Naoyuki Yamamoto
R&D Center,
The Calpis Food
Industry Co., Ltd.,
11-10, 5-Chome, Fuchinobe,
Sagamihara, Kanagawa 229,
Japan

Antihypertensive Peptides Derived from Food Proteins

Abstract: *This paper reviews the angiotensin I converting enzyme inhibitory peptides originated from food materials and enzymatic hydrolysate of different kinds of proteins. Focus was put on the peptides derived from milk casein by the action of the proteolytic system of lactic acid bacteria. Some of the peptides exhibit significant antihypertensive effects in spontaneously hypertensive rats. Some new topics relating to these antihypertensive peptides are introduced. The possible significance of bioactive peptides derived from food in vivo is also discussed.*
 Biopoly ***43:*** *129–134, 1997*

Keywords: *antihypertensive peptides; food proteins; angiotensin I converting enzyme; enzymatic hydrolysate*

INTRODUCTION

In food materials, milk proteins, caseins, and whey proteins are essential for the nutrition of neonates. Although the caseins serve mainly as a nutritional source, i.e., to supply amino acids, recent studies reveal that they also have a biological role. Because of their open, flexible structure, caseins are very susceptible to proteolysis. Enzymatic hydrolysis of milk proteins produces various physiologically functional peptides such as opioid peptides, immunostimulating peptides,[1] and angiotensin I converting enzyme inhibitors.

Lactic acid bacteria growing in milk develop a proteolytic system that releases peptides from casein. Since the beginning of this century, many studies have demonstrated the beneficial effects of lactic acid bacteria and cultured dairy products on nutrition and health status of animals and humans: improvement of intestinal microflora,[2] prevention of intestinal disorder,[3] hypocholesteromic effect,[4] prolongation of life,[5] and cancer therapy and prevention.[6–8] There is also evidence that lactic bacteria and fermented milk might have an immunomodulating effect in animals.[9–19] Recently, an antihypertensive effect related to angiotensin I converting enzyme inhibitory peptides was reported for sour milk produced by a starter containing *Lactobacillus helveticus* and *Saccharomyces cerevisiae*.[20,21] In the present paper, a brief overview of angiotensin I converting enzyme inhibitory peptides from milk casein and other food proteins will be introduced first. Then, the importance of the proteolytic system of lactic acid bacteria in the production of functional peptides, such as the antihypertensive peptides, will be described. Finally, the application of the bioactive peptides for functional foods, based on the study of antihypertensive peptides, will be discussed.

ANGIOTENSIN I CONVERTING ENZYME INHIBITORY PEPTIDES

Among biologically active peptides, antihypertensive peptides have been studied extensively. Angiotensin I converting enzyme (kininase II; EC 3.4.15.1) (ACE) plays an important role in blood

