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Microencapsulation and Modification of Synthetic Peptides of Food Proteins Reduces the Blood Pressure of Spontaneously Hypertensive Rats

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Synthetic peptides were microencapsulated into liposomes, cycled with a disulfide bond or modified with D-phenylglycine (D-phg) at the N-terminal, and their antihypertensive effects as orally administered (0.18 mM/kg body weight) to spontaneously hypertensive rats (SHR) were measured. The microencapsulated Leu-Lys-Pro reduced significantly the systolic blood pressures of SHR by 45 mmHg and showed a prolonged duration, revealing the significant protective effect of encapsulation. D-phg-Leu-Arg-Pro showed a duration about 2 h shorter than that of the peptide without modification. In addition, cyclic Leu-Arg-Pro peptide with a disulfide bond between the N- and C-terminal amino acids reduced the systolic blood pressure of SHR by 35 mmHg and displayed a lengthy duration.

KEYWORDS: Angiotensin-converting enzyme inhibitor; oligopeptide; liposome; modification; spontaneously hypertensive rats (SHR)

INTRODUCTION

Enzymatic hydrolysis of food proteins has produced various biologically active peptides with immunostimulating (*1*, *2*), opioid (*3*, *4*), antithrombotic (*5*), caseino-phosphopeptic (*6*, *7*), bactericidal (*8*), or angiotensin-converting enzyme (ACE) (dipeptidyl carboxypeptidase, EC 3.4.15.1) inhibitory (*9*, *10*) functions and has been the focus of recent research. ACE cleaves angiotensin I to the potent vasodepressor angiotensin II and releases C-terminal dipeptide (*11*). As a consequence, the angiotensin II causes the constriction of blood vessels in the renin−angiotensin system and the release of aldosterone from the adrenal cortex, which leads to an accumulation of sodium ions in the kidneys and increases the systolic blood pressure (*12*). In addition, ACE also hydrolyzes and inactivates bradykinin, a vasodilator, and increases the blood pressure (*13*). Therefore, development of substances that inhibit ACE activity has been a main interest in recent research (*14*, *15*). ACE inhibitors have been synthesized for clinical treatment of hypertension, such as Captopril, which exhibits potent competitive inhibition on ACE (*14*).

Cheung et al. (*16*) and Ariyoshi (*10*) pointed out that peptides with aromatic amino acids, such as tryptophan and phenylalanine, or proline on the C-terminal and valine or isoleucine on the N-terminal exhibited potent ACE inhibitory activity. Kohmura et al. (*17*) synthesized some peptide fragments of human β-casein and found that the length of those peptides had an influence on the ACE inhibitory activity. Namely, peptides composed of 3−10 amino acids with proline on the C-terminal were necessary for ACE inhibitors (*17*, *18*). Blackburn et al. (*19*) tested the synthetic peptides, Ser-Lys-Pro, Ser-Gln-Pro, and Gln-Ser-Pro, and pointed out the importance of the peptide sequence to the ACE inhibitory activity. Thus far, the peptide Leu-Arg-Pro from food protein hydrolysates has been reported to be the most potent natural ACE inhibitor, with an IC_{50} value of 0.27 (*17*) or 1.0 μM (*18*).

However, peptides orally administered to reduce blood pressure are liable to be digested and lose their activities. Microencapsulation using a spray-drying method is an industrially important process for physically coating solids, gases, and liquids with a thin, protective layer or wall of material to inhibit their loss by volatilization and to protect them against chemical deterioration (*22*, *23*). Other encapsulations, such as formation of liposomes, using phospholipids such as lecithin as lipid bilayers have been reported to be effective in encapsulating drugs and other bioactive substances (*24*, *25*). Since liposome is biodegradable (*26*), it is potentially useful as a drug delivery system that would transport desired drugs to a specific site (*27*, *28*).

