Activities of Angiotensin-I Converting Enzyme Inhibition in Proteolytic Hydrolyzate of Food Proteins: In View of Development of Physiologically Functional Peptides

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Summary

ACE inhibitory activity in hydrolyzate of food proteins treated by some proteases was tested. The protein from soybean, when it was hydrolyzed by proteases, had larger ACE inhibitory activity, especially at neutral pH. At lower and higher pH all hydrolyzate did not show high ACE inhibitory activity contrary to our expectations. Without treatment of protease, on the contrary, soybean protein had lower ACE inhibitory activity than those of wheat and corn.

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Angiotensin I-converting enzyme (ACE) is a dipeptidyl carboxy-peptidase which removes a dipeptide from the biologically inactive decapeptide angiotensin-I and produces the potent vasopressor octapeptide angiotensin-II. Thus, ACE plays a pivotal role in blood pressure regulation.[1-3] ACE inhibitory were first discovered in snake venom.[4,5] After then various ACE inhibitors were put forward to a medicinal market as important medical supplies. Captopril, for example, was developed as an orally effective inhibitor, based upon a hypothetical model of the binding site on the enzyme.[6] Peptidic inhibitors for ACE were also presented by many workers. Some of them were prepared in proteolytic hydrolyzate of a food protein, casein, and determined on their amino acid sequences.[7,8]

This report treats whether several food proteins obtain an inhibitory action for ACE when they are hydrolyzed by commercial proteolytic enzymes.

Materials and Methods

Materials The food proteins used were those normally prepared from soybean, wheat and corn. The commercial proteases are shown in each table. Both proteins and proteases were given by Nakano Vinegar Co., Ltd. Other materials used were those of commercial grade.

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Incubation The hydrolytic reaction was achieved by vigorously stirring the reaction mixture in an incubator. The reaction mixture was composed of the following: 10m*l* of 0.2M phosphate buffer at appropriate pH, 500mg of protein and 10mg of enzyme. The normal reaction conditions was temperature at 37°C and reaction time for 24 hours. At the end of the incubation, the reaction was stopped by dipping in water bath at 100°C for 5 min and the mixture was centrifuged at 10,000×g for 10 min. The supernatant was used as the test solution of ACE inhibition.

Assay for ACE inhibitory activity The ACE inhibitory activity was assayed by the use of extract from rabbit lung acetone powder and partially purified ACE from hog kidney cortex. The former was prepared by blending 10g of the powder in 100m*l* of 50mM potassium phosphate buffer, pH 8.3 and centrifuging for 40min at 40,000×g. The clear supernatant obtained is of high ACE activity and stable at 4°C.[9] The latter was prepared by refering to Oshima et al[10]. Fresh fog kidney from a domestic slaughterhouse was washed with saline to get rid of blood. The cortex was cut off thinly and chopped into small pieces. They were suspended in 0.05M Tris-HC1 buffer, pH 7.4 in which sucrose was included of 0.25M and homogenized with Waring blender for 4 min. The homogenate was centrifuged at 5,000×g for 30 min and the supernatant was again centrifuged at 50,000×g for 2 hr. The precipitate was suspended in 0.05M Tris-HC1 buffer, pH 7.4 of 0.26% desoxycholate, mixing with a glass homogenizer. After standing overnight in a refrigerator, the suspension was centrifuged at 50,000×g for 2 hr. Both supernatants obtained above were mixed with DEAE-Cellulose with the same buffer, the adsorbed enzyme on the cellulose was eluted with 5mM Tris-HC1 buffer, pH 7.4 in which NaCl was included of concentration of 0.3M. The eluate was adjusted to pH 6.8 and $Ca_3(PO_4)_2$ was added, following readjustment to pH 6.8. The mixture was stirred for 1 hr and centrifuged at 5,000×g for 10 min. The adsorbed enzyme was extracted with 0.05M sodium phosphate, pH 6.8 and centrifuged. The supernatant was concentrated to about one-tenth by cellulose membrane and dialyzed against 5mM Tris-HCl, pH 7.4, containing 0.1mM $CoCl_2$ for 24 hr. The solution was used as ACE preparation after proper condensason. It was comparably or more active than that from rabbit lung acetone powder.

