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Antihypertensive Peptides Specific to *Lactobacillus helveticus* Fermented Milk

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1. Introduction

Peptides are well known as nitrogen sources to supply various amino acids for many different organisms, and also have many hormonal functions in our body. Previous studies have reported a secondary role for peptides with specific amino acid sequences that possess biological function *in vivo* (1-8). To prepare biologically active peptides (bioactive peptides), food ingredients containing protein are generally hydrolyzed by some proteolytic enzymes. In particular, milk proteins such as bovine casein and whey proteins have been used for preparations of bioactive peptides because inexpensive and safe sources are readily available. Various physiologically functional peptides, such as immunostimulating peptides (1), antimicrobial peptides (2), opioid peptides (3), mineral soluble peptides (4) and antihypertensive peptides (5-8) and have been isolated from enzymatic hydrolyzates of raw food materials and fermented food products.

Among these bioactive peptides, antihypertensive peptides have been extensively studied and reviewed (5-8). Hypertension is a major risk factor in cardiovascular disease, such as heart disease and stroke. In order to reduce the incidence of disease, pharmacological substances can be used to decrease high blood pressure to within the normal range. Angiotensin I-converting enzyme (kininase II; EC 3.4.15.1) (**ACE**) is predominantly expressed as a membrane-bound ectoenzyme in vascular endothelial cells and several other cell types, including absorptive epithelia, neuroepithelia and male germinal cells (9, 10). A dipeptidyl carboxypeptidase, ACE catalyzes the production of a vasoconstrictor, angiotensin II, and inactivates a vasodilator, bradykinin (11, 12). The first competitive inhibitors to ACE were reported as naturally occurring peptides isolated from snake venom (13, 14). Then, inhibitory activities on ACE, which plays an important role in blood pressure regulation are generally assessed for preparation of antihypertensive peptides.

Among lactic acid bacteria, *Lactobacillus helveticus* had the highest extracellular proteinase activity and the highest ability to release the specific antihypertensive peptides in the fermented milk (15). So, this paper mainly reviews processing of the antihypertensive peptides, Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP), by proteolytic enzymes of *L. helveticus*. The antihypertensive effects of these *in vitro* and *in vivo* studies, clinical study, and the mode of action are also reviewed. The antihypertensive effect of the *L. helveticus* fermented milk compared to that produced by various lactic acid bacteria and proteolytic systems of *L.*

helveticus is reviewed for the discussion in more detail. Finally, processing of VPP and IPP specific to the *L. helveticus* is discussed in the context of comparative genome analyses of corresponding proteolytic enzymes in various lactic acid bacteria.

1.1 Antihypertensive peptide in fermented milk

1.1.1 ACE inhibitory peptides from milk proteins

Many kinds of ACE inhibitory (**ACEI**) peptides have been reported from enzymatic hydrolyzates of milk protein, as well as synthetic peptides and fermented products (16-24) (Table 1). Spontaneously hypertensive rat (**SHR**) is a useful animal model to evaluate the antihypertensive activity of ACEI peptides because the systolic blood pressure of SHR reaches over 230 mmHg and is powerful tool for detection of the *in vivo* effect. Some of the orally administered ACEI peptides have demonstrated strong antihypertensive effects in SHR (Table 1).

Peptide	Source	Preparation	*IC50 (μM)	Dose (mg/kg)	***SBP (mm Hg)
<Enzymatic hydrolysate>					
FFVAPFPEVFGK	αs1-casein	Trypsin	77	100	-13.0
AVPYPQR	β-casein	Trypsin	15	100	-10.0
TTMPLW	αs1-casein	Trypsin	16	100	-13.6
LKPNM	Aldolase	Thermolysin	2.4	60	-23
LKP	Aldolase	Chicken muscle	0.32	60	-18
IPA	β-lactogloblin	Proteinase K	141	8	-31
VYPFPG	β-casein	Proteinase K	221	8	-22
GKP	β-microglobulin	Proteinase K	352	8	-26
FP	β-casein, albumin	Proteinase K	315	8	-27
YKVPQL	αs1-casein	Proteinase	22	1	-12.5
<Fermented products>					
RF	Sake lees	Brewing	ND	100	-17
VW	Sake lees	Brewing	1.4	100	-10
YW	Sake lees	Brewing	10.5	100	-28
VY	Sake	Brewing	7.1	100	-31
IYPRY	Sake	Brewing	4.1	100	-19
VPP	β-casein	Fermentation	9	1.6	-20
IPP	β- and κ -casein	Fermentation	5	1	-15.1
YP	αs1, β- and κ-casein	Fermentation	720	1	-27.4

ND: Not described

*IC50: Peptide concentration that shows 50% inhibition of ACE activity

***SBP: systolic blood pressure of spontaneously hypertensive rat

Table 1. Antihypertensive peptides derived from caseins by proteolytic action.