Correspondence to: Naoyuki Yamamoto
 CCC 0006-3525/97/020129-06

Table I Angiotensin I Converting Enzyme Inhibitors from Some Proteins

Peptide	Source	Preparation	IC_{50} (μM)[a]	Reference
Pyr-WPRPTPNIPP	Snake venom	Extraction	2	29, 30
Pyr-WPRPNIPP	Snake venom	Extraction	3	29, 30
Pyr-NWPRPNIPP	Snake venom	Extraction	3	29, 30
Pyr-NWPHPNIPP	Snake venom	Extraction	9	29, 30
Pyr-SWPGPNIPP	Snake venom	Extraction	39	29, 30
Pyr-GGWPRPGPEIPP	Snake venom	Extraction	13	29, 30
Pyr-NWPHPNIPP	Snake venom	Extraction	13	29, 30
FFVAPFPEVFGK	αs1-Casein	Trypsin	77	32
FFVAP	αs1-Casein	Peptidase	6	33
TTMPLW	αs1-Casein	Trypsin	16	35
PLW	αs1-Casein	Synthesis	36	35
LW	αs1-Casein	Synthesis	50	35
VAP	αs1-Casein	Synthesis	2	33
FVAP	αs1-Casein	Synthesis	10	33
FAP	—	Synthesis	3.8	33
AVPYPQR	β-Casein	Trypsin	15	35
IYPFVEPI	h-β-Casein	Synthesis	8	36
IYPFVEPIP	h-β-Casein	Synthesis	12	36
LIYPFVEPIP	h-β-Casein	Synthesis	9	36
IYPFVEPIPY	h-β-Casein	Synthesis	20	36
YPFVEPIPY	h-β-Casein	Synthesis	20	36
PFVEPIPY	h-β-Casein	Synthesis	25	36
FVEPIPY	h-β-Casein	Synthesis	55	36
PIPY	h-β-Casein	Synthesis	30	36
VHLPPP	γ-Zein	Thermolysin	200	37
VHLPP	γ-Zein	Synthesis	18	37
LPP	γ-Zein	Synthesis	9.6	37
VHIPP	—	Synthesis	10	37
VHLAP	—	Synthesis	4.5	37
LRP	α-Zein	Thermolysin	0.27	39
LSP	α-Zein	Thermolysin	1.7	39
LQP	α-Zein	Thermolysin	1.9	39
PTHIKWGD	Tuna muscle	Synthesis	0.9	43, 44
PTHIKWG	Tuna muscle	Synthesis	7.6	44
PTHIKW	Tuna muscle	Synthesis	1.8	44
THIKWGD	Tuna muscle	Synthesis	50	44
HIKWGD	Tuna muscle	Synthesis	50	44
PTHIKWD	Tuna muscle	Synthesis	38	44
PTHIDW	—	Synthesis	13	44
PTHIAW	—	Synthesis	0.39	44
PTHVAW	—	Synthesis	1.5	44
IKPLNY	Tuna muscle	Thermolysin	43	45
IVGRPRHQG	Tuna muscle	Thermolysin	6.2	45
VGRPRHQG	Tuna muscle	Synthesis	5.4	45
GRPRHQG	Tuna muscle	Synthesis	34	45
RPRHQG	Tuna muscle	Synthesis	22	45
IW	Tuna muscle	Synthesis	2	45
IWHHTF	Tuna muscle	Synthesis	2.5	45
IWHHT	Tuna muscle	Thermolysin	5.1	45
WHHTF	Tuna muscle	Synthesis	46	45
HHTF	Tuna muscle	Synthesis	84	45
ALPHA	Tuna muscle	Thermolysin	10	45
LKPNM	Tuna muscle	Thermolysin	17	45
IY	Tuna muscle	Thermolysin	3.7	45
FQP	Tuna muscle	Thermolysin	12	45

Table I (*Continued from the previous page.*)

Peptide	Source	Preparation	IC_{50} (μM)[a]	Reference
DYGLYP	Tuna muscle	Thermolysin	62	45
DMIPAQK	Tuna muscle	Thermolysin	45	45
IKP	Tuna muscle	Synthesis	1.7	45
LNY	Tuna muscle	Synthesis	81	45
LYP	—	Synthesis	6.6	45
VRP	Bonito	Autolysis	2.2	46
IKP	Bonito	Autolysis	2.5	46
LRP	Bonito	Autolysis	1	46
IRP	Bonito	Autolysis	1.8	46
SVAKLEK	Bonito	Autolysis	82	47
ALPHA	Bonito	Autolysis	79	47
GVYPHK	Bonito	Autolysis	1.6	47
IRPVN	Bonito	Autolysis	1.4	47
GVYPHK	Bonito	Synthesis	1.6	47
VYPHK	Bonito	Synthesis	7.6	47
IRP	Bonito	Synthesis	1.8	47
IRPV	Bonito	Synthesis	31	47

[a] The concentration of an ACE inhibitor needed to inhibit 50% of ACE activity.

pressure regulation. It is a dipeptidyl carboxypeptidase that catalyzes both the production of the vasoconstrictor angiotensin II and the inactivation of the vasodilator bradykinin.[22,23] ACE is an unusual zinc-metallopeptidase in that it is activated by chloride and lacks a narrow in vitro substrate specificity.[24] ACE is predominantly expressed as a membrane-bound ectoenzyme in vascular endothelial cells and also in several other types of cells including absorptive epithelial cells, neuroepithelial cells, and male germinal cells.[25–27]