Peptide modification is also one of the available approaches to retain peptide activity. Terada et al. (*29*) compared the

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proteolytic coefficients of linear and cyclic hexapeptides against pepsin and trypsin and concluded that cyclic formation of the peptide was beneficial to its stability. In addition, Wang et al. (*30*) pointed out the advantages of coupling D-phenylglycine (D-phg) to α-methyldopa in an investigation of the concentration dependence of the intestinal bruss-border membrane vesicle (BBMV) uptake of α-methyldopa. They found that the level of D-phg bound to α-methyldopa was linearly related ($r^2 = 0.99$) to the α-methyldopa uptake through the BBMV. In addition, the oral bioavailability of D-phg-α-methyldopa in rabbit blood was 83% when it was orally administered.

In the present research, to retain the activities of peptides in the gastrointestinal tract, peptides were encapsulated in liposomes or modified into cyclic form by a disulfide bond linked between N- and C-terminal amino acids. Subsequently, the changes in systolic blood pressure of spontaneously hypertensive rats (SHR) induced by different types of peptides were measured and compared with the change induced by a potent clinical ACE inhibitor, Captopril.

MATERIALS AND METHODS

Synthesis, Purification, and Mass Analysis of Oligopeptide. All the amino acids referred to in this paper were of L-configuration. The protected N-Fmoc-L-amino acids (Fmoc = fluoren-9-ylmethoxycarbonyl) and D-phg for the solid-phase synthesis (Instruction to Cleavage Techniques, Rainin Instrument Co., Inc., Mack Woburn, MA) were the products of ABI Co. (Foster City, CA). Two oligopeptides, Leu-Lys-Pro and Leu-Arg-Pro, cyclic Leu-Arg-Pro with a disulfide bond between Leu and Pro, and D-phg-Leu-Arg-Pro were synthesized with a PS-3 automated solid-phase peptide synthesizer (Rainin Instrument Co., Inc.) using preloaded proline as the resin (*17*).

After being cleaved from the solid support, the peptides were purified to homogeneity by reversed-phase high-performance liquid chromatography (HPLC) (S-3702 variable-wavelength UV−vis detector, Soma, Tokyo, Japan, with pump PU-980, Jasco, Tokyo, Japan) on a C_{18} column (250 mm in length and 4.6 mm in diameter) (Protein and Peptide C18, Vydac, Hesperia, CA) under the following experimental conditions: eluent A, 5.0% acetonitrile (ACN)/0.1% trifluroroacetic acid (TFA) aqueous solution; eluent B, 95% ACN/0.1% TFA aqueous solution. Purification conditions: 0−100% eluent B; flow rate, 1.0 mL/min; separation time, 20 min. All the solutions were filtered through a 0.45-μm membrane and then deaerated by sonication prior to use.

The purity of each synthetic peptide was further confirmed by mass spectrometric analysis (Finnigan MAT, LCQ, San Jose, CA) to reach a level higher than 95% (data not shown).

Determination of Peptide. The peptide concentration in the sample was determined according to the TNBS (2,4,6-trinitrobenzenesulfonic acid) method described by Adler-Nissen (*31*). Various levels (0.025, 0.0625, 0.125, and 0.25 mM) of L-leucine solutions were used to construct the standard curve ($r^2 = 0.98$) for the calibration of peptide concentration.

