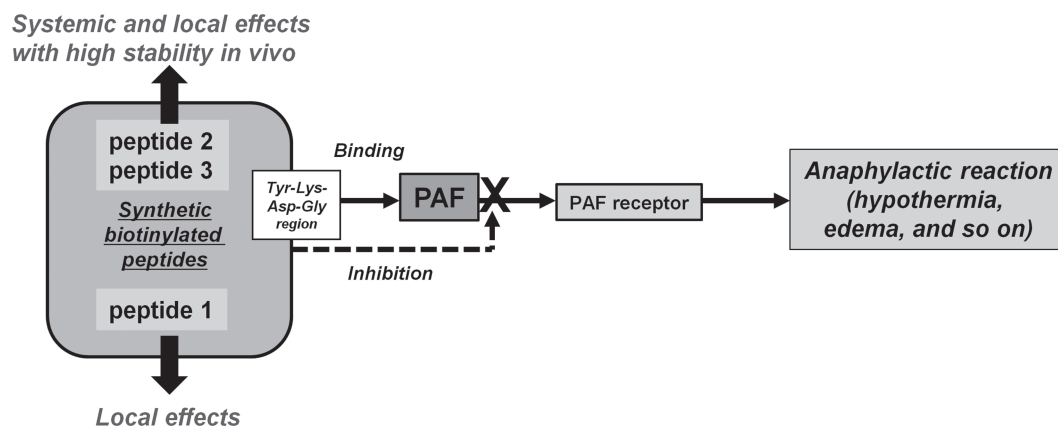


FIGURE 6 Schematic representation of the proposed mechanism of biotinylated peptide-mediated inhibition of anaphylactic reactions targeting PAF

In practice, there are some limitations to this study. First, although we conducted in the present study in vitro, the in vivo pharmacokinetics of biotinylated peptides remain unclear. In general, peptides/proteins can be rapidly metabolized and eliminated by the liver, degraded by peptidases in the plasma, and excreted into the urine, resulting in a short in vivo half-life. Second, in the present study, we examined the inhibitory effects of biotinylated peptides on

anaphylactic hypothermia in vivo; their effects on other systemic anaphylactic reactions such as hypotension and vascular permeability are also needed in vivo. We previously reported that one of the Asp-hemolysin-related biotinylated peptides, BP21, inhibits hypotension and vascular permeability in anaphylactic mice.^{16,17} Moreover, the effects of peptides on PAF-mediated diseases other than anaphylaxis, such as asthma, sepsis, and atherosclerosis, are needed. Therefore,

further studies are needed to investigate the in vivo pharmacokinetics of biotinylated peptides, the effects of the peptides on hypotension and vascular permeability using a mouse model of anaphylaxis, and the effects of biotinylated peptides in a model of experimental models of PAF-mediated diseases.

5 | CONCLUSIONS

The present study shows that two synthetic biotinylated peptides substituted with D-amino acids, peptides **2** and **3**, can be more useful as anti-anaphylactic agents targeting PAF with high stability in plasma in vivo, as compared to peptide **1**, which consists solely of L-amino acids. These findings should lead to the development of treatment strategies for PAF-induced allergic diseases, such as anaphylaxis.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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