International Journal of Food Sciences and Nutrition,
February 2010; 61(1): 52–60

informa
healthcare

Hydroxyproline-containing dipeptides and tripeptides quantified at high concentration in human blood after oral administration of gelatin hydrolysate

SATOMI ICHIKAWA[1], MASASHI MORIFUJI[1], HIROKI OHARA[1], HITOSHI MATSUMOTO[1], YASUO TAKEUCHI[1] & KENJI SATO[2]

[1]*Food and Health R&D Laboratories, Meiji Seika Kaisha, Ltd, Sakado-shi, Saitama, Japan, and,* [2]*Graduate School of Life and Environment Sciences, Kyoto Prefectural University, Kyoto, Japan*

Int J Food Sci Nutr Downloaded from informahealthcare.com by Universite De Sherbrooke on 06/23/14
For personal use only.

Abstract
Several hydroxyproline (Hyp)-containing food-derived collagen peptides were identified in human blood after oral ingestion of gelatin hydrolysates. However, these types of peptides were not quantified in human plasma. In this report, a sensitive LC-MS/MS method was introduced for simultaneous quantitative analysis of Hyp-containing peptides. All peptide concentrations were determined accurately, with all coefficients of determination (r^2) >0.999. The method achieved detection and quantification limits of 0.01 pmol/ml and 12.5–1,000 pmol/ml in plasma, respectively. Concentrations were quantified for nine Hyp-containing peptides in human plasma by this method, identifying Pro-Hyp (C_{max} = 60.65 ± 5.74 nmol/ml) as the major constituent of food-derived collagen peptides, while the minor components were Ala-Hyp-Gly, Ser-Hyp-Gly, Ala-Hyp, Phe-Hyp, Leu-Hyp, Ile-Hyp, Gly-Pro-Hyp, and Pro-Hyp-Gly (C_{max} from 23.84 to 0.67 nmol/ml). Thus a total of nine Hyp-containing peptides in human plasma were successfully quantified by this approach. The concentration of Hyp-containing peptides is substantially higher than that following oral administration of other peptides.

Keywords: *Collagen, hydroxyproline, plasma, hydroxyproline-containing peptide, quantification*

Introduction

Collagen is a major constituent of connective tissues of animals, birds, and fish. Gelatin, a denatured form of collagen, is prepared on an industrial scale from these animals (Shrieber and Seybold 1993). Collagen has a unique triple helix configuration with a repeating sequence $(Gly\text{-}X\text{-}Y)_n$, with X and Y being mostly proline and hydroxyproline (Hyp) (Ramshaw and Shah 1998; Bos et al. 1999). Gelatin-based food derivatives obtained from animals, especially fish and pigs, have been attracting worldwide attention as health-food ingredients. Significant amounts of Hyp-containing peptides were found to be present in the peripheral blood of human volunteers after oral ingestion of porcine skin gelatin hydrolysates (Iwai et al. 2005). Recently, some Hyp-containing peptides were also detected in human blood after ingestion of hydrolysate from fish scales (Ohara et al. 2007a). The major constituents of Hyp-containing

Corespondence: Hiroki Ohara, PhD, Food and Health R&D Laboratories, Meiji Seika Kaisha, Ltd, 5-3-1 Chiyoda, Sakado-shi, Saitama 350-0289, Japan. Tel: 81 49 284 7569. Fax: 81 49 284 7598. E-mail: hiroki_ohara@meiji.co.jp

ISSN 0963-7486 print/ISSN 1465-3478 online © 2010 Informa UK Ltd
DOI: 10.3109/09637480903257711

peptides that remained in the blood were identified as Ala-Hyp, Pro-Hyp, Ala-Hyp-Gly, Ser-Hyp-Gly, Phe-Hyp, Pro-Hyp-Gly, Gly-Pro-Hyp, Ile-Hyp and Leu-Hyp. These collagen-based peptides represent functional peptides involved in various physiological activities. For example, Pro-Hyp and Gly-Pro-Hyp exert chemotactic effects on fibroblasts, peripheral blood neutrophils (Postlethwaite et al. 1978; Laskin et al. 1986) and monocytes (Postlethwaite and King 1976) in cell culture systems. Gly-Pro-Hyp is also suggested to be involved in platelet aggregation (Knight et al. 1999). Recently, Shigemura et al. (2009) indicated that Pro-Hyp enhanced mice fibroblast cell proliferation. Therefore, it could be assumed that food-derived collagen peptides in blood may be involved in some of the biological activities suggested by animal and human experiments.