Assay of ACE Inhibitory Activity. The synthetic peptides were assayed in vitro for their capacity to inhibit the ACE activity with the method described by Cushman and Cheung (*12*) and Maruyama and Suzuki (*32*). To 300 μL of 5.0 mM hippuryl-L-histidyl-L-leucine (Sigma, St. Louis, MO)/300 mM NaCl/100 mM borate buffer solution (pH 8.3) was added 30 μL of peptide, and the mixture was then preincubated in a water bath at 37 ± 1 °C for 30 min. Next, 50 μL (8.0 mU) of ACE (Sigma)/300 mM NaCl/100 mM borate buffer solution (pH 8.3) was mixed with the above substrate solution to initiate the enzyme reaction. After incubation of the enzyme−substrate mixture in a water bath at 37 ± 1 °C for 30 min, 1 N hydrochloric acid was added to terminate the reaction. The liberated hippuric acid was extracted with 1.5 mL of ethyl acetate by vortex mixing for 15 s. After a brief centrifugation (3600*g*, 5 min), a 1.0-mL aliquot of each acetyl acetate layer was transferred to a test tube and then dried in a boiling water bath. Distilled water (1.0 mL) was added to dissolve the hippuric acid, and the absorbance of the solution at 228 nm was monitored by a spectrophotometer. Sample with hydrochloric acid added before mixing with ACE was used as a blank, while the peptide sample replaced by the same volume of distilled water was treated as a control. The inhibitory ratio (%) of ACE was calculated as $(C - A)/(C - B) \times 100\%$, where A is A_{228} of the inhibitor, B is A_{228} of the blank, and C is A_{228} of the control.

The concentration of the peptide sample in the reaction mixture with substrate was controlled to exhibit ACE inhibitory activity between 40 and 60% (*12*, *32*). Each peptide sample was tested at a minimum of three concentrations to construct the standard cure for the determination of the IC_{50} value (concentration of inhibitor required to inhibit 50% of the ACE activity). Each peptide sample was tested in triplicate.

Preparation of Liposome. Liposomes were prepared according to the method described by Shimizu et al. (*33*) and Chang et al. (*25*) with dehydration−rehydration processes. Initially, egg lecithin and cholesterol (Sigma) in a ratio of 1:0.25 (total lipid, 300 μmol) were dissolved in 5 mL of chloroform, and the mixtures were dried (50 ± 2 °C) under reduced pressure in round-bottom flasks with a rotary evaporator (model Re-111, Buchi, Flawil, Switzerland) to form a thin film on the flasks. Subsequently, 2 mL of distilled water was added to the flask, and the mixture was agitated with glass beads (0.5 cm in diameter) at 60 ± 2 °C for 5 min, followed by incubation at ambient temperature for 10 min. Next, 2 mL of Leu-Lys-Pro [5.0 mg/mL in 0.01 M phosphate buffer saline (PBS), pH 7.2] was thoroughly mixed with the above suspension and dehydrated in a freeze-dryer (Eyela FD-5N, Rikakikai Co., Tokyo, Japan). The freeze-dried material was rehydraded by adding 2 mL of distilled water, and the mixture was centrifuged (30000*g*, 30 min, 5 °C) after incubation at ambient temperature (25−28 °C) for 10 min. The supernatants thus obtained were sampled to determine the encapsulation efficiency (EE, %) (*33*) of the peptide by the TNBS method described above: EE (%) of peptide = [total peptide (mg) in solution − peptide (mg) in supernatant of liposomal suspension/total peptide (mg) in solution] × 100%. Each sample was tested in triplicate.

Antihypertensive Activity of Peptides. The effect of orally administered peptides on the systolic blood pressure was determined according to the method described by Maruyama and Suzuki (*32*) with minor modification. Twenty male spontaneously hypertensive rats (6 week of age) (Experimental Animal Center, National Taiwan University) were raised in an air-conditioned room (24 °C). All rats had free access to Lab Diet (5001 Rodent Diet, PMI, St. Louis, MO) and water. After being raised for eight more weeks, SHR were randomly divided into four groups (five rats/group) and warmed for 10 min in a 39 ± 1 °C thermostated box before each measurement of blood pressure. Tail systolic blood pressure was measured four times at each the desired time using a indirect blood pressure meter (BP-98-A meter, Softron Co. Ltd., Tokyo, Japan) for each treatment.