The first competitive inhibitors to ACE were reported from naturally occurring peptides in snake venom.[28,29] Ferreira et al.[28] isolated nine biologically active peptides from *Bothrops jararaca.* The peptides contain 5–13 amino acid residues per molecule. Among them, the peptide that had a sequence of pyrrolodonecarboxyl-Lys-Trp-Ala-Pro showed the highest ACE inhibitory (ACEI) activity. Ondetti et al.[29] reported other strong ACEI peptides from the venom of *Bothrops jararaca* (Table I). Thereafter, many other ACE inhibitors have been discovered from enzymatic hydrolysates or the related synthetic peptides of bovine casein,[30–34] human casein,[35] zein,[36–38] gelatin,[39] soy sauce,[40] soybean, corn, wheat, and other food proteins. Many studies have also been performed on fish products, such as sardine muscle,[41] tuna muscle,[42–44] and bonito.[45,46] The most active ACEI peptides found in these studies are summarized in Table I. These peptides contain 2–12 amino acid residues, several of them with proline in the C-terminus. These peptides show potent ACEI activities in vitro. Whereas some specific inhibitors of ACE have been proven to be useful as antihypertensive drugs,[47] the physiological importance of these bioactive peptides is not yet understood.

ANTIHYPERTENSIVE PEPTIDES PRODUCED BY THE PROTEINASE OF LACTIC ACID BACTERIA

Antihypertensive effects of some peptides have been demonstrated in spontaneously hypertensive rate (SHR) by oral administration (Table II). Some peptides show strong antihypertensive effects in SHR with a low dose of peptides by oral administration. For the peptides that showed the antihypertensive effects with a high administration dose, the effects were initially tested at lower doses of peptides.[48,49]

Lactic acid bacteria have extracellular proteinase and release peptides from casein.[50–54] The hydrolysate of casein by the extracellular proteinase demonstrated antihypertensive activity in SHR at a dosage of 15 mg/kg by oral administration, but control tryptic digested peptides did not show the effect.[55] A potent antihypertensive peptide, Lys-Val-Leu-Pro-Val-Pro-Gln, was purified and identified from casein hydrolysate produced by a proteinase from *L. helveticus* CP790[56] (Table II). Unexpectedly, the antihypertensive peptide did not show strong ACEI activity. However, a shorter peptide, Lys-Val-Leu-

Table II Antihypertensive Peptides Derived from Caseins by Proteolytic Action of Lactic Acid Bacteria

Peptide	Source	Preparation	IC_{50} (μM)[a]	Dose (mg/kg)	SBP (mm Hg)	Reference
Enzymatic hydrolysate						
FFVAPFPEVFGK	αs1-Casein	Trypsin	77	100	−13.0[b]	32
AVPYPQR	β-Casein	Trypsin	15	100	−10.0[b]	35
TTMPLW	αs1-Casein	Trypsin	16	100	−13.6[b]	35
KVLPVPQ	β-Casein	Proteinase	> 1000	1	−24.1[c]	56
KVLPVP	β-Casein	Digestive enzyme	5	1	−32.2[c]	56
YKVPQL	αs1-Casein	Proteinase	22	1	−12.5[c]	56
Fermented products						
RF	Sake lees	Brewing	—	100	−17[c]	48
VW	Sake lees	Brewing	1.4	100	−10[c]	48
YW	Sake lees	Brewing	10.5	100	−28[c]	48
VY	Sake	Brewing	7.1	100	−31[c]	48
IYPRY	Sake	Brewing	4.1	100	−19[c]	49
VPP	β-Casein	Fermentation	9	1.6	−20[c]	20, 21
IPP	β- and κ-casein	Fermentation	5	1	−15.1[c]	20, 21

[a] The concentration of an ACE inhibitor needed to inhibit 50% of ACE activity.
[b] Changes of systolic blood pressure in SHR at 3 h after oral administration.
[c] Changes of systolic blood pressure in SHR at 6 h after oral administration.