However, no quantitative analysis of peptides has been reported in an earlier human absorption study. Previous methods to quantify these types of peptides involved subtraction of free Hyp and Hyp-containing peptide concentrations after determining the Hyp concentration in plasma using reverse-phase high-performance liquid chromatography (Iwai et al. 2005; Aito-Inoue et al. 2006; Ohara et al. 2007a). Thus, quantification of food-derived Hyp-containing peptides has been evaluated by semi-quantitative methods such as determining the recovery of Hyp in each peptide peak. Moreover, it has been difficult to detect and isolate small amounts of food-derived peptides that do not have any marker amino acids or modified amino acids from animal and human blood after oral ingestion.

To overcome this problem, a sensitive and convenient liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS) method was introduced for simultaneous analysis of Hyp-containing peptides in human plasma after oral ingestion of fish-scale gelatin hydrolysate. Recently, digested mixtures of collagen type II and type I containing many specific peptides and common peptides were analyzed by MS/MS sequencing (Zhang et al. 2006). However, only tetrapeptides to nonapeptides were analyzed to define the collagen type, whereas specific dipeptides and tripeptides from human blood samples were not analyzed.

The goal of the present study was to quantify food-derived Hyp-containing peptides in a complex matrix such as human plasma.

Int J Food Sci Nutr Downloaded from informahealthcare.com by Universite De Sherbrooke on 06/23/14
For personal use only.

Materials and methods

Gelatin hydrolysate

Enzymatic hydrolysate of fish-scale gelatin was a kind gift from Nitta Gelatin, Ltd (Osaka, Japan). This preparation was of food grade and it can be obtained commercially. The average molecular weight of peptides in this gelatin hydrolysate, which did not contain the free form of Hyp, was about 5,000 Da.

Chemicals

Acetonitrile (high-performance liquid chromatography grade), pentafluoropropionic acid, and trichloroacetic acid were purchased from Wako Pure Chemical Industries (Osaka, Japan). Ala-Hyp, Ala-Hyp-Gly, Ser-Hyp-Gly, Pro-Hyp-Gly, Ile-Hyp, Leu-Hyp, and Phe-Hyp were purchased from Kokusan Chemical (Tokyo, Japan), and Pro-Hyp and Gly-Pro-Hyp were purchased from Bachem (Bubendort, Germany).

Preparation of standard samples

Standards prepared for nine Hyp-containing peptides (Ala-Hyp, Pro-Hyp, Ala-Hyp-Gly, Ser-Hyp-Gly, Phe-Hyp, Pro-Hyp-Gly, Gly-Pro-Hyp, Ile-Hyp and Leu-Hyp) were dissolved in blank human plasma or water, mixed and diluted to 1 nmol/ml, 5 nmol/ml, 10 nmol/ml, 25 nmol/ml, 50 nmol/ml and 100 nmol/ml. They were then mixed with equal amounts of 5% (w/v) trichloroacetic acid. After filtration with a 4-mm, 0.22-μm PVDF filter (Millipore, Bedford, MA, USA), 5 μl of the resulting filtrate was injected into the LC-MS/MS system.

Human study design

The present study was performed according to the Helsinki Declaration and was approved by the Ethical Committee of Meiji Seika Kaisha, Ltd, Food and Health R&D Laboratories. Five healthy male volunteers with no incidence of gelatin allergy (33.0 ± 5.6 years old and 69.8 ± 7.4 kg body weight) participated in the study. Subjects did not consume any food or beverages except for water in the 12-h period prior to the experiment. On the morning of the experiment, the subjects were fasting and each subject orally ingested the fish-scale gelatin hydrolysate concentrate (0.385 g/kg body weight) in water (20% w/v). Three hours after ingestion of the gelatin hydrolysate preparation, the subjects were served a collagen-free lunch, consisting of only a rice ball with salt. Approximately 5 ml venous blood was collected from the cubital vein before (0 h) and 0.5 h, 1 h, 2 h, 4 h, and 7 h after ingesting the hydrolysate. Plasma was obtained after blood centrifugation at 880 x *g* for 10 min at 4°C and stored in tubes at −80°C until analysis was performed.