The tested peptides (Leu-Lys-Pro, Leu-Arg-Pro, cyclic Leu-Arg-Pro, and D-phg-Leu-Arg-Pro) were dissolved in 1.0 mM normal saline before administration. After being warmed up, rats were orally administered (0.18 mmol/kg bw) with peptides or normal saline (control). Tail systolic blood pressures were measured at 2-h intervals (0, 2, 4, 6, 8, 10, and 12 h) after the administration. Captopril [(2*S*)-*N*-(3-mercapto-2-methylpropionyl)-L-proline] (Sigma), a potent ACE inhibitor for clinical hypertension treatment, was used as a positive control (0.18 m mol/kg bw) in vivo for comparison with the synthetic peptides.

Statistical Analysis. Analysis of variances of results was carried out using the General Linear Model Procedure of SAS Statistical Software, version 6.11 (SAS Institute, Cary, NC, 1995) (*31*). Multiple comparisons of means were carried out by Duncan's multiple range tests at $p < 0.05$ (*) and $p < 0.01$ (**).

RESULTS AND DISCUSSION

IC_{50} Values of Synthetic Peptides. The potent ACE inhibitor Leu-Arg-Pro, with IC_{50} values of 0.27 and 1.0 μM, has previously been isolated from protein hydrolysates of α-zein (*20*) and fish (*21*), respectively. However, the synthetic Leu-Arg-Pro (**Table 1**) showed an IC_{50} value of about 1.17 μM, higher than the reported values. Variations in determining the IC_{50} value could be responsible for the difference. On the other

Table 1. IC_{50} Values[a] of Modified Oligopeptides[b] and Encapsulation Efficiency (EE)[c] of Leu-Lys-Pro in Liposome[d]

oligopeptide[b]	IC_{50} (μM)[a]	EE (%)[c]
Leu-Lys-Pro	0.87 ± 0.13	61.8 ± 2.7
Leu-Arg-Pro	1.17 ± 0.24	[e]
D-phg-Leu-Arg-Pro	4.55 ± 0.22	[e]

[a] Concentration of an inhibitor required to inhibit 50% of ACE activity. [b] See the methods in the text. [c] (Weight of added peptide − weight of uncapsulated peptide)/weight of added peptide × 100%. [d] Lecithin/cholesterol = 1:0.25; total lipid content in liposome was 300 μmol/mL; rehydration temperature 60 ± 2 °C. [e] Not determined.

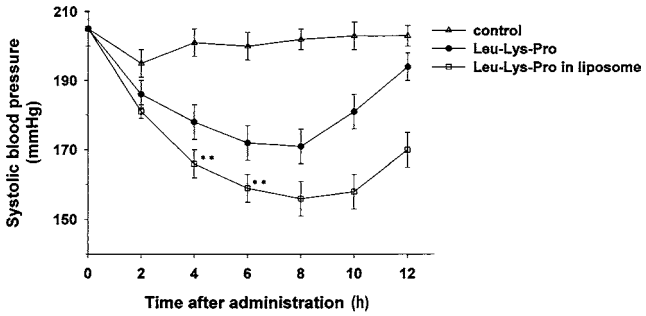

Figure 1. Antihypertensive effect of synthetic oligopeptides on spontaneously hypertensive rats ($p < 0.01$, **). Fourteen-week old rats were randomly divided into groups (five rats/group) and orally administered (0.18 mmol/kg bw). The control group received the same volume of normal saline.

hand, the synthetic Leu-Lye-Pro showed an IC_{50} value of 0.87 μM, similar to that for the protein separated from food proteins (*35*), revealing no diversity of bioactivity in synthetic and hydrolyzed proteins (*36*).

Antihypertensive Activity of Encapsulated Peptides. Leu-Lys-Pro was encapsulated in liposomes with an EE of about 62%. The EE of immunoglobulin in yolk in liposomes was reported to be 30% (*33*) and 70% (*25*), depending upon the level and composition of lipids as well as the methods used (*33*, *37*). An increase in freeze-drying−rehydration cycles was favorable for increasing EE (*25*, *38*).