Lactic acid bacteria have proteolytic systems that can hydrolyze milk protein and have been reported to utilize the peptides released from the milk protein casein (25-27). Among lactic acid bacteria, *Lactobacillus helveticus* had the highest extracellular proteinase activity and the

ability to release the largest amount of peptides in the fermented milk (Table 2). As a result, among various kinds of fermented milk, the antihypertensive effect was specific to the *L. helveticus* fermented milk (15). In our study, an antihypertensive effect related to ACEI peptides was found in sour milk produced by *L. helveticus* (19, 28). Two ACE inhibitory peptides were purified from sour milk and identified as VPP and IPP. The ACEI activity of the two peptides was very high, 9 μM and 5 μM, compared to other reported peptides (Table 1). The amino acid sequences of VPP and IPP were found in the primary structure of bovine β-casein (84-86) (74-76) and κ-casein (108-110), respectively. These peptides were produced during fermentation (16), but were not found in the hydrolyzate of casein after digestion with an extracellular proteinase of *L. helveticus* (29). Oral administration of *L. helveticus* fermented milk containing VPP and IPP to SHR, with a single dose of 5 ml/kg body weight, significantly decreased systolic blood pressure between 4 and 8 h after administration (28). The antihypertensive effect of these two chemically synthesized peptides was also observed between 2 and 8 h after administration and the effects were dose-dependent. Furthermore, a dose-dependent antihypertensive effect of these two chemically synthesized peptides was also observed from 0.1 to 10 mg/kg of body weight (28).

Strain	**Peptide conc. (%)**	**Proteinase act. (U/ml)**	***ACEI act. (U/ml)**	*****Change in SBP (mmHg)**
Non-fermented milk (Lactobacilli)	0.00	-	0	- 5.0 ± 7.3
L. helveticus CP790	0.19	230	58	- 27.4 ± 13.3 * *
L. helveticus CP611	0.25	367	70	- 20.0 ± 9.6 * *
L. helveticus CP615	0.18	420	51	- 23.0 ± 13.4 * *
L. helveticus JCM1006	0.15	182	26	- 15.2 ± 9.3 *
L. helveticus JCM1120	0.10	112	34	- 6.5± 10.8
L. helveticus JCM1004	0.21	186	48	- 29.3 ± 13.6 * *
L. delbrueckii subsp. *bulgaricus* CP973	0.19	105	22	- 0.8 ± 8.2
L. delbrueckii subsp. *bulgaricus* JCM1002	0.11	124	28	- 4.5 ± 4.0
L. casei CP680	0.01	35	3	- 0.2 ± 6.6
L. casei JCM1134	0.00	28	9	- 7.0 ± 11.2
L. casei JCM1136	0.09	25	18	- 9.6 ± 7.2
L. acidophilus JCM1132	0.00	28	8	- 8.7 ± 7.8
L. delbrueckii subsp. *lactis*JCM1105 (Streptococci)	0.08	18	16	- 3.3 ± 3.5
S. thermophilus CP1007 (Lactococci)	0.02	25	3	- 2.4 ± 8.1
L. lactis subsp. *lactis* CP684	0.00	35	4	- 7.3 ± 10.5
L. lactis subsp. *cremoris* CP312	0.02	18	4	- 5.8 ± 13.9

Significant differences from the control, $^{**}p < 0.01$, $^{*}p < 0.05$.

*ACEI activity: Peptides that show 50% inhibition of ACE activity was defined as one unit.

***SBP: systolic blood pressure of spontaneously hypertensive rat

Table 2. Antihypertensive effects in spontaneously hypertensive rats and ACE inhibitory activities of various fermented milk.

