Short Paper

Inhibitory Activity against Angiotensin I-Converting Enzyme of Peptides Originating from Fish and Shellfish Muscle*¹

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Food proteins are changed into small peptides by digestive enzymes and some of the resultant peptides were found to have physiological activities.¹⁾ We have observed *in vitro* inhibitory activity against angiotensin I-converting enzyme (EC 3.4.15.1) of basic peptides originating from the muscle of sardine and hair tail²⁾ and *in vivo* blood pressure lowering and vasodilatory effects of these peptides.³⁾ In this paper we report the inhibitory activity against angiotensin I-converting enzyme and the amino acid compositions of muscle peptides from 12 kinds of fish and 3 kinds of shellfish.

Five hundred grams of various fish and shellfish muscles were homogenized in 1 l of deionized water. Ten grams of pepsin (EC 3.4.23.1, Merck Co.) was added to the homogenates whose pH had been adjusted to 2.0 with 1 N-HCl, and the mixtures were incubated for 20 h at 37°C with stirring. After the incubation, the reaction mixtures were immediately subjected to ultrafiltration through Diaflo-membrane of type YM 10 (φ 76 mm). The filtrate was applied to a column (4.2 × 15 cm) of Dowex 50 W-X4 (50–100 mesh, H⁺ form) cation exchange resin. After the column was washed sufficiently with deionized water

to remove some impurities, the desired peptides were eluted with 2 l of 2N-NH₄OH. After being concentrated to 100 ml under vacuum, the eluate was applied to a column (2.5 × 150 cm) of Sephadex G-25 (medium). Required fractions were collected and concentrated to dryness to give peptide powder.

Inhibitory activity against angiotensin I-converting enzyme was measured as previously described.²⁾ ID₅₀ value was defined as mg of peptide powder, when enzyme activity was reduced by 50%. Peptide (10 μg) was hydrolyzed with 200 μl of 6N-HCl in an evacuated sealed glass tube for 24 h at 108°C. Amino acid analyses were performed on a PICO-TAGTM amino acid analyzer (Waters Associates).⁴⁾ The inhibitory activity (ID₅₀) and amino acid compositions of the fish and shellfish peptides are shown in Table 1. ID₅₀ values of sardine and skipjack peptides were lower than those of the others. Amino acid analyses showed that fish peptides were rich in aspartic acid, glutamic acid, arginine, proline, isoleucine and lysine and that shellfish peptides were rich in aspartic acid, glutamic acid and lysine. Isolation of these peptides and determination of their amino acid sequences are under investigation.

References

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Table 1. Inhibitory activity (ID₅₀) against angiotensin I-converting enzyme and amino acid compositions*¹ of fish and shellfish muscle peptides

Fish name	(ID ₅₀)* ²	ASP	GLU	SER	GLY	HIS	ARG	THR	PRO
Black porgy	68.7	6.87	7.85	2.62	5.67	2.22	8.12	1.65	7.14
Skipjack	29.6	7.68	8.14	3.09	5.64	3.51	7.90	3.15	7.76
Marlin	59.1	6.38	7.50	2.63	4.78	4.50	7.09	2.48	9.94
Pacific mackerel	56.7	8.34	9.40	4.43	5.51	2.21	8.92	2.46	10.14
Jack mackerel	48.2	6.51	8.38	3.24	4.74	2.04	6.93	3.10	7.72
Flounder	54.4	7.02	10.37	2.34	3.95	4.28	8.91	2.56	8.73
Spanish mackerel	84.9	9.02	9.76	2.19	2.88	2.50	8.26	2.08	6.88
Atka mackerel	66.5	8.47	9.43	2.33	4.03	2.15	7.11	1.87	6.98
Flying fish	53.3	8.02	11.37	2.68	4.90	2.26	7.89	2.98	7.48
Sting ray	63.4	7.38	10.01	3.37	5.15	2.42	8.02	2.88	6.54
Sardine	27.1	9.68	15.31	2.56	2.39	3.02	6.85	2.97	5.57
Hair tail	56.6	9.89	9.26	2.58	3.17	2.94	6.13	2.74	5.22
Pearl oyster	28.8	12.8	16.3	5.6	6.1	1.7	7.4	5.5	2.3
Blue mussel	45.4	14.6	16.4	5.3	5.7	1.4	6.9	4.6	2.8
Little clam	31.0	12.7	14.7	4.9	5.3	1.3	6.4	4.3	2.3

Fish name	ALA	TYR	VAL	MET	CYS	ILEU	LEU	PHE	LYS
Black porgy	4.07	1.42	3.20	1.65	0.89	6.09	7.39	1.26	31.89
Skipjack	3.42	1.74	2.75	1.67	0.65	5.91	8.47	1.49	27.03
Marlin	3.11	1.69	3.94	2.00	0.65	5.82	6.50	1.72	29.27
Pacific mackerel	3.48	0.90	3.01	3.11	0.82	6.27	9.65	0.71	20.64
Jack mackerel	4.55	1.17	1.02	2.76	1.08	6.89	6.89	0.93	31.39
Flounder	3.02	1.53	1.98	2.07	0.42	7.38	7.42	0.90	27.12
Spanish mackerel	2.91	1.40	1.05	1.15	1.05	5.61	6.38	0.79	36.09
Atka mackerel	2.13	1.53	1.84	2.31	1.84	6.46	7.99	0.67	34.39
Flying fish	2.67	1.58	1.65	1.09	0.91	7.23	10.62	0.90	25.79
Sting ray	2.43	1.83	1.76	2.03	0.70	5.98	9.27	0.78	29.45
Sardine	2.05	1.53	1.76	1.08	1.23	6.32	8.33	0.62	28.73
Hair tail	1.87	0.98	1.90	1.23	0.66	8.03	8.42	0.44	36.54
Pearl oyster	6.1	4.2	4.9	1.1	0.8	10.9	6.9	3.1	9.5
Blue mussel	6.4	4.3	4.4	1.4	0.7	9.3	7.4	3.5	10.0
Little clam	5.9	4.0	4.1	1.1	0.6	8.6	6.9	3.2	9.4

*¹ Expressed as g/100 g of peptides.

*² Expressed as mg of peptides when angiotensin I-converting enzyme activity was reduced by 50%.

*¹ 魚貝類筋肉由来ペプチドのアンジオテンシンI変換酵素阻害能について.

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