Pre-treatment of blood sample for LC-MS/MS

The plasma was de-proteinized by adding equal amounts of 5% (w/v) trichloroacetic acid. The supernatant was then centrifuged at 14,010 x *g* for 10 min at 4°C. After filtering through a 4-mm, 0.22-μm PVDF filter, 5 μl of the resulting filtrate was injected into the LC-MS/MS system.

LC-MS/MS analysis

Samples were analyzed by LC-MS/MS. The LC analysis was performed using an ACQUITY UPLC system (Waters, Milford, MA, USA). A particular Octa Decyl Sillica (ODS) column that retains polar compounds tightly was better adapted to this analysis than the conventional ODS column that was used previously. Therefore an ACQUITY UPLC HSS T3 column (2.1 x 50 mm, 1.7 μm; Waters) was used for the separation. Gradient elution was carried out with 0.05% (v/v) pentafluoropropionic acid and acetonitrile at a constant flow rate of 0.3 ml/min. The gradient profile with the following proportions (v/v) of acetonitrile was applied (*t* (min), % acetonitrile): (0 min, 0%), (4 min, 0%), (9 min, 25%), (9.01 min, 80%), (10 min, 80%) (3 min: time was required to reach initial conditions). The column temperature was maintained at 40°C. The Quattro Premier XE tandem quadrupole mass spectrometer was used in positive ion electrospray mode. The ion source was operated at 120°C with a capillary voltage of 3.5 kV. Nitrogen was employed for the desolvation gas at 400°C and 850 l/h. The mode of acquisition was multiple reaction monitoring (MRM) at an argon collision gas pressure of 5.0 x 10^{-3} mbar. The list of peptides and the MRM transitions, along with

Int J Food Sci Nutr Downloaded from informahealthcare.com by Universite De Sherbrooke on 06/23/14
For personal use only.

Table I. MRM method parameters.

Peptide	Retention time (min)	MRM transition
Ala-Hyp	1.7	203.3 > 132.1
Pro-Hyp	2.7	229.2 > 70.2
Ala-Hyp-Gly	2.1	260.3 > 189.0
Ser-Hyp-Gly	1.9	276.3 > 189.1
Phe-Hyp	7.8	279.3 > 119.9
Pro-Hyp-Gly	4.0	286.3 > 189.0
Gly-Pro-Hyp	5.6	286.3 > 154.7
Ile-Hyp	7.0	245.3 > 131.9
Leu-Hyp	7.2	245.3 > 131.9

Cone voltage: Pro-Hyp, 25 V; others, 20 V. Collision energy, 15 eV.

Int J Food Sci Nutr Downloaded from informahealthcare.com by Universite De Sherbrooke on 06/23/14
For personal use only.

the retention times, cone voltages, and collision energies for the method, are presented in Table I. The data were acquired using MassLynx Software version 4.1 (Waters) and were processed using the TargetLynx application manager.

Pharmacokinetic analysis

Analysis of blood concentration–time data was carried out with a non-compartment model using WinNonlin Professional (version 5.2.1; Pharsight Co., Mountain View, CA, USA). The total area under the concentration–time curve ($AUC_{0-7\ h}$) was calculated by the trapezoidal rule based on the plasma concentrations up to the time of final measurement using the WinNonlin Professional program.

Results

Analysis of standards

Figure 1 shows typical MRM chromatograms of the nine Hyp-containing peptide standards. The total run-time per sample was only 13 min. The sensitivity of the method was evaluated by determining the limit of detection (LOD) and the limit of quantification (LOQ). The LOD was defined as the concentration of the nine Hyp-containing peptides with a signal-to-noise ratio of 3, for the chromatographic peaks from 0.01 pmol/ml to 100 nmol/ml, stepwise. The LOQ was the lowest standard concentration with a signal-to-noise ratio of 10. The LOD and LOQ for a 5 µl injection, coefficients of determination and recovery for each of the nine Hyp-containing peptides in plasma are presented in Table II. The method achieved detection and quantification limits of 0.01 pmol/ml and 12.5–1,000 pmol/ml in plasma, respectively. The LOQ was as follows: Ala-Hyp, 225 pmol/ml; Ser-Hyp-Gly, 125 pmol/ml; Ala-Hyp-Gly, 200 pmol/ml; Pro-Hyp, 1000 pmol/ml; Pro-Hyp-Gly, 125 pmol/ml; Gly-Pro-Hyp, 75 pmol/ml; Ile-Hyp, 50 pmol/ml; Leu-Hyp, 12.5 nmol/ml; and Phe-Hyp; 150 pmol/ml.