1.2 Clinical effects of the fermented milk

Hypertension is a major risk factor in cardiovascular diseases, such as heart disease and stroke. In order to reduce the incidence of disease, pharmacological substances can be used to decrease high blood pressure to within the normal range. In the first Japanese study with the fermented milk, hypertensive subjects were randomly assigned to two groups: the one group ingested 95 ml of the milk, containing 3.4 mg of VPP and IPP, daily for 8 wk; the other group ingested the same amount of artificially acidified milk as a placebo, for 8 wk (30). In the fermented milk group, systolic blood pressure decreased significantly between 4 and 8 wk after the beginning of ingestion, but not in the placebo group (30). Moreover, clinical tests were performed for Japanese subjects with different blood pressure levels, which confirmed the mild and prolonged effects for the hypertensive subjects following oral administration of bioactive milk (30-33) (Fig. 1). In a pilot study conducted in Finland, the antihypertensive effect was also observed in the group ingesting *L. helveticus* fermented milk containing the two tripeptides (34, 35). Moreover, a recent study indicated significant beneficial effects of hypertensive patients ingesting the *L. helveticus* fermented milk over a long period of time for 21 wk (35). There was a significant decrease in systolic blood pressure (6.7 ± 3.0 mmHg) by comparative study with placebo group.

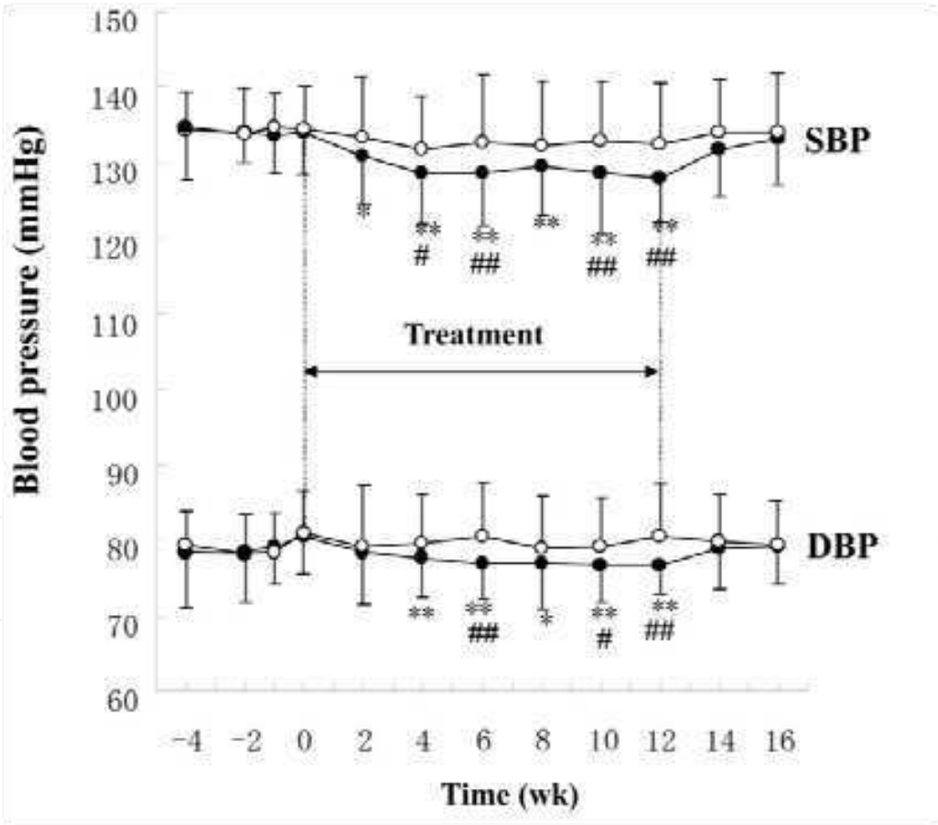

Fig. 1. Blood pressure lowering effect of *Lactobacillus helveticus* fermented milk product for subjects with high-normal hypertension (Nakamura *et al.*, J. Nutritional Food (in Japanese) 2004, 7, 123-137). Significant difference from initial value (*t*-test): $^{*}p$ <0.05, $^{**}p$ <0.01. Significant difference from placebo group (Bonferroni test): # p<0.05, ## p<0.01.

