CHAPTER 16

Marine Fish-Derived Bioactive Peptides as Potential Antihypertensive Agents

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Contents

Abstract

Hypertension is the most widespread risk factor for many serious cardiovascular diseases. Angiotensin-converting enzyme (ACE) plays a crucial role in cardiovascular physiological regulation by converting angiotensin I to a potent vasoconstrictor, angiotensin II. Hence, the inhibition of ACE is a key target for antihypertensive activity. Recently, potent antihypertensive peptides have been purified widely by enzymatic hydrolysis of muscle protein, skin collagen, and gelatin of many different kinds of marine fishes. Marine fish-derived bioactive peptides can be developed as antihypertensive components in functional foods or nutraceuticals. This contribution presents an overview of the ACE inhibitory peptides derived from marine fishes and discusses their future prospects to be used as potential drug candidates for preventing and treating high blood pressure.

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I. INTRODUCTION

Hypertension (high blood pressure) is increasingly prevalent in developed countries and is one of the major independent risk factors for myocardial infarction, congestive heart failure, arteriosclerosis, stroke, and end-stage renal disease. Angiotensin-converting enzyme (ACE, a dipeptidyl carboxypeptidase) plays an important role in the regulation of blood pressure as well as cardiovascular function by converting the decapeptide angiotensin I to the vasoconstricting octapeptide angiotensin II. Moreover, ACE inactivates bradykinin, a vasodilatory peptide, which leads to increase blood pressure (Shahidi and Zhong, 2008).

ACE inhibitors are considered to be useful therapeutic approaches to treat hypertension, heart failure, stroke, and myocardial infarction. Therefore, in the development of drugs to control high blood pressure, three kinds of synthetic ACE inhibitors were designed; they are grouped by their ligand for the active site on ACE. Captopril, the key representative of this group, has a sulfhydryl moiety; enalapril and lisinopril have a carboxyl moiety; and fosinopril has a phosphorus group. Many studies have been attempted in the synthesis of ACE inhibitors, which are currently used as clinical antihypertensive drugs (Wijesekara and Kim, 2010). However, these synthetic drugs are believed to have adverse side effects such as cough, taste disturbances, dizziness, headache, skin rashes, and angioneurotic edema. Therefore, it is necessary to search safer, more economical, more innovative, and no-side-effect ACE inhibitors in the treatment of essential hypertension and heart failure in humans (Kamath *et al.*, 2007).

Recently, marine fish-derived bioactive peptides have been shown to possess many physiological functions including antihypertensive, antioxidant, antimicrobial, antiproliferative, antitumor, anticoagulant, and immunomodulatory activities. Among these, antihypertensive peptides act as ACE inhibitors are of particular interest for prevention and treatment of hypertension (Kobayashi *et al.*, 2008).

Marine fishes are rich sources of structurally diverse bioactive compounds including polyunsaturated fatty acids, polysaccharides, minerals, vitamins, antioxidants, enzymes, and bioactive peptides (Kim *et al.*, 2008). Marine fish-derived ACE inhibitory peptides have been purified from enzymatic digestion of various fish materials from Alaska pollack (Nakajima *et al.*, 2009), bonito (Fujita *et al.*, 2000; Hideaki *et al.*, 1993; Yokoyama *et al.*, 1992), tuna (Hwang, 2010), salmon (Ohta *et al.*, 1997), shark (Wu *et al.*, 2008), and sardine (Bougatef *et al.*, 2008; Otani *et al.*, 2009). Hence, a great interest has been developed nowadays to obtain bioactive compounds, which act as ACE inhibitors from marine fishes due to their numerous health beneficial effects. This chapter discusses the marine fish-derived antihypertensive peptides and their potential applications as ingredients in functional foods and nutraceuticals to prevent hypertension in humans.