Inhibitory activity was determined as percentage of inhibition of assay sample to activity for HHL (hippuryl-L-histidyl-L-leucine).[10] Each 500*μl* aliquot contained the following components: 250*μl* of 100mM potasium phosphate buffer, pH 7.4; 50*μl* of 3M NaCl; 50*μl* of 50mM HHL; 50*μl* of distilled water; 50*μl* of ACE solution; 50*μl* of sample solution (or 50% methanol for blank). The assay mixture was incubated for 45 min (in an incubator) at 37°C. The reaction was

terminated by addition of 500μl of 1N HCl. The mixture was shaked vigorously with 3ml of ethylacetate for *ca* 10 seconds and centrifuged at 2,000rpm for 10 min. One ml of the upper layer was dried at 120°C in an oil bath. After dryness, 3ml of distilled water was added and shaked. The solution thus obtained was measured at 228nm.

Results and Discussion

Tables 1, 2 and 3 show percent inhibition of hydrolyzate of soybean-, wheat- and corn-protein respectively at pHs of 2.3, 7.2 and 12.3. The results demonstrated that with pH larger ACE inhibitory activity was given at pH around neutral, although there was different aspect within three proteins. Especially soybean protein was more potent for ACE inhibitory activity. At both lower

Table 1 ACE inhibitory activity of soybean-protein-hydrolyzate (%)

Enzyme	Buffer pH		
	2.3	7.2	12.3
1. Protease A-Amano (6～10)	55	91	0
2. Protease M-Amano (3～6)	55	92	0
3. Protease N-Amano (6～8)	15	98	0
4. Protease P-Amano (7～9)	38	89	0
5. A 1-Amano, Miso (7)	52	90	0
6. A 2-Amano, Miso (7)	37	88	0
7. Nulase F (3～7)	51	70	0
8. Papain W-40 (3～12)	43	95	0
9. Proleather (9～12)	11	96	0
10. Bromelain F (6～8)	52	100	6
11. Sumityme LP20 (5～7)	60	90	0
12. Sumityme LPL (5～7)	50	88	0
13. Sumityme MP (6～9)	47	89	0
14. Denapsin 2P (2～5)	52	65	0
15. Denatyme AP (6～8)	58	87	0
16. Bioplase PN4 (6～9)	26	100	0
17. Protin PC10 (5～7)	15	100	0
18. Thermoase PC10 (6～9)	8	91	10
19. No enzyme	40	0	85

1-10 : product of Amano Pharmaceuticals Co., Ltd.
11-13 : product of Shinnippon Chemicals Co., Ltd.
11-16 : product of Nagase Biochemicals Co., Ltd.
17, 18 : product of Daiwakasei Co., Ltd.
The values in parentheses are the nominal enzyme activity range.
All the percentages are subtracted the blank (no enzyme).

Table 2 ACE inhibitory activity of wheat-protein-hydrolyzate (%)

Enzyme	Buffer pH		
	2.3	7.2	12.3
1. Protease A-Amano	17	39	60
2. Protease M-Amano	24	43	40
3. Protease N-Amano	16	51	39
4. Protease P-Amano	17	38	18
5. A 1-Amano, Miso	21	45	0
6. A 2-Amano, Miso	11	37	0
7. Nulase F	33	16	0
8. Papain W-40	27	44	0
9. Proleather	16	45	0
10. Bromelain F	22	33	0
11. Sumityme LP20	18	40	35
12. Sumityme LPL	26	44	39
13. Sumityme MP	15	45	0
14. Denapsin 2P	0	0	28
15. Denatyme AP	18	41	0
16. Bioplase PN4	14	37	14
17. Protin PC10	20	44	48
18. Thermoase PC10	13	32	0
19. No enzyme	62	48	20

All the percentages are subtracted the blank (no enzyme).

and higher pH, each protein resulted in lower ACE inhibitory activity than that at neutral one. The limiting pH value may cause these results, that is, it may deactivate ACE in part. It is very interesting that at high and low pH values of inhibitory activity were reverse for soybean and wheat respectively. Table 4 shows the temperature dependence on percent inhibition, using three types of enzyme which have a typical pH range of activity. There was no noteworthy acceleration of percent value at both high and low temteratures. We tried to hydrolyze as different protein concentrations (2.5-15%, w/v) and obtained almost same values. This should be attributed to stirring way of the mixture of protein and enzyme, because protein content in the mixture changes the viscosity of solution. The large content of protein makes the viscosity high, thus the effect of stirring should be minimized. Anyway hydrolyzation of food protein by proteolytic enzyme demonstrated to be capable of showing some interesting aspects for ACE inhibitory activity.