1.3 Processing of antihypertensive peptides in *L. helveticus*

1.3.1 Proteolytic system in lactic acid bacteria

Many kinds of proteolytic enzymes have been reported from lactic acid bacteria, and were reviewed extensively (26, 27, 36, 37). The components of the proteolytic systems of lactic acid bacteria are divided into three groups, including the extracellular proteinase that catalyzes casein breakdown to peptides, peptidases that hydrolyze peptides to amino acids and a peptide transport system. The number of proteinases was reported from lactococci, which are mainly used in cheese making. The extracellular proteinase activity is linked to cell growth in milk and seemed to be essential for utilization of milk protein for growth. The gene encoding the proteinases, named *prtP*, *prtH* and *prtH2*, has been sequenced and characterized from several of *L. lactis* strains (38-40). These proteinases are processed to active enzymes by removal of N-terminal polypeptides from the pre-proteinase with the help of the maturation protein, PrtM.

1.4 Processing by a cell wall-associated proteinase

The first step in casein decomposition is typically caused by an extracellular proteinase, and further digestion to amino acids is catalyzed by many kinds of intracellular peptidases (25, 36). Among the lactic acid bacteria, *L. helveticus* has the highest extracellular proteinase activity and the ability to release the highest amount of peptides in fermented milk (Table 2). *L. helveticus* strains were classified into 2 types based on differences in the extracellular proteinase (41). One type has an enzyme with a molecular weight of 170 kDa with homology to the lactococcus enzyme, and other has an enzyme with a molecular weight of 45 kDa (29, 42). Polycloncal antibodies raised against the mature 170 kDa proteinase reacted not only with the 170 kDa enzyme but also the 53 kDa protein (Table 3). The 53 kDa protein was thought to be a degradation product from the 170 kDa active enzyme. A gene encoding a proteinase with a homology to the *Lactococcu*s proteinase was cloned and sequenced from *L. helveticus* CNRZ32 (43). A gene encoding *PrtM* gene was also cloned from *L. helveticus* CNRZ32. On the other hand, the gene encoding a small type of proteinase with a molecular weight of 45 kDa was cloned from *L. hel*veticus CP790 strain (44). A 46 kDa pre-proteinase was activated to the 45 kDa active proteinase by release of 7 amino acids from the N-terminus with the help of a maturation protein (44, 45).

Moreover, a slight difference in the specificity of the two types of proteinases toward casein was suggested for the two types of *L. helveticus* strains (46). However, there were no clear relationships between proteinase specificity and the antihypertensive effects and the ACEI activities of the fermented milk, which depended on the strain of *L. helveticus* used. The extracellular proteinase activity of each *L. helveticus* strain was almost correlated with ACEI activity in the fermented milk (Table 1). These results strongly suggest that the proteolysis of casein by the extracellular proteinase is the most important parameter in the processing of active components. This possibility is also supported by the fact that the *L. helveticus* CM4, which was selected for its strong proteinase activity compared to other *L. helveticus* strains, has the ability to release ACE inhibitory peptides in the fermented milk (47). The proposed importance of the proteinase was also supported by the fact that a proteinase negative mutant was not able to generate antihypertensive peptides in the fermented milk, whereas the wild-type strain had the ability to release strong antihypertensive peptides in the fermented milk (16). Strain CM4, which had the highest antihypertensive peptide

No	Strain	Subspecies	Reactivity (CP790)1	Reactivity (CP53)2	Type
1	*L. helveticus* CP39	J3	45 kDa	45 kDa	A
2	*L. helveticus* CP53	H4	ND5	53, 170 kDa	B
3	*L. helveticus* CP209	J	45 kDa	45 kDa	A
4	*L. helveticus* CP210	J	45 kDa	45 kDa	A
5	*L. helveticus* CP293	J	45 kDa	45 kDa	A
6	*L. helveticus* CP510	J	45 kDa	45 kDa	A
7	*L. helveticus* CP611	J	45 kDa	45 kDa	A
8	*L. helveticus* CP615	J	45 kDa	45 kDa	A
9	*L. helveticus* CP617	J	45 kDa	45 kDa	A
10	*L. helveticus* CP789	J	45 kDa	45 kDa	A
11	*L. helveticus* CP790	J	45 kDa	45 kDa	A
12	*L. helveticus* JCM1004	H	ND	53, 170 kDa	B
13	*L. helveticus* JCM1006	J	45 kDa	45 kDa	A
14	*L. helveticus* JCM1007	J	ND	53, 170 kDa	B
15	*L. helveticus* JCM1062	J	ND	53, 170 kDa	B
16	*L. helveticus* JCM1103	H	ND	53, 170 kDa	B
17	*L. helveticus* JCM1120	H	ND	53, 170 kDa	B