II. DEVELOPMENT OF MARINE FISH-DERIVED ANTIHYPERTENSIVE PEPTIDES

Bioactive peptides can be purified from enzymatic hydrolysis of different marine fish sources using appropriate proteolytic enzymes. Proteolytic enzymes derived from plants, animals, and microbes can be used for the hydrolysis process of marine fish products to develop bioactive peptides. The physicochemical conditions of the reaction media, such as temperature and pH of the protein solution, must then be adjusted to optimize the activity of the enzyme used (Slizyte *et al*., 2009). Kim *et al*. (1997) used the crude proteinase which was extracted from the pyloric ceca of tuna for enzymatic hydrolysis of cod frame protein under optimal conditions in order to obtain a maximum yield. Further, α-chymotrypsin, papain, neutrase, and trypsin have been used for the hydrolysis of tuna dark muscle under optimal pH and temperature conditions of the respective enzymes. Moreover, one of the most important factors in producing bioactive peptides with desired functional properties is the molecular weight of the bioactive peptide. Therefore, for the efficient recovery and to obtain bioactive peptides with both a desired molecular size and a functional property, a suitable method is the use of an ultrafiltration membrane system. This system has the main advantage that the molecular weight distribution of the desired peptide can be controlled by adoption of an appropriate ultrafiltration membrane. In order to obtain functionally active peptides, it is a suitable method to use a three-enzyme system for sequential enzymatic digestion. Moreover, it is possible to obtain serial enzymatic digestions in a system using a multistep recycling membrane reactor combined with ultrafiltration membrane system to separate marine fish-derived bioactive peptides (Kim and Mendis, 2006). This membrane bioreactor technology equipped with ultrafiltration membranes is recently emerging for the development of bioactive compounds and considered as a potential method to utilize marine fish proteins as value-added nutraceuticals with beneficial health effects.

III. ANTIHYPERTENSIVE ACTIVITY OF BIOACTIVE PEPTIDES DERIVED FROM MARINE FISHES

Nowadays, ACE inhibitory peptides have been isolated from meat, remaining muscle proteins, skin collagen and gelatin, bone, and internal organs of fishes such as Alaska pollack, bonito, tuna, salmon, shark, and sardine. Table 16.1 provides a partial summary of ACE inhibitory peptides derived from marine fish sources, their amino acid sequence, the enzyme used for hydrolysis, and IC_{50} values. The IC_{50} value is the concentration of peptide that inhibits 50% of ACE activity.

TABLE 16.1 ACE inhibitory peptides derived from marine fish: source, enzyme used for hydrolysis, amino acid sequence and IC_{50} value

Source	Enzyme	Amino acid sequence	IC_{50} (μM)	Reference
Alaska pollack skin	Alcalase+pronase+ collagenase	GPL	2.6	Byun and Kim (2002)
Alaska pollack skin		LGP	0.72	Byun and Kim (2002)
Alaska pollack skin		GLP	1.62	Byun and Kim (2002)
Alaska pollack skin		PLG	4.74	Byun and Kim (2002)
Alaska pollack skin		LPG	5.73	Byun and Kim (2002)
Alaska pollack skin		PGL	13.93	Byun and Kim (2002)
Alaska pollack frame	Pepsin	FGASTRGA	14.7	Je *et al*. (2004)
Bonito muscle	Thermolysin	IKPLNY	43	Yokoyama *et al*. (1992)
Bonito muscle	Thermolysin	DYGLYP	62	Yokoyama *et al*. (1992)
Bonito bowels	Autolysis	LRP	1	Matsumura *et al*. (1993b)
Bonito liver	Autolysis	GVYPHK	1.6	Hideaki *et al*. (1993)
Bonito intestine	Autolysis	IRPVE	1.4	Hideaki *et al*. (1993)
Bonito muscle	Thermolysin	LKP	0.32	Fujita and Yoshikawa (1999)
Bonito muscle	Thermolysin	ILP	6.9	Fujita and Yoshikawa (1999)
Bonito muscle	Thermolysin	LKPNM	2.4	Fujita and Yoshikawa (1999)
Bonito muscle	Thermolysin	IWHHT	5.1	Fujita *et al*. (2000)
Bonito meat	Pepsin	HERDPTHIKWGD	8	Hasan *et al*. (2006)
Bonito meat	Pepsin	PTHIKWGD	8	Hasan *et al*. (2006)
Bonito protein		IKW	0.4	Hasan *et al*. (2007)
Bonito protein		IKY	1	Hasan *et al*. (2007)
Bigeye tuna muscle	Pepsin	WPEAAELMMEVDP	21.6	Qian *et al*. (2007)
Bigeye tuna frame	Pepsin	GDLGKTTTVSNWSPPKYKDTP	11.28	Lee *et al*. (2010)
Salmon muscle	Thermolysin	VW	2.5	Ono *et al*. (2003)