Pro-Val-Pro, was liberated from Lys-Val-Leu-Pro-Val-Pro-Gln by pancreatic digestion, and the shorter peptide had very potent ACEI activity (IC_{50} = 5 μM). This ACEI peptide may be generated from heptapeptide by gastrointestinal digestion and therefore may exhibit antihypertensive activity in SHR. These results suggest that breakdown of the ingested peptides by the gastrointestinal enzyme, in the intestine, affects the biological reactions in animals.

ANTIHYPERTENSIVE PEPTIDES FROM FERMENTED MILK

Single oral administration of a sour milk fermented by a starter containing *L. helveticus* and *S. cerevisiae* to SHR with a dosage of 5 mL/kg of body weight significantly decreases the systolic blood pressure from 6 to 8 h after administration.[21] Two kinds of antihypertensive peptides, Val-Pro-Pro and Ile-Pro-Pro, were purified from the sour milk.[20] Antihypertensive effect of these two peptides were also observed from 2 to 8 h after administration and the effects were dose dependent. These peptides were produced during fermentation,[21] but were not found in the hydrolysate of casein by an extracellular proteinase of *L. helveticus.* These peptides seemed to be processed from the casein molecule by extracellular proteinase followed by some peptidase action during fermentation. Among lactic acid bacteria, *L. helveticus* has generally higher proteolytic activity than other species and the peptide content in the milk medium is higher after fermentation.[57] The antihypertensive effect in SHR was specific to the milk fermented by *L. helveticus* strain. In contrast, the milk fermented with *L. helveticus* CP791, a variant defective for proteinase activity, was not effective on systolic blood pressure (SBP) of SHR.[55]

On the other hand, Furushiro et al. reported an antihypertensive effect of a cell extract from *Lactobacillus casei.*[58] The antihypertensive compounds purified from an extract of *L. casei* cell lysate were polysaccharide–glycopeptide complexes, and showed a molecular weight of 180,000.[59] Sawada et al. suggested that the antihypertensive effect of these complexes is not caused by inhibition of ACE but rather by enhancement of prostaglandin I_2 biosynthesis and the subsequent decrease in peripheral vascular resistance.[60]

ABSORPTION OF BIOACTIVE PEPTIDE

To show the antihypertensive function in the human body, the active peptide has to be absorbed from the intestine as active form. It is known that small peptides, such as di- and tripeptides, are easily adsorbed in the intestine.[61,62] However, there have been no reports about the adsorption of a functional peptide and detection of it in the target organ. In SHR fed with the sour milk, ACE activities of aorta, heart, liver, testes, kidney, lung, and brain were

measured.[63] In ACE activity of the various organs measured, the activity in aorta was significantly lower in the sour milk group than the control group. Ikemoto et al.[64] reported that the ACE activity of aorta in stroked-prone SHR was significantly higher than in normotensive rats. The importance of the decrease of ACE activity in the aorta caused by the expression of antihypertenseive activity of the sour milk in SHR was suggested.[63] Moreover, the major antihypertensive peptides in the sour milk, Val-Pro-Pro and Ile-Pro-Pro, were detected in a heat-treated solubilized fraction from the abdominal aorta of rats given the sour milk, but not in the rats given unfermented milk.[65] Masuda et al.[65] suggested that these peptides are absorbed directly without being decomposed by digestive enzymes. After that they reach the abdominal aorta, inhibit the ACE, and show the antihypertensive effect in SHR.

CLINICAL TEST OF THE ANTIHYPERTENSIVE EFFECT

The antihypertensive effect in humans has not been proven for most of the peptides obtained by processing of food proteins. The antihypertensive effect of a *L. casei* cell extract was confirmed in patients with hypertension.[66] Recently, the antihypertensive effect of a sour milk that contained two peptides, Val-Pro-Pro and Ile-Pro-Pro, was tested in hypertensive patients.[67] These patients were randomly assigned to two groups. One group ingested daily 95 mL of the sour milk for 8 weeks, and another group ingested the same amount of an artificially acidified milk, as a placebo, for 8 weeks. In the sour milk group, systolic blood pressure decreased significantly at 4 and 8 weeks after the beginning of ingestion, but not in placebo group. Merit of the antihypertensive peptide for mild pharmacological effect in the human body can be suggested.