(Yamamoto et al., 1998, Biosci. Biotech. Biochem. 58, 776-778)

[1]Monoclonal antibody to the proteinase from L. helveticus CP790

[2]Polyclonal antibody to the proteinase from L. helveticus CP53

[3]Classified as L. helveticus biovar jugurti

[4]Classified as L. helveticus biovar helveticus

[5]Not detected

Table 3. Immunological Difference of Proteinases of *L. helveticus* Strains with Two Types of Antibodies.

production in fermented milk, seemed to have the potential for use as a functional food product. Currently, we completed the whole genome sequence of *L. helveticus* CM4, and the results revealed the presence of 2,171 open reading frames in 2,028,493 bp of whole DNA sequence (unpublished data). Then, whole genome sequences of CM4 and DPC4571 (48) were compared as shown in Table 4 for full understanding of the intracellular processing to VPP and IPP (49). As shown in Table 4, three genes for cell-wall associated proteinase genes, *prtY*, *prtH2* and *prtM2*, and 23 kinds of intracellular peptidases were detected in the CM4 sequence. The genes of *prtH2* and *prtM2* were detected both in CM4 and DPC4571, but the *prtM2* was considered to be pseudo-genes for extracellular proteinase in the previous study (50). However, no *prtH1* and *prtM1* genes reported in CNRZ32 (51) were detected in sequences in both CM4 and DPC4571 strains. The genes of *prtH2* and *prtM2* were detected both in CM4 and DPC4571, but the *prtM2* was considered to be pseudo-genes for extracellular proteinase in the previous study. However, no *prtH1* and *prtM1* genes reported in CNRZ32 (51) were detected in sequences in both CM4 and DPC4571 strains. On the other hand, one of the cell wall-associated proteinase gene (*prtY*) corresponding to a 45 kDa proteinase previously detected in *L. helveticus* CP790 strain (41, 42) was detected in CM4 strain but not in DPC4571 strain (Table 1). These results reveal the cell wall-associated extracellular proteinase which plays key role in decomposition of casein might be different

among detected proteolytic enzymes between CM4 and DPC4571. On the other hand, the large size of the proteinase gene (*prtH*) corresponding to a 200 kDa proteinase and its maturation gene (*prtM*) reported by Pederson *et al.* (43) were not detected in the CM4 and DPC4571 genes. This observation supports our previous results that there are two types of extracellular proteinases, and *L. helveticus* can be classified by the proteinase type as shown in Table 4. This result also suggested that the extracellular proteinase might play a key role to release high amount of VPP and IPP from the comparative analysis of both proteolytic genes (Table 4).

Proteolytic enzyme	Gene	Molecular weight (kDa)		Protein ID	Identity (%)
		CM4	DPC4571	DPC4571	
Proteinase	*prtY*	47.0	ND	-	-
	prtH2	181.6	180.871*	-	99.2
	prtM	33.7	32.7	*ABX27563*	98.0
Aminopeptidase	*pepC1*	51.4	51.4	*ABX26582*	98.9
	pepC2	52.9	50.2	*ABX27065*	98.6
	pepN	95.8	95.9	*ABX27731*	99.4
	pepN2	57.2	57.2	*ABX27544*	100.0
	pepA	41.3	40.1	*ABX26758*	99.4
XPDAP	*pepX*	90.5	90.6	*ABX27419*	99.6
Endopeptidase	*pepE*	50.0	50.0	*ABX26466*	99.8
	pepE2	51.4	50.3	*ABX26457*	99.8
	pepF	68.1	68.1	*ABX27686*	99.3
	pepO	73.6	73.5	*ABX27358*	99.4
	pepO2	73.8	73.5	*ABX27211*	98.6
	pepO3	73.1	72.6	*ABX26433*	99.7
Tripeptidase	*pepT*	47.1	46.7	*ABX27305*	99.3
	pepT2	48.8	48.4	*ABX27165*	99.5
Dipeptidase	*pepD1*	54.0	54.1	*ABX27625*	99.4
	pepD2	54.9	54.9	*ABX27375*	99.8
	pepD3	53.5	53.5	*ABX27723*	100.0
	pepV	51.5	51.5	*ABX27224*	98.9
	pepDA	53.5	53.5	*ABX26492*	99.6
Prolidase	*pepQ*	41.2	41.2	*ABX26664*	99.5
	pepQ2	41.4	41.1	*ABX27405*	99.7
Prolinase	*pepPN*	35.0	35.0	*ABX27633*	99.7
Proline iminopeptidase	*pepI*	33.9	33.8	*ABX26375*	99.3