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Table 3 ACE inhibitory activity of corn-protein-hydrolyzate(%)

Enzyme	Buffer pH		
	2.3	7.2	12.3
1. Protease A-Amano	11	37	0
2. Protease M-Amano	7	18	0
3. Protease N-Amano	8	61	0
4. Protease P-Amano	3	55	0
5. A1-Amano, Miso	7	30	0
6. A 2-Amano, Miso	8	12	0
7. Nulase F	10	17	0
8. Papain W-40	11	60	0
9. Proleather	0	56	0
10. Bromelain F	13	57	0
11. Sumityme LP20	13	49	0
12. Sumityme LPL	10	23	0
13. Sumityme MP	8	64	0
14. Denapsin 2P	12	8	0
15. Denatyme AP	4	35	0
16. Bioplase PN4	0	44	0
17. Protin PC10	4	37	0
18. Thermoase PC10	4	38	0
19. No enzyme	73	27	100

All the percentages are subtracted the blank (no enzyme).

Table 4 ACE inhibitory activity of soybean-protein-hydrolyzate (%) : Temperature dependence

Enzyme	Temp (°C)	Buffer pH		
		2.3	7.2	12.3
Protin PC10	15	0	67	29
	37	15	100	0
	60	5	58	14
Denapsin 2P	15	32	9	6
	37	52	65	0
	60	32	7	0
Proleather	15	27	70	15
	37	11	96	0
	60	13	61	14

All the percentages are subtracted the blank (no enzyme).

References

1) R.L. Soffer, *Ann. Rev. Biochem.*, 45, 73 (1976).
2) M.J. Peach, *Physiol. Rev.*, 57, 313 (1977).
3) I.A. Reid, B.J. Morris and W.F. Ganong, *Ann. Rev. Physiol.*, 40, 377 (1978).
4) M.A. Ondetti, N.J. Williams, E.F. Sabo, J. Pluscec, E.R. Weaver and O. Kocy, *Biochemistry*, 10, 4033 (1971).
5) H. Kato and T. Suzuki, *Biochemistry*, 10, 937 (1971).
6) D.W. Cushman, H.S. Cheung, E.F. Sabo and M.A. Ondetti, *Biochemistry*, 16, 5484 (1977).
7) S. Maruyama and H.Suzuki, *Agric. Biol. Chem.*, 46, 1393 (1982).
8) S. Maruyama, K. Nakagomi, N. Tomizuka and H. Suzuki, *Agric. Biol. Chem.*, 49, 1405 (1985).
9) D.W. Cushman and H.S. Cheung, *Biochem. Pharmacol.*, 20, 1637 (1971).
10) G. Oshima, K. Nagasawa and J. Kato, J. Biochem., 80, 477 (1976).

食品タンパク質のプロテアーゼ分解物中における
アンジオテンシン—I変換酵素阻害活性:
生理機能性ペプチドの開発をめざして

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最近，機能性食品の名のもと，種々の生理的機能を持たせた食品が市場にだされている。ここではその研究の一環として，食物タンパク質をプロテアーゼで限定加水分解することにより，その分解物中に抗高血圧活性物であるアンジオテンシン-I変換酵素（ACE）阻害剤の存否を探索した。食物タンパク質として，大豆，小麦，トウモロコシからのものを用い，プロテアーゼは市販の18種を用いた。殆どの条件で各タンパク質とも，酵素処理によりACE阻害活性は高められた。しかしタンパク質間には異なった特性がみられ，種々の様相を呈したが，中性 pH での大豆タンパク質において最も大きい阻害活性が認められた。

これらの結果から，この方法は機能性食品を効果的に得る可能性を示唆するものであった。