The linearity of the method was investigated by spiking blank human plasma (obtained before collagen ingestion) with known concentrations of the nine Hyp-containing peptides at six concentration levels ranging from 1 to 100 nmol/ml. The linearity of measurement over the calibration curve range was good for all peptides measured, and all coefficients of determination (r^2) were >0.999. Furthermore, the recovery of standards added to blank human plasma (obtained before collagen ingestion) was investigated with 25 nmol/ml of the nine Hyp-containing peptides, and their recovery rates were 97–100%

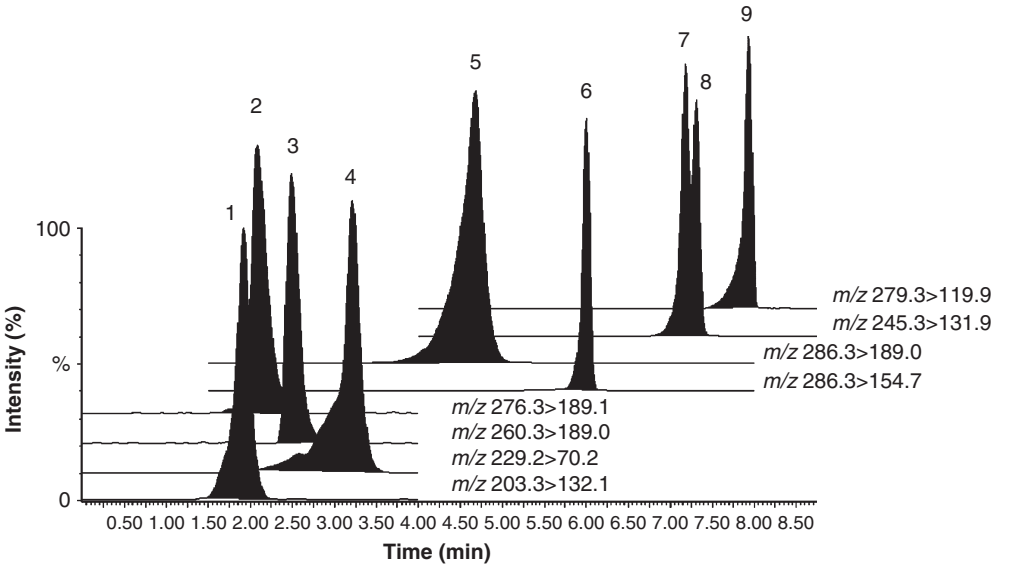

Figure 1. MRM chromatogram of nine Hyp-containing peptides. Peak 1, Ala-Hyp (*m/z* 203.3 > 132.1); peak 2, Ser-Hyp-Gly (*m/z* 276.3 > 189.1); peak 3, Ala-Hyp-Gly (*m/z* 260.3 > 189.0); peak 4, Pro-Hyp (*m/z* 229.2 > 70.2); peak 5, Pro-Hyp-Gly (*m/z* 286.3 > 189.0); peak 6, Gly-Pro-Hyp (*m/z* 286.3 > 154.7); peak 7, Ile-Hyp (*m/z* 245.3 > 131.9); peak 8, Leu-Hyp (*m/z* 245.3 > 131.9); peak 9, Phe-Hyp (*m/z* 279.3 > 119.9).

(Table II). In addition, other concentrations of the nine Hyp-containing peptides at 1 nmol/ml, 5 nmol/ml, 10 nmol/ml, 50 nmol/ml and 100 nmol/ml were investigated. Their recovery rates were 94–107% (data not shown). Therefore, this method is adequate to detect these nine Hyp-containing peptides.