Figure 1 represents the changes in tail systolic blood pressures of SHR orally administered (0.18 mmol/kg bw) Leu-Lys-Pro. It was observed that the blood pressure remarkably declined at the fourth hour after administration. The maximal reduction of blood pressure of SHR administered with this peptide without encapsulation was about 30 mmHg. However, the encapsulated peptide in liposomes exhibited more significant ($p < 0.01$, **) reduction effects on blood pressure at the fourth and sixth hours and, furthermore, showed a maximal reduction effect of 45 mmHg between the sixth and tenth hours after the administration. Therefore, it was clear that the cholesterol−lecithin bilayers of liposomal structure facilitated the peptide stability against proteases in vivo. Some similar results showed the efficiency of liposomal structures in protecting IgY specific against certain antigens or amylase against the pepsin hydrolysis in strong acidic pH conditions (*25*, *33*, *38*). In addition, emulsion formation of sardine hydrolysates by 30% egg yolk were also reported to be effective in enhancing the antihypertensive effect on SHR (*39*).

Antihypertensive Activity of Modified Peptides. The clinical ACE inhibitor, Captopril, exhibited a fast and potent antihypertensive effect, reducing the systolic blood pressure of SHR from about 200 to 160 mmHg at the second hour, and then maintained the blood pressure at about 170 mmHg from

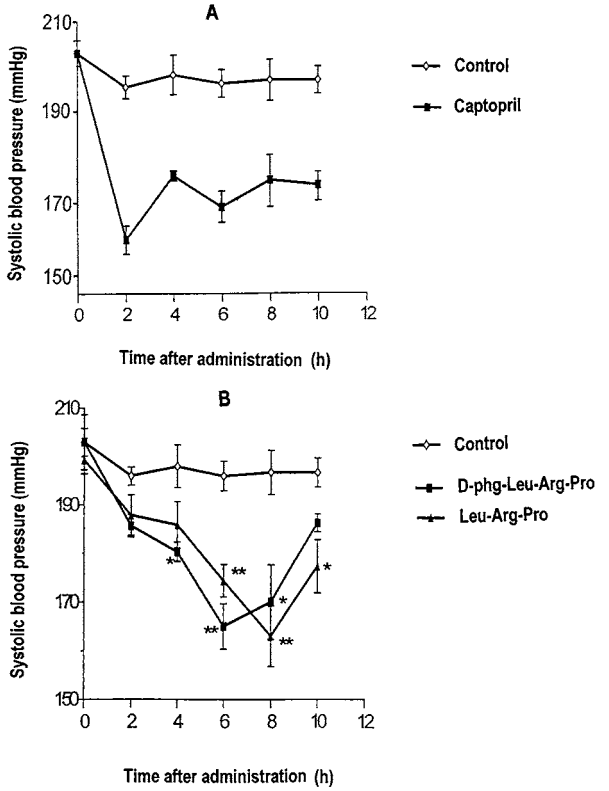

Figure 2. Changes in systolic blood pressure of spontaneously hypertensive rats orally administered (0.18 mmol/kg bw) with synthetic peptide ($p < 0.05$, *; $p < 0.01$, **). Captopril was used as the positive control. The control group received the same volume of normal saline.

the fourth to the tenth hours after oral administration (**Figure 2A**). However, Leu-Arg-Pro peptide reduced the blood pressure by about 15 mmHg at the fourth hour and showed a maximal reduction effect of about 35 mmHg at the eighth hour after oral administration. In contrast, the D-phg-Leu-Arg-Pro peptide showed a significant ($p < 0.05$, *) reduction effect (20 mmHg) of blood pressure at the fourth hour and reached the maximal reduction effect of about 35 mmHg ($p < 0.01$, **) at the sixth hour after oral administration. It was observed that the duration of this modified peptide was about 2 h shorter than that of the unmodified peptide (**Figure 2B**). Therefore, the D-phg group appeared to facilitate the absorption of the modified peptide, as it did in increasing the uptake and bioactivity of α-methyldopa through the BBMV of rabbits (*30*). D-phg has been used as a "seeing-eye dog" and, coupled with drugs with amino acid structure, as a type of prodrug, which is hydrolyzed to release drug after absorption in small intestine through the oligopeptide transporter system (*30*).