Salmon muscle	Thermolysin	IW	4.7	Ono *et al*. (2003)
Salmon muscle	Thermolysin	MW	9.9	Ono *et al*. (2003)
Salmon muscle	Alcalase+papain	IW	1.2	Enari *et al*. (2008)
Shark meat	Protease	FE	1.45	Wu *et al*. (2008)
Shark meat	Protease	CF	1.98	Wu *et al*. (2008)
Shark meat	Protease	EY	2.68	Wu *et al*. (2008)
Shark meat	Protease	MF	0.92	Wu *et al*. (2008)
Sardine muscle	Alcalase	KW	1.63	Matsufuji *et al*. (1994)
Sardine muscle	Alcalase	AKK	3.13	Matsufuji *et al*. (1994)
Sardine muscle	Alcalase	GWAP	3.86	Matsufuji *et al*. (1994)
Sardine muscle	Alcalase	VY	26	Kawasaki *et al*. (2000)
Sardine head	Proteases	nd	1.2*	Bougatef *et al*. (2008)
Sardine viscera	Proteases	nd	0.81*	Bougatef *et al*. (2008)

nd, not detected.
* mg/ml.

The competitiveness against ACE activity of different antihypertensive peptides has been determined kinetically using Lineweaver–Burk plots (Zhao *et al.*, 2009). Generally, the mechanism of action of antihypertensive peptides is different from that of synthetic drugs. The synthetic drugs basically, indiscriminately block ACE by interfering with its action, while ACE inhibitory peptides interact much differently by competing with ACE. ACE converts angiotensin I to angiotensin II by cleaving off a small peptide. Synthetic drugs work by directly blocking the action of ACE. ACE actually reacts with the antihypertensive peptides instead of attacking angiotensin I. Antihypertensive peptides relax the arterial walls and reduce fluid volume by inhibiting the formation of angiotensin II. Therefore, antihypertensive peptides actually improve heart function and increase blood and oxygen flow to the heart, liver, and kidneys. Many studies have shown that tryptophan, tyrosine, phenylalanine, or proline at the C-terminal and branched-chain aliphatic amino acids at the N-terminal were suitable for a peptide binding to ACE as a competitive inhibitor (Ahhmed and Muguruma, 2010).

In addition, a noncompetitive mechanism has also been observed in some peptides, and this means that the peptide can combine with an enzyme molecule to produce a dead-end complex, regardless of whether a substrate molecule is bound or not. For example, LIY (Nakagomi *et al.*, 2000) and YLYEIAR (Nakagomi *et al.*, 1998) have been found to act as noncompetitive inhibitors. The hydrophobicity of the N-terminus, which is one of the common features of ACE inhibitory peptides, may contribute to the inhibitory activity. ACE inhibitory peptides are generally short-chain peptides, often carrying polar amino acid residues like proline. Further, structure–activity relationships among various peptide inhibitors of ACE indicate that binding to ACE is strongly influenced by the C-terminal tripeptide sequence of the substrate, and it is suggested that peptides, which contain hydrophobic amino acids at these positions, are potent inhibitors (Rho *et al.*, 2009).

Proteolytic digestion of gelatin extracts from Alaska pollack (*Theragra chalcogramma*) skin brought about a high ACE inhibitory activity. Gelatin extracts were hydrolyzed by serial protease treatments in the order of alcalase, pronase E, and collagenase using a three-step recycling membrane reactor. The isolated peptide was composed of GPL and showed an IC_{50} value of 2.6μM (Byun and Kim, 2002). In addition, the peptides GLP, LGP, LPG, PGL, PLG, GP, and PL, which consisted of glycine, proline, and leucine, were synthesized by the solid-phase method from Alaska pollack skin. The IC_{50} values of each dipeptide—namely, GP and PL—were 252.6 and 337.3μM, respectively. The IC_{50} values of each tripeptide—namely, LGP, GLP, PLG, LPG, and PGL—were 0.72, 1.62, 4.74, 5.73, and 13.93μM, respectively. The ACE inhibitory activity of these tripeptides was higher than that of dipeptides. Among these tripeptides, LGP

and GLP had higher inhibitory activity than GPL. Among the different types of tripeptides that were examined, the highest ACE inhibitory activity was observed for LGP. LGP has the highest ACE inhibitory activity among the different types of tripeptides derived from Alaska pollack skin. It had the leucine residue at the N-terminal and proline residue at the C-terminal. Further, Je *et al*. (2004) purified a novel ACE inhibitory peptide from Alaska pollack frame protein hydrolyzed with pepsin with an IC_{50} value of 14.7 μM, and the sequence of the peptide was FGASTRGA. In addition, the ACE inhibition pattern of the peptide was found to be noncompetitive.