ND: Not detected, *size of the reported pseudo-gene

Table 4. Genes encoding proteolytic enzymes reported in *Lactobacillus helveticus* strains

By the proteolytic action of the extracellular proteinase in CP790 (and CM4), a long β-casein peptide with a 28 amino acid residue including VPP and IPP sequences was generated (29) (Fig. 2). The proteinase activity is easily repressed by accumulated peptides by the proteinase in the fermented milk. Moreover, the enzyme activity is inactivated by pH drop during the fermentation. So, the first degradation of casein by the extracellular proteinase would be occurred mostly at the beginning of the fermentation.

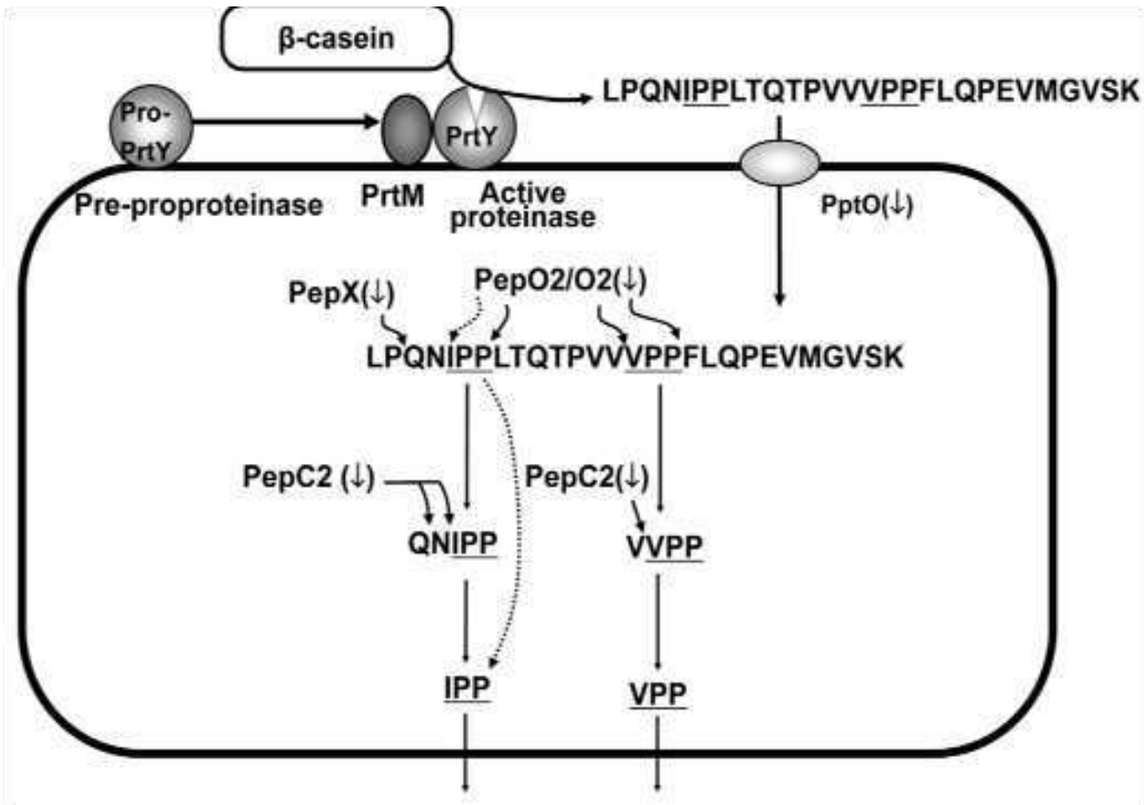

Fig. 2. Postulated proteolytic system for Val-Pro-Pro and Ile-Pro-Pro processing in *Lactobacillus helveticus*. PrtY: cell-wall proteinase, PrtM: maturation protein, PptO: oligopeptide transporter, PepO: endopeptidase O, PepO2: endopeptidase O2, PepC2: aminopeptidase C2, PepX: X-prolyl dipeptidyl aminopeptidase. Up- and down-regulation and amount of release are indicated by arrows as (↑) and (↓), respectively.