Figure 3 shows the reduction profile of blood pressure of SHR induced by cyclic Leu-Arg-Pro peptide. It was obvious that the maximal reduction effect of blood pressure induced by this cyclic peptide was about 35 mmHg, similar to that induced by linear peptide; however, a prolonged duration of modified peptide was observed. Terada et al. (*29*) indicated that the cyclic hexapeptide (-Gly_2-Phe_2-Gly-Lys-) was not hydrolyzed at all by pepsin at 37 °C and at pH values between 1.5 and 4.0. However, it showed a proteolytic coefficient as low as 0.015−0.025 when incubated with 2.0 mg of trypsin/mL at 30 °C and pH values between 7.0 and 9.0. In the present study, Leu-Arg-Pro peptide was cycled with a disulfide bond between N- and C-terminal amino acids and displayed a marked improvement in duration, revealing the stability of the cyclic peptide (**Figure 3**).

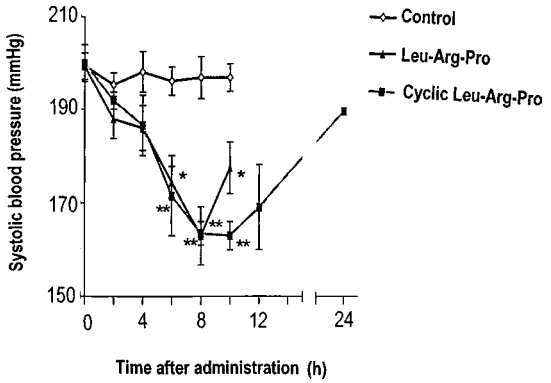

Figure 3. Influence of orally administered (0.18 mmol/kg bw) cyclic Leu-Arg-Pro on the systolic blood pressure of spontaneously hypertensive rats ($p < 0.05$, *; $p < 0.01$, **). Cyclic peptide was linked with a disulfide bond between N- and C-terminal amino acids. The control group received the same volume of normal saline.

In conclusion, potent ACE inhibitors of food protein hydrolysates were synthesized and encapsulated or modified to enhance their antihypertensive activity. Results in vivo showed the efficiency of those treatments. It was noteworthy that the encapsulation of peptides by liposome formation was significantly effective in increasing the maximal effect of reducing blood pressure.

ABBREVIATIONS USED

SHR, spontaneously hypertensive rat; ACEI, angiotensin-converting enzyme inhibitor; Leu, leucine; Lys, lysine; Pro, proline; Arg, arginine; D-phg, D-phenylglycine.

LITERATURE CITED

(1) Parker, F.; Migliore-Samour, O.; Floch, F.; Zerial, A.; Werner, G. H.; Jolles, J.; Casaretto, M.; Zahn, H.; Jolles, P. Immunostimulating hexapeptide from human case in amino acid sequence, synthesis and biological properties. *Eur. J. Biochem.* **1984**, *145*, 677−682.

(2) Fiat, A. M.; Daniele, M. S.; Pierre, J. Biological active peptides from milk proteins with emphasis on two examples concerning antithrombotic and immunomodulating activities. *J. Dairy Sci.* **1993**, *76*, 301−310.

(3) Zioudrou, C.; Streaty, R. A.; Klee, W. A. Opioid peptides derived from food proteins. *J. Biol. Chem.* **1979**, *254*, 2446−2449.

(4) Ramabadran, K.; Bansinath, M. Pharmacology of β-casomorphins, opioid peptides derived from milk protein. *Asia Pac. J. Pharmacol.* **1989**, *4*, 45−49.