Ten ACE inhibitory peptides were isolated from bonito bowels autolysate (Matsumura *et al*., 1993a,b) IC_{50} of ACE inhibitory peptides—namely, YRPY, GHF, VRP, IKP, LRP, IRP, SVAKLEK, ALPHA, GVYPHK, and IRPVQ—were estimated to be 320, 1100, 2.2, 2.5, 1.0, 1.8, 82, 79, 1.6, and 1.4 μM, respectively. C-terminal amino acids were considered to be essential for their expression of ACE inhibition, while the N-terminal tripeptide IRP was presumed to inhibit ACE after the removal of a dipeptide from IRPVQ with ACE digestion. In addition, LKPNM (IC_{50} = 2.4 μM) from bonito fish product has found to be hydrolyzed by ACE to produce LKP (IC_{50} = 0.32 μM), which had eightfold higher ACE inhibitory activity compared with the initial peptide (Fujita and Yoshikawa, 1999). Further, two peptides HERDPTHIKWGD and PTHIKWGD from bonito muscle in an artificial gastric juice were purified and their IC_{50} values were about 8 μM (Hasan *et al*., 2006). Tripeptides IKW and IKY derived from bonito protein showed high ACE inhibitory activities with IC_{50} of 0.4 and 1.0 μM, respectively, and acted as competitive inhibitors (Hasan *et al*., 2007).

A novel ACE inhibitory peptide (PTHIKWGD) was purified from acid extract of tuna muscle. Further, this peptide has inhibited ACE activity by noncompetitively with K_i values of 1.7 and 5.7 μM with substrates hippuryl-L-histidyl-L-leucine and angiotensin I, respectively (Kohama *et al*., 1989). The structure of ACE inhibitory peptide from pepsin hydrolysate of bigeye tuna dark muscle, *Thunnus obesus* was identified to be WPEAAELMMEVDP, and the IC_{50} value of the peptide was 21.6 μM (Qian *et al*., 2007). Numerous *in vivo* studies of marine fish-derived antihypertensive peptides in spontaneously hypertensive rats (SHR) have shown potent ACE inhibitory activity, and their systolic blood pressure (SBP) has reduced significantly after oral administration of peptides. According to Lee *et al*. (2010), a single oral administration (10 mg/kg of body weight) of the peptide derived from tuna frame protein hydrolysate exhibited *in vivo* activity by lowering blood pressure in SHR, and this antihypertensive activity was similar with captopril, a commercial antihypertensive drug. Further, they have reported that no side effects observed on rats after administration of antihypertensive peptide derived from bigeye tuna.

Thermolysin hydrolysate of defatted upstream chum salmon muscle showed a high inhibitory activity against ACE, with an IC_{50} value of 27.9 μg/ml *in vitro*. After fractionation, six dipeptides containing tryptophan residue were identified as WA, WM, MW, LW, IW, and VW, with an IC_{50} value of 277.3, 96.6, 9.9, 17.4, 4.7, and 2.5 μM, respectively. When orally administrated, the hydrolysate significantly lowered blood pressure for up to 8 h after administration with a maximum decrease 4 h after administration (Ono *et al.*, 2003). Collagen extracted from Atlantic salmon (*Salmo salar* L.) skin was hydrolyzed with alcalase and papain and treated by multistage separation. After fractionation, the ACE inhibitory activities of AP (IC_{50} = 0.060 mg/ml) and VR (IC_{50} = 0.332 mg/ml) were found to be approximately 20-fold and 4-fold higher than that of initial salmon skin collagen peptide (1.165 mg/ml), respectively (Gu *et al.*, 2011). In addition, the antihypertensive effect of the salmon peptide on SHR was examined. After the single intravenous administration of the salmon peptide at a dose of 30 mg/kg body weight, the SBP was significantly reduced against the control. Further, a double-blind, placebo-controlled, parallel-group study determined the efficacy of the salmon peptide in mild hypertensive subjects. The SBP, after a 1.0 g of salmon peptide intake, was significantly reduced at 4 weeks after the intake, and 2 weeks after the intake finished, compared to the value before ingestion. IW had the strongest ACE inhibitory activity (IC_{50} = 1.2 μM) *in vitro* (Enari *et al.*, 2008).