Levels of nine Hyp-containing peptides in human plasma

Figure 2 shows the amounts of the nine Hyp-containing peptides in human plasma after oral ingestion of fish-scale gelatin hydrolysate. Only negligible amounts of each peptide were observed before the ingestion of fish-scale gelatin hydrolysate. In all subjects, the nine Hyp-containing peptides in the plasma increased after oral ingestion and reached a

Table II. Correlation coefficient, recovery, limit of quantification, and detection data obtained from LC-MS/MS analysis of nine Hyp-containing peptides in human plasma (n = 6).

Peptide	Correlation coefficient	Percentage recovery (% relative standard deviation)	LOQ (pmol/ml)	LOD (pmol/ml)
Ala-Hyp	0.999	100 (2)	225	0.01
Ser-Hyp-Gly	0.999	99 (1)	125	0.01
Ala-Hyp-Gly	0.999	99 (3)	200	0.01
Pro-Hyp	0.999	100 (5)	1,000	0.01
Pro-Hyp-Gly	0.999	98 (4)	125	0.01
Gly-Pro-Hyp	0.999	99 (2)	75	0.01
Ile-Hyp	0.999	97 (1)	50	0.01
Leu-Hyp	0.999	99 (1)	12.5	0.01
Phe-Hyp	0.999	99 (3)	150	0.01

Int J Food Sci Nutr Downloaded from informahealthcare.com by Universite De Sherbrooke on 06/23/14
For personal use only.

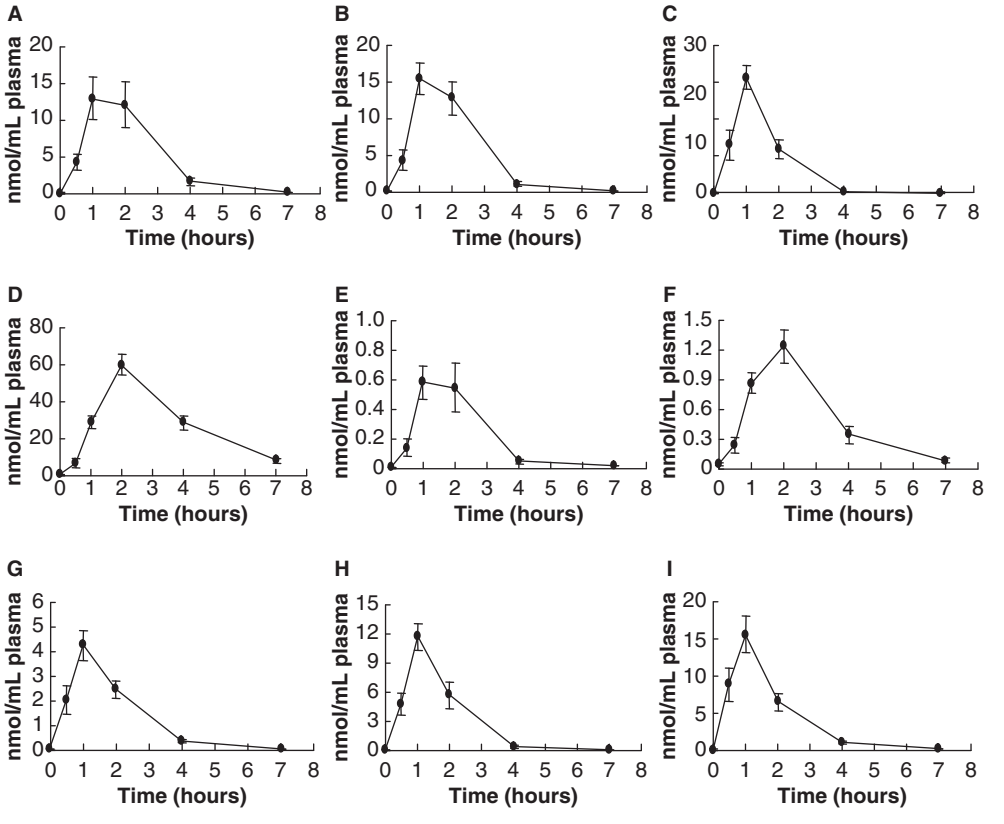

Figure 2. Plasma levels of nine Hyp-containing peptides after oral ingestion of fish-scale gelatin hydrolysate. (a) Ala-Hyp; (b) Ser-Hyp-Gly; (c) Ala-Hyp-Gly; (d) Pro-Hyp; (e) Pro-Hyp-Gly; (f) Gly-Pro-Hyp; (g) Ile-Hyp; (h) Leu-Hyp; (i) Phe-Hyp. Values presented as the mean ± standard error, $n = 5$ subjects.