(5) Scarborough, R. H.; Rose, J. W.; Hsu, M. H.; Phillips, D. R.; Fried, V. A.; Campbell, A. M.; Manniaai, L.; Charo, I. F. Barbourin AGpIIb-IIIa specific integrin antagonist from the venom of sistrurus M, Barbouri. *J. Biol. Chem.* **1991**, *266*, 9359−9360.

(6) Maubois, J. L.; Leonil, J. Peptides du lait a activite biologique. *Lait* **1989**, *69*, 245−269.

(7) Fox, P. F.; Mulvihill, D. M. Developments in milk protein processing. *Food Sci. Technol. Today* **1993**, *7*, 152−161.

(8) Bellamy, W. R.; Wakabayashi, H.; Takase, M.; Kawase, K.; Shimamura, S.; Tomita, M. Role of cell-binding in the antibacterial mechanism of Lactoferricin B. *J. Appl. Bacteriol.* **1993**, *75*, 478−484.

(9) Ehlers, M. R.; Riordan, J. F. Angiotensin-converting enzyme new concepts converting its biological role. *Biochemistry* **1989**, *28*, 5311−5317.

(10) Ariyoshi, Y. Angiotensin-converting enzyme inhibitors derived from food proteins. *Trends Food Sci. Technol.* **1993**, *4*, 139−143.

(11) Byers, L. D.; Wolfenden, R. Binding of the by-product analog benzylsuccinic acid by carboxypeptidase A. *Biochemistry* **1973**, *12*, 1070−2078.

(12) Cushman, D. W.; Cheung, H. S. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochem. Phamacol.* **1971**, *20*, 1637−1648.

(13) Studdy, P. R.; Lapworth, R.; Bird, R. Angiotension-converting enzyme and its clinical significance—a review. *J. Clin. Pathol.* **1983**, *36*, 938−947.

(14) Salvetti, A. Newer ACE inhibitors. A look at the future. *Drugs* **1990**, *40*, 800−826.

(15) Yamamoto, N. Antihypertensive peptides derived from food proteins. *Biopolymers* **1997**, *43*, 129−134.

(16) Cheung, H. S.; Wang, F. L.; Ondetti, M. A.; Sabo, E. F.; Cushman, D. W. Binding of peptide substrates and inhibitors of angiotensin-converting enzyme. *J. Biol. Chem.* **1980**, *255*, 401−407.

(17) Kohmura, M.; Nio, N.; Kubo, K.; Minoshima, Y.; Munekata, E.; Ariyoshi, Y. Inhibition of angiotensin-converting enzyme by synthetic peptide fragments of human β-casein. *Agric. Biol. Chem.* **1989**, *53*, 2107−2114.

(18) Kohmura, M.; Nio, N.; Ariyoshi, Y. Inhibition of angiotensin-converting enzyme by synthetic peptide fragments of human κ-casein. *Agric. Biol. Chem.* **1990**, *54*, 835−836.

(19) Blackburn, C.; Pingali, A.; Kehoe, T.; Herman, H. W.; Kates, S. A. Libraries of angiotensin converting enzyme inhibitors: Solid-phase synthesis and affinity selection. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 823−826.

(20) Miyoshi, S.; Kaneko, T.; Yoskizawa, Y.; Fukui, F.; Tananka, H.; Maruyama, S. Hypertensive activity of enzymatic α-zein hydrolysate. *Agric. Biol. Chem.* **1991**, *5*, 1407−1408.

(21) Matsumura, N.; Fujii, M.; Takeda, Y.; Shimizu, T. Angiotensin I-converting enzyme inhibitory peptides derived from bonito bowels autolysate. *Biosci. Biotech. Biochem.* **1993**, *57*, 1743−1744.

(22) Balassa, L. L.; Fanger, G. O. Microencapsulation in the food industry. *CRC Crit. Rev. Food Technol.* **1971**, *7*, 245−261.

