CHAPTER 15

Development of Bioactive Peptides from Fish Proteins and Their Health Promoting Ability

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Contents

Abstract

Great amount of marine fish species have been identified with potential nutraceutical and medicinal values. Consequently, a number of bioactive compounds have been identified including fish muscle proteins, peptides, collagen and gelatin, fish oil, fish bone. Bioactive peptides derived from various fish muscle proteins have shown various biological activities including antihypertensive, antibacterial, anticoagulant, anti-inflammatory, and antioxidant activities, and hence they may be a potential material for biomedical and

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food industries. Further, they are commonly used in medical and pharmaceutical industries as carrier molecules for drugs, proteins, and genes. Hence, fish muscle protein-derived peptides are valuable natural resources that can be potential material for biomedical, nutraceutical, and food industries.

I. INTRODUCTION

The marine environment has a tremendous biodiversity, and it is a source of a plethora of bioactive compounds with great potential for food and pharmaceutical areas. Ocean covers 70% of the earth and around 70% of creatures are living in it. Recent studies have confirmed that most of these species possess active compounds but still underutilized sources for various applications. Fish used as human food accounts for 78% of the total fish catch in developing and developed countries (Vannuccini, 2004). Fish catch is mainly used for human consumptions and other minor uses such as meal production and bait. Moreover, many studies are conducting with the fish wastes discarded from fisheries industries. Great amount of researches have been initiated to investigate an increased utilization of fish muscle protein and found that they possess potential bioactive materials such as gelatin, collagen, peptides, and various fatty acids including EPA and DHA in various industries (Wassawa *et al*., 2007).

Recent studies have reported a number of bioactive peptides from various fish sources. They possess short sequences with approximately 2–20 amino acids in length that exert physiological benefits when consumed *in vivo*. They can be produced by proteolytic hydrolysis using commercially available enzymes including trypsin, chymotrypsin, alcalase, or proteolytic microorganisms and fermentation methods (Vercruysse *et al*., 2005). After digestion, bioactive peptides can be absorbed in the intestine and enter the blood stream directly, which ensures their bioavailability *in vivo* and a physiological effect at the target site (Erdmann *et al*., 2008). A growing number of literatures demonstrated that peptides from marine organisms had a wide spectrum of effects, such as antihypertensive, immunomodulating, antithrombotic, antioxidative, anticancer, and antimicrobial (Je *et al*., 2007; Vercruysse *et al*., 2005; Yang *et al*., 2010). A number of bioactive compounds have been isolated from remaining fish muscle proteins, collagen and gelatin, fish oil, fish bone, and internal organs (Je *et al*., 2005; Jeon and Kim, 2002; Kim *et al*., 2001). Generally, a far better profitability is obtained by producing human consumables, and the highest profitability is currently expected from bioactive compounds. These bioactive compounds can be extracted and purified with technologies varying from simple to complex, and such compounds may include preparation and isolation of bioactive peptides

for biotechnological and pharmaceutical applications. Further, some of these bioactive compounds have been identified to possess nutraceutical potentials that are beneficial in human health promotion. Therefore, much attention must be paid for the development of new technologies in search of novel bioactive compounds from marine fish protein. This chapter discusses trends in developing bioactive peptides from marine fish protein and their biological activities relating to human health.

II. STRUCTURE–ACTIVITY RELATIONSHIP OF FISH PEPTIDES

Peptide quantitative structure–activity relationship modeling has been used for predicting peptide structures with high ACE inhibitory activity. Using physicochemical descriptors, Pripp *et al*. (2004) emphasized that increased side chain hydrophobicity at the carboxy-terminus and decreased side chain length of the penultimate amino acid exemplified ACE inhibitory potential of peptides up to six amino acids in length. Moreover, ACE appears to be strongly influenced by the C-terminal sequence of the peptides. Hence, it has been suggested that proline, lysine, or arginine are the preferred amino acids at the C-terminal residue and thus contribute to ACE-I-inhibitory potency (Meisel, 1997). Further, studies with SHR revealed that dipeptides with a C-terminal tyrosine residue produced a slow but prolonged decrease in systolic blood pressure compared to dipeptides with phenylalanine at the C-terminal. In contrast, dipeptides with a C-terminal phenylalanine caused a more rapid reduction in systolic blood pressure and a shorter duration of action (Suetsuna, 1998).