CONCLUSION

In this report, some ACEI peptides derived from food proteins, especially from milk caseins, mainly studied in our laboratory, were introduced. For some of ACEI peptides, significant antihypertensive effects in rats have been demonstrated. Ingested peptides are generally hydrolyzed to small peptides by digestive enzymes. To demonstrate the function in vivo, peptides (a) have to be adsorbed efficiently through the intestine in active form without degradation, (b) have to reach the target organ and (c) have to express the activity. Furthermore, it is necessary to study the mechanism of the antihypertensive effect in vivo. Finally, there is the possibility of pharmaceutical and commercial use of the antihypertensive peptides because of their mild effect and safety with respect to the human body.

I thank Masafumi Maeno and Toshiaki Takano for a critical reading the manuscript, and Robert C. O'Brien for revising the English of the document.

REFERENCES

1. Parker, F., Migliore-Amour, D., Floc'h, F., Zerial, A., Werner, G. G., Jollès, J., Caseretto, M., Zahn, H. & Jollès, P. (1984) *Eur. J. Biochem.* **145,** 677–682.
2. Gurr, M. I. (1987) *FEMS Microbiol. Rev.* **46,** 337–342.
3. Clements, M. L., Levine, M. M. Ristaino, P. A., Daya, V. E. & Hughes, P. T. (1983) *Prog. Food Nutr. Sci.* **7,** 29–37.
4. Mann, G. V. (1977) *Artherosclerosis* **26,** 335–348.
5. Metchnikoff, E. (1908) *The Prolongation of Life,* 1st ed., Putnam's Sons, New York.
6. Bogdanov, I. G., Dalev, P. G., Gurevich, A. I., Kolosov, M. N., Mal'kova, V. P., Plemyannikova, L. A. & Sorokina, I. B. (1975) *FEBS Lett.* **57,** 259–261.
7. Ohashi, T., Ninamishima, Y., Yokokura, T. & Mutai, M. (1989) *Biotherapy* **1,** 89–95.
8. Shahani, K. M. & Shandan, R. C. (1979) *J. Dairy Sci.* **62,** 1685–1694.
9. Fernandes, C. F. & Shahani, K. M. (1990) *J. Food Protect.* **53,** 704–710.
10. Adachi, S. (1992) *Lactic Acid Bacteria in Health and Disease,* Wood, Ed., Elsevier, Barking, pp. 233–262.
11. Tomioka, H. & Saito, H. (1992) *Lactic Acid Bacteria in Health and Disease,* Wood, Ed., Elsevier, Barking, pp. 263–296.
12. Perdigon, G. & Alvarez, S. (1992) *Probiotics, the Scientific Basis,* Fuller, R., Ed., Chapman & Hall, London, pp. 145–180.
13. Perdigon, G., Alvarez, S., Nader de Macas, M., Roux, M. E. & de Ruiz Holgado, A. P. (1990) *J. Food Protect.* **53,** 404–410.
14. De Simone, C., Vesely, R., Negri, R., Bianchi Salvadori, B., Tzantzoglou, S., Cilli, A. & Lucci, L. (1987) *Immunopharmacol. Immunotoxicol.* **10,** 399–415.
15. Kato, I., Yokokura, T. & Mutai, M. (1984) *Microbiol. Imunol.* **28,** 209–217.
16. Sato, K. (1980) *Infect. Immunol.* **44,** 445–451.
17. Sato, K., Saito, H., Tomioka, H. & Yokokura, T. (1988) *Microbiol. Imunol.* **32,** 689–698.
18. Yokokura, T., Nomoto, K., Shimizu, T. & Nomoto, K. (1986) *Infect. Immunol.* **52,** 156–160.
19. Namba, Y., Hidaka, Y., Taki, K. & Morimoto, T. (1981) *Infect. Immunol.* **31,** 580–583.