(23) Rosenberg, M.; Young, S. L. Whey proteins as microencapsulating agents. Microencapsulation of anhydrous milkfat—structure evaluation. *Food Struct.* **1993**, *12*, 31−41.

(24) Koide, K.; Karel, M. Encapsulation and stimulated release of enzymes using lecithin vesicles. *Int. J. Food Sci. Technol.* **1987**, *22*, 707−723.

(25) Chang, H. M.; Lee, Y. C.; Chen, C. C.; Tu, Y. Y. Microencapsulation protects immunoglobulin in yolk (IgY) specific against *Helicobactor pylori* urease. *J. Food Sci.* **2002**, *67*, 15−20.

(26) Rustum, Y. M.; Dave, C.; Mayhew, E.; Papahadjopoulos, D. Role of liposome type and route of administration in the antitumor activity of liposome-entrapped 1-β-D-arabinofuranosylcytosine against mouse L1210 leukemia. *Cancer Res.* **1979**, *39*, 1390−1395.

(27) Gregoriadis, G. *Liposome Technology*; CRC Press: Boca Roton, FL, 1984; Vol. 3, pp 1−282.

(28) Kikuchi, H.; Inoue, K. Liposomes: properties and applications. *Yukagaku* **1985**, *34*, 784−798.

(29) Terada, S.; Kato, T.; Izumiya, N. Synthesis and hydrolysis by pepsin and trypsin of a cyclic hexapeptide containing lysin and phenylalanine. *Eur. J. Biochem.* **1975**, *52*, 273−282.

(30) Wang, H. P.; Lu, H. H.; Lee, J. S.; Cheng, C. Y.; Mah, J. R.; Ku, C. Y.; Hsu, W.; Yen, C. F.; Lin, C. J.; Kuo, H. S. Intestinal absorption studies on peptide mimetic alpha-methyldopa prodrugs. *J. Pharm. Pharmacol.* **1996**, *48*, 271−278.

(31) Adler-Nissen, J. Control of the proteolytic reaction and of the level of bitterness in protein hydrolysis processes. *J. Chem. Tech. Biotechnol.* **1984**, *34*, 215−222.

(32) Maruyama, S.; Suzuki, H. A peptide inhibitor of angiotensin I converting enzyme in the tryptic hydrolysate of casein. *Agric. Biol. Chem.* **1982**, *46*, 1393−1394.

(33) Shimizu, M.; Miwa, Y.; Hashimoto, K.; Goto, A. Encapsulation of egg yolk immunoglobulin G (IgY) by liposomes. *Biosci. Biotech. Biochem.* **1993**, *57*, 1445−1449.

(34) SAS Institute, Inc. *SAS User's Guide. Statistics*, Version 6.11; SAS Institute, Inc.: Cary, NC, 1995.
(35) Fujita, H.; Yoshikawa, M. LKPM: a prodrug-type ACE-inhibitory peptide derived from fish protein. *Immunopharmacology* **1999**, *44*, 123−127.
(36) Maruyama, S.; Miyoshi, S.; Tanaka, H. Angiotensin I-converting enzyme inhibitors derived from *Ficus carica*. *Agric. Biol. Chem.* **1989**, *53*, 2763−2767.
(37) Szoka, F. C.; Papahadjopoulos, D. Comparative properties and methods of preparation of lipid vesicle-liposomes. *Annu. Rev. Biophys. Bioeng.* **1978**, *9*, 467−508.
(38) Hsieh, Y. F.; Chen, T. L.; Wang, Y. T.; Chang, J. H.; Chang, H. M. Properties of liposomes prepared with various lipids. *J. Food Sci*. **2002**, *67*, 2808−2813.
(39) Suetsuna, K.; Osajima, K. Blood pressure reduction and vasodilatory effects in vivo of peptides originating from sardine muscle. *Nihon Eiyo Shokuryo Gakkaishi* **1989**, *42*, 47−54.

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