Antimicrobial peptides are biomolecules employed for the defense against bacteria by animals as well as plants (Brogden, 2005). These short-chain positively charged peptides secreted by organisms are composed of 12–45 amino acid residues. When the antimicrobial peptides binding onto the outermost leaflet of negatively charged bacterial membrane by the aid of hydrophobic and electrostatic interactions, they fold to amphipathic secondary structures, typically α-helices and β-sheets. Those peptides can kill the bacteria via physical, chemical, or biological processes. Many models are suggested to explain the antibacterial mechanisms of peptides including Shai–Matsuzaki–Huang model, barrel–stave model, carpet model, and toroidal-pore model. Moreover, many theoretical and experimental models have been used to explain the antimicrobial mechanisms of cell lysis by antimicrobial peptides including, molecular mean-field theory (La Rocca *et al*., 1999), dynamics simulations (Leontiadou *et al*., 2006), and coarse-grained simulations (Lopez *et al*., 2005). Most of these approaches support the SMH model that a physical

hole in the membrane is stable and is an effective mechanism of antimicrobial activity.

Several mechanisms have been presented to explain the antioxidative activity of peptides including metal ion chelating ability, radical scavenging activity, and aldehyde adduction (Zhou and Decker, 1999). Most of the food-derived peptides possess low molecular weight with hydrophobic residues including Lue and Val in their N-terminus and Pro, His, Tyr, Trp, Met, and Sys in their sequences (Chen *et al*., 1995). Those amino acid residues can increase the presence of peptides at the water–lipid interface and promote the radical scavenging activity at the lipid phase (Ranathunga *et al*., 2006). The mechanism of His containing peptides could be attributed to the hydrogen donating ability, lipid peroxyl radical-trapping ability, and the metal chelating ability of the imidazole group (Chan and Decker, 1994).

III. SOURCES OF FISH PEPTIDES

Peptides can be isolated from various fish parts, or various parts of marine fishes possess peptides.

A. Muscle protein peptides

Discarded fish bones and cutoffs may contain considerable amounts of muscle proteins. These muscle proteins are nutritionally valuable and easily digestible with well-balanced amino acid composition (Venugopal *et al*., 1996). Therefore, fish proteins derived from seafood processing by-products can be hydrolyzed enzymatically to recover protein. Protein hydrolysates from several marine species have been analyzed for their nutritional and functional properties, and researches have mainly explored the possibility of obtaining biologically active peptides (Benkajul and Morrissey, 1997). Moreover, skipjack tuna muscle (Kohama *et al*., 1988), sardine muscle (Bougatef *et al*., 2008), and shark meat (Wu *et al*., 2008) have been used to separate potential peptides.

B. Peptides from fish skin collagen and gelatin

Gelatin is derived from the fibrous protein collagen, which is the principal constituent of animal skin, bone, and connective tissue. Fish skin waste could be used as a potential source to isolate collagen and gelatin. Zhu *et al*. (2010) evaluated the effect of collagen peptides on markers of metabolic nuclear receptors.

C. Fish bone as potential peptide source

The organic component of fish bone represents 30% of collagen, while inorganic component mainly consists of calcium phosphate and hydroxylapatite and it was around 60–70% (Aronove *et al*., 2007; Barakat *et al*., 2008).

D. Peptides from other body parts

Antimicrobial peptides mainly present in the mucous layer and eliminate the pathogenic bacteria before they enter the skin barrier. Several antimicrobial peptides have been isolated from marine fishes, such as Pleurocidins from winter flounder, *Pleuronectes americanus* (Walbaum), *American plaice*, and *Hippoglossoides platessoides* (Fabricius), and Atlantic halibut, *Hippoglossus hippoglossus* (Cole *et al*., 1997; Douglas *et al*., 2003). Intestinal antimicrobial peptides were derived from hagfish, *Eptatretus burgeri* (Girard) (Hwang *et al*., 1999), chrysophsins from gills of red sea bream (Iijima *et al*., 2003), and piscidin or moronecidin from gills and skin of striped bass (Lauth *et al*., 2002; Silphaduang and Noga, 2001).