Shark meat hydrolyzed with protease SM98011 showed high ACE inhibitory activity, with an IC_{50} value of 0.4 mg/ml (He *et al.*, 2007). Four peptides with high ACE inhibitory activity were purified from shark meat hydrolysate. Their amino acid sequences were CF, EY, MF, and FE, and their IC_{50} values were 1.98, 2.68, 0.92, and 1.45 mM, respectively. They may have potential in the treatment of hypertension or in clinical nutrition (Wu *et al.*, 2008).

ACE inhibitory peptides derived from sardine and hair tail meat were made by Suetsuna and Osajima (1986). They reported that protease hydrolysates of sardine contained ACE inhibitory peptides with IC_{50} values *in vitro* of 3.79 and 9.01 mg/L. The ACE inhibitory activities of protein hydrolysates prepared from heads and viscera of sardine (*Sardinella aurita*) by treatment with the alkaline protease extract from the viscera of sardine were investigated. The IC_{50} values for ACE inhibitory activities of sardinelle by-products protein hydrolysates and fraction P_4 were 1.2±0.09 and 0.81±0.013 mg/ml, respectively. Fraction P_4 was rich in phenylalanine, arginine, glycine, leucine, methionine, histidine, and tyrosine. The peptide prepared from sardine muscle by *Bacillus licheniformis* alkaline protease displayed the ACE inhibitory activity with an IC_{50} of 260 μg/ml (Matsui *et al.*, 1993). This activity is about 2.4-fold higher than that of a peptic hydrolysate (620 μg/ml) of sardine muscle. The ACE inhibitory activity of an alkaline protease hydrolysate from sardine

muscle did not change after being treated by gastrointestinal proteases ($IC_{50}=82\mu g/ml$). Eleven new ACE inhibitory peptides have been isolated with IC_{50} values mostly below $100\mu M$; the maximal ACE inhibitory activity has been observed for KW ($IC_{50}=1.63\mu M$) (Matsufuji *et al.*, 1994). VY with potent ACE inhibitory activity were intravenously administered to SHR, and a significant reduction of diastolic blood pressure has been determined (Matsufuji *et al.*, 1995). Further, a randomized, double-blind, placebo-controlled study has carried out on 29 volunteers. VY presented a significant antihypertensive effect on mild hypertensive subjects via ACE inhibition, as well as SHR, but no adverse effects could be detected at all (Kawasaki *et al.*, 2000). Ohba *et al.* (2003) studied the physiological functions of enzymatic hydrolysates of collagen or keratin contained in livestock and fish waste. The enzymatic hydrolysate of meat meal, a collagen-waste, showed strong ACE inhibitory activity with IC_{50} values ranging from 600 to $2800\mu g/ml$.

IV. CONCLUSION

Recently, much attention has been paid by consumers toward natural bioactive compounds as functional ingredients, and hence it can be suggested that marine fish-derived ACE inhibitors are alternative tools that can contribute to consumer's well-being, by being a part of novel nutraceuticals or pharmaceuticals replacing synthetic drugs. Food bioactive compounds are often effective in promoting health and lead to the reduction of disease risk. Especially, bioactive compounds derived from marine fishes have served as rich sources of health-promoting components. Among them, bioactive peptides are rich sources of natural health enhancers, and this fact implies their potential use as a functional ingredient in future nutraceutical and pharmaceutical products. Until now, most of these ACE inhibitory activities have been observed *in vitro* or in mouse model systems. Therefore, further research studies are needed in order to investigate their activity in human subjects. In conclusion, it can be suggested that marine fish-derived ACE inhibitory bioactive peptides are potential therapeutic candidates for preventing hypertension and their involvement in the future pharmaceuticals is promising.

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