20. Nakamura, Y., Yamamoto, N., Sakai, K., Okubo, A., Yamazaki, S. & Takano, T. (1995) *J. Dairy Sci.* **78,** 777–783.
21. Nakamura, Y., Yamamoto, N., Sakai, K. & Takano, T. (1995) *J. Dairy Sci.* **78,** 1253–1257.
22. Skeggs, L. T., Jr., Kahn, J. R. & Shumway, N. P. (1956) *J. Exp. Med.* **103,** 295–299.
23. Yang, H. Y. T., Erdös, E. G. & Levin, Y. (1970) *Biochem. Biopys. Acta* **214,** 374–376.
24. Erdös, E. G. & Skidgel, R. A. (1987) *Lab. Invest.* **56,** 345–348.
25. Caldwell, P. R., Seegal, B. C., Hsu, K. C., Das, M. & Soffer, R. L. (1976) *Science* **191,** 1050–1051.
26. Defendini, R., Zimmerman, E. A., Weare, J. A., Alhenc-Gelas, F. & Erdös, E. G. (1983) *Neuroendocrinology* **37,** 32–40.
27. El-Dorry, H. A., Bull, H. G., Iwata, K., Thornberry, N. A., Cordes, E. H. & Sofffer, R. L. (1982) *J. Biol. Chem.* **257,** 14128–14133.
28. Ferreira, S. H., Bartet, D. C. & Greene, L. J. (1970) *Biochemistry* **9,** 2583–2593.
29. Ondetti, M. A., Williams, N. J., Sabo, E. F., Pluvec, J., Weaver, E. R. & Kocy, O. (1971) *Biochemistry* **10,** 4033–4039.
30. Karaki, H., Doi, K., Sugino, S., Uchiwa, H., Sugai, R., Murakami, U. & Takemoto, S. (1990) *Comp. Biochem. Physiol.* **96,** 367–371.
31. Maruyama, S. & Suzuki, H. (1982) *Agricul. Biol. Chem.* **46,** 1393–1394.
32. Maruyama, S., Mitachi, H., Tanaka, H., Tomizuka, N. & Suzuki, H. (1987) *Agricul. Biol. Chem.* **51,** 1581–1586.
33. Maruyama, S., Mitachi, H., Awaya, J. & Suzuki, H. (1987) *Agricul. Biol. Chem.* **51,** 2557–2561.
34. Maruyama, S., Nakagome, K., Tomizuka, N. & Suzuki, H. (1985) *Agricul. Biol. Chem.* **49,** 1405–1409.
35. Kohmura, M., Nio, N., Kubo, K., Minoshima, Y., Munekata, E. & Ariyoshi, Y. (1989) *Agricul. Biol. Chem.* **53,** 2107–2114.
36. Maruyama, S., Miyoshi, S., Kaneko, T. & Tanaka, H. (1989) *Agricul. Biol. Chem.* **53,** 1077–1081.
37. Miyoshi, S., Kaneko, T., Yoshizawa, Y., Fukui, F., Tanaka, H. & Maruyama, S. (1991) *Agricul. Biol. Chem.* **55,** 1407–1408.
38. Miyoshi, S., Ishikawa, H., Kaneko, T., Fukui, F., Tanaka, H. & Maruyama, S. (1991) *Agricul. Biol. Chem.* **55,** 1313–1318.
39. Oshima, G., Shimabukuro, H. & Nagasawa, K. (1979) *Biochim. Biophys. Acta.* **566,** 128–137.
40. Kinoshita, E., Yamakoshi, J. & Ikuchi, M. (1983) *Biosci. Biotech. Biochem.* **57,** 1107–1110.
41. Matsui, T., Matsufuji, H., Seki, E., Osajima, K., Nakashima, M. & Osajima, Y. (1993) *Biosci. Biotech. Biochem.* **57,** 922–925.
42. Kohama, Y., Matsumoto, S., Oka, H. & Teramoto, T. (1988) *Biochem. Biophys. Res. Commun.* **155,** 332–337.