maximum 1–2 h after ingestion. The T_{max} (h), C_{max} (nmol/ml), and AUC (h nmol/ml) of the nine Hyp-containing peptides are presented in Table III. The T_{max} values for Pro-Hyp and Gly-Pro-Hyp were reached 2 h after oral ingestion of fish-scale gelatin hydrolysate. On the other hand, the T_{max} values for the seventh through ninth Hyp-containing peptides were from 1 to 1.6 h after oral ingestion of the hydrolysate. The C_{max} in plasma was 60.65 ± 5.74 nmol/ml plasma, and the C_{max} of Pro-Hyp was higher than that of the other eight Hyp-containing peptides. The calculated $AUC_{0-7\,h}$ of each Hyp-containing peptide was as follows: Ala-Hyp, 34.55 ± 8.48 h nmol/ml; Ser-Hyp-Gly, 36.25 ± 5.26 h nmol/ml; Ala-Hyp-Gly, 37.72 ± 3.98 h nmol/ml; Pro-Hyp, 201.17 ± 18.78 h nmol/ml; Pro-Hyp-Gly, 1.49 ± 0.31 h nmol/ml; Gly-Pro-Hyp, 3.62 ± 0.57 h nmol/ml; Ile-Hyp, 9.06 ± 1.19 h nmol/ml; Leu-Hyp, 21.30 ± 3.36 h nmol/ml; and Phe-Hyp, 28.85 ± 4.50 h nmol/ml. This result indicated that Pro-Hyp was the major Hyp-containing peptide in plasma after oral ingestion of fish-scale gelatin hydrolysate, as reported earlier (Ohara et al. 2007a).

Discussion

Several Hyp-containing food-derived collagen peptides were identified in human blood after oral ingestion of gelatin hydrolysates. However, none of these peptides were

Int J Food Sci Nutr Downloaded from informahealthcare.com by Universite De Sherbrooke on 06/23/14
For personal use only.

Table III. $AUC_{0-7\ h}$ of nine Hyp-containing peptides in human plasma after oral ingestion of fish-scale gelatin hydrolysate.

Peptide	T_{max} (h)	C_{max} (nmol/ml)	$AUC_{0-7\ h}$
Ala-Hyp	1.60 ± 0.24	13.70 ± 2.78	34.55 ± 8.48
Ser-Hyp-Gly	1.40 ± 0.24	16.58 ± 1.72	36.25 ± 5.26
Ala-Hyp-Gly	1.00 ± 0.00	23.84 ± 2.44	37.72 ± 3.98
Pro-Hyp	2.00 ± 0.00	60.65 ± 5.74	201.17 ± 18.78
Pro-Hyp-Gly	1.40 ± 0.24	0.67 ± 0.14	1.49 ± 0.31
Gly-Pro-Hyp	2.00 ± 0.00	1.24 ± 0.17	3.62 ± 0.57
Ile-Hyp	1.00 ± 0.00	4.26 ± 0.60	9.06 ± 1.19
Leu-Hyp	1.00 ± 0.00	11.71 ± 1.35	21.30 ± 3.36
Phe-Hyp	1.00 ± 0.00	15.61 ± 2.46	28.85 ± 4.50

Values presented as the mean ± standard error, $n = 5$ subjects.

Int J Food Sci Nutr Downloaded from informahealthcare.com by Universite De Sherbrooke on 06/23/14
For personal use only.

quantified in human plasma. In this report, a LC-MS/MS method was introduced to quantify Hyp-containing peptides in human plasma after oral ingestion of fish-scale gelatin hydrolysate. The recovery of standards added to plasma was quantified, confirming that this method could be used to measure concentrations of Hyp-containing peptides without derivatization. In addition, the linearity of the measurements was evaluated, and results confirmed that it was accurate over the calibration curve range for all peptides. Previous approaches to measuring peptides containing Hyp were based on their derivatization with phenyl isothiocyanate (Iwai et al. 2005; Ohara et al. 2007a; Aito-Inoue et al. 2006).