IV. PRODUCTION OF PEPTIDES FROM VARIOUS FISH BODY PARTS

It is a key step to develop methods to separate peptides with different molecular weights. An ultrafiltration membrane system equipped with the appropriate molecular weight cutoff has been effectively used in separating peptides having desired molecular weights (Jeon *et al*., 2000). In order to obtain functionally active peptides, it is a common method to use the type of enzymes letting sequential enzymatic digestions.

Antimicrobial peptides have been purified from the gill filaments of sea beams by acid extraction method (Iijima *et al*., 2003). Gill filaments were crushed in liquid nitrogen and resulting powder was boiled in water. Then the extraction was performed with hydrochloric acid, trifluoroacetic acid, formic acid, and NaCl. Finally, this extract was purified in a Sep-Pack C18 cartridge, SP-Sephadex C-25 resin, gel filtration, reversed-phase HPLC, ESI–MS/protein sequencer, cation exchange HPLC, and MLC, respectively. Moreover, several studies have used acid extraction method to isolate antimicrobial peptides from various body parts of fish (Lauth *et al*., 2002; Zhang *et al*., 2009). However, extraction medium containing sodium acetate, Triton X-100, and phenylmethylsulfonyl fluoride also have been used for the extraction of antimicrobial peptides from fish (Park *et al*., 1998).

Collagen is generally extracted with acid treatment and solubilized without altering its triple helix. However, thermal treatment cleaves

hydrogen and covalent bonds that stabilizes the triple helix configuration of collagen and converts its helical conformation into coiled conformation resulting in gelatin state (Djabourov *et al.*, 1993). Fish skin gelatin is processed into peptides by enzymatic hydrolysis. In many studies, many commercial proteases, such as alcalase (Dong *et al.*, 2008) and flavourzyme (Thiansilakul *et al.*, 2007a,b), were used to obtain antioxidative peptides by hydrolyzing various fish protein sources.

A. Health promoting ability of fish peptides

Peptides isolated from various fish protein hydrolysates have shown a different biological activities such as antihypertensive, antioxidative, antithrombotic, and immunomodulatory activities.

1. Antioxidant activity

Peptides derived from fish proteins have shown the ability of exerting potent antioxidative activities (Table 15.1) in different oxidative systems (Rajapakse *et al.*, 2005). Currently, an increasing interest exists to explore natural antioxidative substances without side effects and the identified antioxidative activities have potential to develop safe and nonhazardous natural antioxidants for the complications arose from oxidation of biomolecules. Je *et al.* (2007) purified an antioxidative peptide from tuna backbone protein and identified as VKAGFAWTANQQLS (1519 Da) which was very important regarding the functional foods. It was reported that the derived peptides were good radical scavengers and antioxidant.

In another study, an antioxidant peptide has been isolated from hoki frame protein by gastrointestinal digestion (Kim *et al.*, 2007). A number of enzymes have been used for the hydrolysis process, and the isolated peptides showed strong antioxidant activities in various model systems.

Gelatin peptides have repeated unique Gly–Pro–Ala sequence in their structure, and it is presumed that the observed antioxidative properties of gelatin peptides can be associated with their unique amino acid compositions.

A dipeptide Met-Tyr, derived from sardine muscle (Matsufuji *et al.*, 1994), stimulates expression of the antioxidant defense protein HO-1 in a concentration-dependent manner. Previous findings revealed that HO-1 protein expression is accompanied by the induction of a secondary antioxidant protein, ferritin. In a present study, the effect of Met-Tyr on the expression of the antioxidant stress proteins, heme oxygenase-1 (HO-1), and ferritin in endothelial cells derived from the human umbilical vein and their contribution to the decrease in radical formation that occurs under the influence of this dipeptide were studied and reported potential activity (Erdmann *et al.*, 2006).