1.4.1 Intracellular processing by some peptidases

Next, the long peptide was thoroughly hydrolyzed to shorter peptides by intracellular peptidases. Intracellular peptidases of *L. helveticus* and these genes were well reviewed, previously (37), however, there was no clear explanation for the processing of VPP and IPP. Long peptide containing two kinds of tri-peptide sequences released by the extracellular proteinase from β-casein will be incorporated into the cell by the oligopeptide transporter (**PptO**), and processed intracellularly to VPP and IPP by some peptidases (Fig. 2). Carboxyl peptidase most likely needs for the release of C-terminal amino acid from Pro-Pro-X sequence. However, recently, a key enzyme that can catalyzed carboxyl terminal processing to produce VPP and IPP was detected and purified from the CM4 strain (Fig. 2) (52). The enzyme had a homology to an endopeptidase (**PepO**) from *L. helveticus* CNRZ32 by amino terminal sequence analysis of the purified enzyme (52) and a homology with the deduced amino acid sequence of the gene (53). The enzyme can catalyze C-terminal processing of VPPFL and IPPLT to VPP and IPP.

Based on the previous reported characteristics of many peptidases from *L. helveticus*, processing of the N-terminal sequence of VPP and IPP are presumed and summarized in

Fig. 2. Generally, amino peptidase shows broad specificity toward amino acid at N-terminal end, however, amino terminal processing seems to terminate if proline residue is present at the N-terminal end in the peptide. However, X-prolyl dipeptidyl aminopeptidase (**XPDAP**) is able to release the di-peptide with a sequence of X-Pro, from the N-terminus. On the other hand, aminopeptidase may stop the hydrolysis at a Xaa-Pro-Pro- sequence if it is present. Therefore, the N-terminal processing to release VPP and IPP may be catalyzed by specific aminopeptidases, such as pepC2 and XPDAP as shown in Fig. 2. However, for more detailed understanding of these peptide processing in *L. helveticus*, productivities of these peptides in transformant strains expressing the each postulated peptidase gene or disrupting of these corresponding peptidase genes from wild type strain should be studied.

1.5 Comparison of the *L. helveticus* proteolytic system to those in other lactic acid bacteria

The unprocessed proteinase of *L. lactis* consists of about 1950 amino acid residues, and the mature proteinase of *L. lactis* is a serine type enzyme with a molecular mass between 180-190 kDa (38-40). The gene encoding the proteinase, named *prtP*, has been cloned and sequenced from several of *L. lactis* strains (38-40). These proteinases are processed to active enzymes by removal of N-terminal polypeptides from the pre-proteinase with the help of the maturation protein, PrtM. Recent decay, whole genome sequences for more than 36 kinds of lactobacilli have been reported. The comparative analysis of those genes to other species revealed that the CM4 peptidases were more homologous to those in *Lactobacillus acidophilus* NCFM strain (54)(Table 5). Moreover, homologies of CM4 peptidases were detected with peptidases in *Lactobacillus gasseri* ATCC33323 (55) and *Lactobacillus johnsonii* (NC533) (56). These observations suggest that the *L. acidophilus* group has similar peptidases, endopeptidase, XPDAP, and aminopeptidase to *L. helveticus* CM4 and might have the ability to process VPP and IPP if the initial decomposition of casein by an extracellular proteinase is accelerated in the fermentation process.