43. Kohama, Y., Oka, H., Kayamori, Y., Tsujikawa, K., Mimura, T., Nagase, Y. & Satake, M. (1991) *Agricul. Biol. Chem.* **55,** 2169–2170.
44. Yokoyama, K., Chiba, H. & Yoshikawa, M. (1992) *Biosci. Biotech. Biochem.* **56,** 1541–1545.
45. Matsumura, N., Fujii, M., Takeda, Y., Sugita, K. & Shimizu, T. (1993) *Biosci. Biotech. Biochem.* **57,** 695–697.
46. Matsumura, N., Fujii, M., Takeda, Y. & Shimizu, T. (1993) *Biosci. Biotech. Biochem.* **57,** 1743–1744.
47. Rubin, B., Antonaccio, M. J., Goldberg, M. E., Harris, D. N., Itkin, A. G., Horovitz, Z. P., Panasevich, R. E. & Laffan, R. J. (1987) *Eur. J. Pharm.* **51,** 377–388.
48. Saito, Y., Wanezaki, K., Kawato, A. & Imayasu, S. (1994) *Biosci. Biotech. Biochem.* **58,** 812–816.
49. Saito, Y., Wanezaki, K., Kawato, A. & Imayasu, S. (1994) *Biosci. Biotech. Biochem.* **58,** 1767–1771.
50. Smid, E. J., Poolman, B. & Konings, W. N. (1991) *Appl. Environ. Microbiol.* **57,** 2447–2452.
51. Thomas, T. D. & Mills, O. E. (1981) *Neth. Milk Dairy J.* **35,** 255–263.
52. Pritchard, G. G. & Coolbear, T. (1993) *FEMS Microbiol. Rev.* **12,** 179–206.
53. Tan, P. S. T., Poolman, B. & Konings, W. N. (1993) *J. Dairy Res.* **60,** 269–286.
54. Yamamoto, N., Akino, A. & Takano, T. (1993) *J. Biochem.* **114,** 740–745.
55. Yamamoto, N., Akino, A. & Takano, T. (1994) *J. Dairy Sci.* **77,** 917–922.
56. Maeno, M., Yamamoto N. & Takano T. (1996) *J. Dairy Sci.* **79,** 1316–1321.
57. Yamamoto, N., Akino, A. & Takano, T. (1994) *Biosci. Biotech. Biochem.* **58,** 776–778.
58. Furushiro, M., Sawada, H., Hirai, K., Motoike, M., Sansawa, H., Kobayashi, S., Watanuki, M., & Yokokura, T. (1990) *Agricul. Biol. Chem.* **54,** 2193–2198.
59. Furushiro, M., Hashimoto, S., Hamura, M. & Yokokura, T. (1993) *Biosci. Biotech. Biochem.* **57,** 978–981.
60. Sawada, H., Furushiro, M., Hirai, K., Motoike, M., Watanabe, T. & Yokokura, T. (1990) *Agricul. Biol. Chem.* **54,** 3211–3219.
61. Adibi, S. A. (1971) *J. Cln. Invest.* **50,** 2266–2275.
62. Hara, H., Funabiki, R., Iwata, M. & Yamazaki, K. (1984) *J. Nutr.* **114,** 1122–1129.
63. Nakamura, Y., Masuda, O. & Takano, T. (1996) *Biosci. Biotech. Biochem.* **60,** 488–489.
64. Ikemoto, F., Tanaka, M., Ito, S., Song, G. B., Tominaga, M., Hiruma, M., Takada, T., Nakamura, N. & Yamamoto, K. (1986) *J. Cardiovasc. Pharacol.* **8,** 69–74.
65. Masuda, O., Nakamura, Y. & Takano, T. (1996) *J. Nutr.* **126,** 3063–3068.
66. Nakajima, K., Hata, Y., Osano, Y., Hamura, M., Kobayashi, S. & Watanuki, M. (1995) *J. Clin. Biochem. Nutr.* **18,** 181–187.
67. Hata, Y., Yamamoto, M., Ohni, M., Nakajima, K., Nakamura, Y. & Takano, T. (1996) *Am. J. Cln. Nutr.* **64,** 767–771.