TABLE 15.1 Potential bioactive antioxidative peptides derived from various fish proteins

Name of organism and substrate	References
Alaska pollak skin gelatin	Kim *et al*. (2001)
Hoki frame protein	Je *et al*. (2005)
Alaska pollak frame protein	Je *et al*. (2005)
Conger eel muscle protein	Ranathunga *et al*. (2006)
Hoki frame protein	Kim *et al*. (2007)
Jumbo squid skin gelatin	Mendis *et al*. (2005)

2. Antihypertensive activity

ACE inhibitory peptides have been separated from the skin of skate by Lee *et al*. (2011). The purified peptides showed IC_{50} values of 95 and 148 μM, respectively, for both peptides isolated from the skin of skate. Further, Lineweaver–Burk plots indicated that the peptides act as noncompetitive inhibitor against ACE. Recently, many inhibitory peptides against ACE are reported (Table 15.2) as natural alternative biofunctional peptides that are safer than that of the existing artificial ACE inhibitory compounds in the market that show some side effects. Various peptides have been isolated from seafood by-products from the fisheries industry such as backbones from tuna (Lee *et al*., 2010). Tuna backbone has hydrolyzed using various proteases such as alcalase, α-chymotrypsin, neutrase, papain, pepsin, and trypsin to obtain an antioxidant peptide (Je *et al*., 2007).

Gelatin peptides have repeated unique Gly–Pro–Ala sequence in their structure, and it is presumed that the observed antihypertensive properties of gelatin peptides can be associated with their unique amino acid compositions.

3. Antimicrobial activity

Antimicrobial peptides are widely distributed throughout the animal kingdom and show potential antimicrobial activities (Table 15.3). Three isoforms of a novel C-terminally amidated peptides, such as chrysophsin-1, chrysophsin-2, and chrysophsin-3, consist of 25, 25, and 20 amino acids, respectively, and are highly cationic, containing an unusual C-terminal RRRH sequence was isolated from the gills of red sea bream, *Chrysophrys* (*Pagrus*) *major* (Iijima *et al*., 2003). These peptides show potent antimicrobial activity against Gram-negative and Gram-positive bacteria and act as nonspecific defense substances in fish skin. The antimicrobial peptides fold to amphipathic secondary structures, typically α-helices and β-sheets. Such peptides can kill the bacteria via physical, chemical, or biological processes. A novel 25-residue linear antimicrobial peptide

TABLE 15.2 Potential bioactive antimicrobial peptides derived from various fish proteins

Fish name	Body part	Peptide name	Reference
Large yellow croaker	Head and kidney	Hepadin	Zhang *et al.* (2009)
Red sea bream	Gills	Chrysophsin-1, 2, and 3	Iijima *et al.* (2003)
Loach (mud fish)	Whole body	Misgurin	Park *et al.* (1997)
Catfish	Proteinaceous epithelial muscosal layer	Parasin 1	Park *et al.* (1998)
Winter flounder	Skin mucous secretion	Pleurocidin	Cole *et al.* (1997)
Striped bass	Skin and gill	Moronecidin	Lauth *et al.* (2002)
Bony fish (winter flounder) Atlantic salmon	Hepcidin-like		Douglas *et al.* (2003)

TABLE 15.3 Other potential biological activities from peptides derived from various fish proteins

Name of organism and substrate	Bioactivity	Reference
Fish protein hydrolysate	Anticoagulant	Rajapakse *et al.* (2005)
Alaska pollack back bone	Calcium bioavailability	Jung *et al.* (2006)
Fish protein hydrolysate	Breast cancer	Picot *et al.* (2006)
Tilapia muscle	Anticancer	Chang *et al.* (2011)
Anchovy sauce, whole fish	Human lymphoma cancer	Lee *et al.* (2003)
Tuna dark muscle	Breast cancer	Hsu *et al.* (2011)

named as pleurocidin found in the skin mucous secretions of the winter flounder was isolated (Cole *et al.*, 1997). Pleurocidin is predicted to assume an amphipathic α-helical conformation similar to many other linear antimicrobial peptides and exhibited antimicrobial activity against *Escherichia coli* in a bacterial cell lysis plate assay. Moreover, a study with

skate skin included the production of collagen and the purification of an antimicrobial peptide (Cho *et al*., 2004).

4. Anticoagulant effect

Enzymatically prepared fish muscle peptides also have shown anticoagulant and antiplatelet properties tested *in vitro*, and the results have suggested the capability of fish peptides to inhibit coagulation factors in the intrinsic pathway of coagulation (Rajapakse *et al*., 2005). A peptide with 12.2 kDa molecular weight was purified from yellowfin sole that inhibited activated coagulation factor XII by forming an inactive complex regardless of Zn^{2+} mediation in their study. In addition, this anticoagulant peptide acts to antagonize platelet membrane glycoprotein integrin to arrest platelet aggregation.