1.6 Repression of proteolytic systemg

For growth of lactic acid bacteria in milk, the proteolytic system is activated in the milk medium because of a limited amount of amino acids. However, during fermentation in the milk medium, the proteolytic system of lactic acid bacteria is repressed by accumulated peptides in the fermented milk. The amount of VPP and IPP in the *L. helveticus* fermented milk was also repressed if amino acids were added to the fermented milk (57). Microarray analysis of the whole *L. helveticus* CM4 genome suggested extracellular proteinase, endopeptidases, XPDAP, some aminopeptidases and some kinds of peptide transporters might be suppressed by addition of amino acids and be involved in the processing of the VPP and IPP (Fig. 2). Regulatory systems (57) that repress the proteolytic enzymes and transporters in the presence of amino acids was reported as codY system in *Lactococcus lactis* (58, 59). However, there is no codY-like protein in the *Lactobacillus* genome that has been reported. Thus, a novel type regulatory system for the proteolytic system must exist in lactobacilli and strongly affect on the release of VPP and IPP in *L. helveticus*.

	Proteinase		Aminopeptidase			XPDAP	Endopeptidase					
	prtY	prtH	pepN	pepC1	pepC2	pepX	pepE	pepE2	pepF	pepO	pepO2	pepO3
***Lb. acidophilus* NCFM**	-	-	90	91	87	91	89	90	88	85	61	-
***Lb. gasseri* ATCC 33323**	-	-	67	83	76	72	70	83	77	63	-	77
***Lb. johnsonii* NCC533**	-	-	66	82	75	72	69	73	76	65	-	78
***Lb. delbrueckii* subsp. *bulgaricus* ATCC BAA-365**	-	-	71	-	53	70	71	-	-	58	-	68
***Lb. casei* subsp. *casei* ATCC334**	-	-	62	59	-	-	-	-	54	-	-	-
***Lc. lactis* IL1403**	-	-	-	-	-	-	-	-	-	-	-	-

Lb: Lactobacillus; Lc: Lactococcus

All values are shown as % homology. Values below 50% were omitted (-).

Table 5. Proteolytic enzymes in various lactic acid bacteria with homology to enzymes in *Lactobacillus helveticus* CM4, which are expected to have roles in processing Val-Pro-Pro and Ile-Pro-Pro

2. Conclusion

In this paper, we showed the potential of bioactive peptides to maintain blood pressure in the normal range. About 30% of Japanese people are estimated to be at risk for hypertension. Generally, hypertension has been improved by medication and partly by controlling the diet. Recently, some food products containing antihypertensive peptides and proven antihypertensive effects in clinical studies were recognized as functional foods, Foods for Specified Health Use (**FOSHU**) in Japan. Biologically functional peptides exerting a mild influence on hypertensive subjects without adverse effect have enormous potential in reducing the risk of cardiovascular disease.

Among many kinds of commercially available lactic acid bacteria, *L. helveticus* has ability to release functional peptides such as antihypertensive peptides in the fermented milk. These potential is strongly depends on the activities of proteolytic enzymes of *L. helveticus*. So, the isolation and mutation breeding of a new *L. helveticus* strain having strong ability to release peptides in the fermented milk is very important to develop industrially useful fermented milk. For the understanding of the antihypertensive peptides, based on comparative analysis with other *Lactobacillus* proteolytic enzymes, the extracellular proteinase and endopeptidase enzymes seemed to be unique to *L. helveticus* and seemed at least partially explain the *L. helveticus* specific release of VPP and IPP from fermented milk. For a full understanding of protein processing, the genomic information and the analysis of the *L. helveticus* will be a very useful tool and might be needed in future studies.

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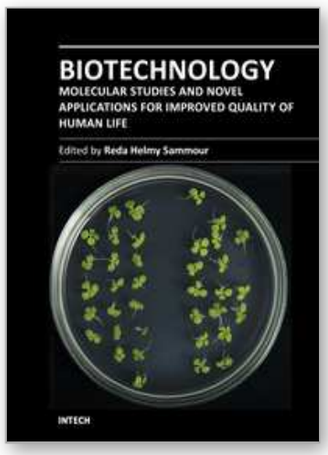

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This book deals with the importance of application of molecular biology as an approach of biotechnology for improvement of the quality of human life. One of the interesting topics in this field, is the identification of the organisms that produce bioactive secondary metabolites. It also discusses how to structure a plan for use and preservation of those species that represent a potential source for new drug development, especially those obtained from bacteria. The book also introduces some novel applications of biotechnology, such as therapeutic applications of electroporation, improving quality and microbial safety of fresh-cut vegetables, producing synthetic PEG hydro gels to be used as an extra cellular matrix mimics for tissue engineering applications, and other interesting applications.

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