The major constituent of food-derived collagen peptides remaining in blood was confirmed to be Pro-Hyp ($AUC_{0-7\ h}$ = 201.17 ± 18.78 h nmol/ml), while the minor components were Ala-Hyp-Gly, Ser-Hyp-Gly, Ala-Hyp, Phe-Hyp, Leu-Hyp, Ile-Hyp, Gly-Pro-Hyp, and Pro-Hyp-Gly ($AUC_{0-7\ h}$ from 37.72 to 1.49 h nmol/ml). This result indicated that Pro-Hyp was the major Hyp-containing peptide in plasma after oral ingestion of fish-scale gelatin hydrolysate, as reported earlier (Ohara et al. 2007a). In the present study, Pro-Hyp reached its maximum concentration in plasma 2 h after oral ingestion of fish-scale gelatin hydrolysate, while Ala-Hyp and Ala-Hyp-Gly reached their maximum concentrations 1 h after ingestion of the hydrolysate. Another study reported that more than 75% of Pro-Hyp remained 24 h after being added *in vitro* to human serum (Iwai et al. 2005). Therefore, Pro-Hyp can be considered indigestible by human blood.

It is well known that the abundance of the oligopeptide transporter (PEPT-1) in the brush-border membrane of the intestinal epithelium is the principal mechanism for regulation of transport of products of protein digestion (dipeptides and tripeptides). Gly-Pro-Hyp can be partially hydrolyzed by the brush-border membrane-bound aminopeptidase N to remove Gly, and the resulting Pro-Hyp may be transported into small intestinal epithelial cells via the H^+-coupled PEPT-1 (Aito-Inoue et al. 2007). It therefore may be possible for Hyp-containing dipeptides or tripeptides to be absorbed transcellularly, at least partly, via this peptide transporter (Adibi 2003).

After peptide ingestion, dipeptides were detected in human blood, but their concentrations were quite low. Matsui et al. (2002) reported that the dipeptide Val-Tyr was observed in plasma 2 h after oral peptide administration. The maximal Val-Tyr concentration in plasma was 2,041 ± 148 fmol/ml. Morifuji et al. (2009) reported the plasma levels of Val-Leu, Ile-Leu and Leu-Leu after ingestion of soy and whey protein hydrolysate. The maximal Val-Leu, Ile-Leu and Leu-Leu concentrations in

plasma were 25 nmol/l, 40 nmol/l and 6 nmol/l, respectively. In the present study, Pro-Hyp was the major Hyp-containing peptide in plasma after oral ingestion of fish-scale gelatin hydrolysate, and the maximal level in plasma was 60.65 ± 5.74 nmol/ml plasma. The C_{max} of Pro-Hyp was higher than that of Val-Tyr. Stimulation of human fibroblast proliferation and hyaluronan synthesis by Pro-Hyp has been achieved at a concentration of 100 nmol/ml (Ohara et al. 2007b). The amount of Pro-Hyp in plasma 2 h after oral ingestion of fish-scale gelatin hydrolysate is approximately 60 nmol/ml plasma. Therefore, the total Pro-Hyp content in plasma or skin is estimated to reach approximately 100 nmol/ml. This suggests that oral ingestion of collagen can result in biological activities that depend on food-derived Hyp-containing peptides.

Conclusions

Concentrations of nine Hyp-containing peptides were determined in human plasma after oral ingestion of fish-scale gelatin hydrolysate. Pro-Hyp was the major constituent of food-derived collagen peptides, while the minor components were Ala-Hyp-Gly, Ser-Hyp-Gly, Ala-Hyp, Phe-Hyp, Leu-Hyp, Ile-Hyp, Gly-Pro-Hyp, and Pro-Hyp-Gly. The concentration of Hyp-containing peptides is substantially higher than that following oral ingestion of other peptides.

References

Adibi SA. 2003. Regulation of expression of the intestinal oligopeptide transporter (Pept-1) in health and disease. Am J Physiol Gastrointest Liver Physiol 285:G779–G788.

Aito-Inoue M, Ohtsuki K, Nakamura Y, Park EY, Iwai K, Morimatsu F, Sato K. 2006. Improvement in isolation and identification of food-derived peptides in human plasma based on precolumn derivatization of peptides with phenyl isothiocyanate. J Agric Food Chem 54:5261–5266.

Aito-Inoue M, Lackeyram D, Fan MZ, Sato K, Mine Y. 2007. Transport of a tripeptide, Gly-Pro-Hyp, across the porcine intestinal brush-border membrane. J Pept Sci 13:468–474.

Bos KJ, Rucklidge GJ, Dunbar B, Robins SP. 1999. Primary structure of the helical domain of porcine collagen X. Matrix Biol 18:149–153.

Iwai K, Hasegawa T, Taguchi Y, Morimatsu F, Sato K, Nakamura Y, Higashi A, Kido Y, Nakabo Y, Ohtsuki K. 2005. Identification of food derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. J Agric Food Chem 53:6531–6536.

Knight CG, Morton LF, Onley DJ, Peachey AR, Ichinohe T, Okuma M, Farndale RW, Barnes MJ. 1999. Collagen–platelet interaction: Gly-Pro-Hyp is uniquely specific for platelet Cp VI and mediates platelet activation by collagen. Caradiovasc Res 41:450–457.

Laskin DL, Kimura T, Sakakibara S, Riley DJ, Berg RA. 1986. Chemotractic activity of collagen-like polypeptides for human peripheral blood neutrophils. J Leukoc Biol 39:255–266.

Matsui T, Tamaya K, Seki E, Osajima K, Matsumoto K, Kawasaki T. 2002. Absorption of Val-Tyr with in vivo angiotensin I-converting enzyme inhibitory activity into the circulating blood system of mild hypertensive subjects. Biol Pharm Bull 25:1228–1230.

Morifuji M, Ishizaka M, Baba S, Matsumoto H, Koga J, Higuchi M. Unpublished data. Food and Health R&D Laboratories. Saitama, Japan: Meiji Seika Kaisha Ltd.

Ohara H, Matsumoto H, Ito K, Iwai K, Sato K. 2007a. Comparison of quantity and structures of hydroxyproline-containing peptides in human blood after oral ingestion of gelatin hydrolysates from different sources. J Agric Food Chem 55:1532–1535.

Ohara H, Saito S, Matsumoto H, Akiyama M, Fujimoto N, Kuroda K. 2007b. Effects of collagen-derived hydroxyproline containing peptides in cultures human dermal fibroblasts. J Dermatol Sci 47:102.

Postlethwaite AE, King A. 1976. Collagen and collagen like peptide-induced chemotaxis of human blood monocytes. J Exp Med 143:1299–1307.

Postlethwaite AE, Seyer JM, Kang AH. 1978. Chemototacitic attraction of human fibroblasts to type I, II and III collagens and collagen derived peptides. Proc Natl Acad Sci USA 75:871–875.

Int J Food Sci Nutr Downloaded from informahealthcare.com by Universite De Sherbrooke on 06/23/14
For personal use only.

Ramshaw JAM, Shah NK. 1998. Brodsky, B. Gly-X-Y tripeptide frequencies in collagen: A context for host–guest triple helical peptides. J Struct Biol 122:86–91.

Shigemura Y, Iwai K, Morimatsu F, Iwamoto T, Mori T, Oda C, Taira T, Park EY, Nakamura Y, Sato K. 2009. Effect of prolyl-hydroxyproline (Pro-Hyp), a food-derived collagen peptide in human blood, on growth of fibroblasts from mouse skin. J Agric Food Chem 57:444–449.

Shrieber R, Seybold U. 1993. Gelatin production, the six steps to maximum safety. Dev Biol Stand 80: 195–198.

Zhang G, Sun A, Li W, Liu T, Su Z. 2006. Mass spectrometric analysis of enzymatic digestion of denatured collagen for identification of collagen type. J. Chromatogr A 1114:274–277.

Int J Food Sci Nutr Downloaded from informahealthcare.com by Universite De Sherbrooke on 06/23/14
For personal use only.