5. Anticancer effect

Proteins, peptides, and amino acids have been reported to show antitumor or antiproliferative activities. However, the antiproliferative activity of peptides derived from fish protein was rarely studied. Picot *et al*. (2006) reported that hydrolysates obtained from three blue whiting, three cod, three plaice, and one salmon showed significant inhibition against two human breast cancer cell lines, MCF-7/6 and MDA-MB-231. The hydrolysates contained a complex mixture of free amino acids, peptides with various sizes ranging up to 7 kDa, and in a lower proportion, lipids and sodium chloride. Moreover, two antiproliferative peptides were purified from tuna dark muscle by enzymatic hydrolysis with papain and protease against human breast cancer cell line MCF-7 (Hsu *et al*., 2011). The amino acid sequences of the two antiproliferative peptides were Leu-Pro-His-Val-Leu-Thr-Pro-Glu-Ala-Gly-Ala-Thr (1206 Da) and Pro-Thr-Ala-Glu-Gly-Gly-Val-Tyr-Met-Val-Thr (1124 Da), and they showed significant activities in a dose-dependent manner. Further, antiproliferative hydrophobic peptide (440.9 Da) derived from anchovy fish source was able to induce apoptosis in human U937 lymphoma cells through the increase of caspase-3 and caspase-8 activity (Lee *et al*., 2003, 2004). Tilapia (*Oreochromis mossambicus*) hepcidin TH2-3 was evaluated in several tumor cell lines. The results indicated that TH2-3 inhibited human fibrosarcoma cell (HT1080 cell line) proliferation and migration. Chang *et al*. (2011) also investigated an antimicrobial peptide, TH1-5 with the view of finding antitumor activity in cancel cells, including human cervix adenocarcinoma cell (HeLa), human hepatocellular carcinoma cell (HepG2), human fibrosarcoma cell (HT1080), *Cercopithecus aethiops* kidney cell (COS-7), and human kidney cell (WS-1).

6. Ca absorbing and bone mineralization ability

Kim *et al*. (1999) and Jung *et al*. (2005) reported that fish peptides are capable of accelerating calcium absorption. Further, researchers have identified that fish protein hydrolysates possess hormone-like peptides and growth factors to accelerate calcium absorption (Fouchereau-Peron *et al*., 1999). These peptides are capable of binding to cell surface receptors on osteoclasts and involved mainly in calcium metabolism by decreasing the number of osteoclasts. Therefore, these peptides could be used in the treatment of osteoporosis and Paget's disease (Table 15.3).

In addition, gelatin peptides have shown to accelerate absorption of dietary calcium in animal models increasing calcium bioavailability (Kim *et al*., 1998). Jung *et al*. (2006) reported that fish bone peptides (FBP) II could inhibit the formation of insoluble Ca salts in neutral pH. During the experimental period, Ca retention was increased and loss of bone mineral was decreased by FBP II supplementation in ovariectomized rats. The levels of femoral total Ca, bone mineral density, and strength were also significantly increased by the FBP II diet to levels similar to those of the casein phosphopeptide diet group.

7. Others activities

Further, acidic peptide fractions from Atlantic cod hydrolysate have shown strong immunostimulatory effects, and treatment of these peptides has stimulated the oxidative burst of Atlantic salmon leucocytes (Gildberg *et al*., 1996). Basically, immunomodulators that enhance the production of oxygen metabolites in macrophages that are responsible for these oxygen metabolites determine the oxidative burst. Oxidative burst reactions are of major importance for the bactericidal power of phagocytes.

Some peptides isolated from various fishes and fermented fish have been reported to show prolyl endopeptidase inhibitory activity (Sørensen *et al*., 2004). The peptides obtained from muscles from salmon, trout, and cod as well as Norwegian fermented trout muscle have been investigated for the potential inhibitory activity for prolyl endopeptidase and found that they are good sources.

V. FUTURE TRENDS OF PEPTIDES FROM FISH PROTEINS

Technological advances have created a cost- and time-efficient platform to screen a large number of compounds for their pharmacological and medicinal potency. These technologies give insightful indications on the feasibility of transforming a new chemical entity into a drug and also reduce the requirement for more extensive clinical trials. Due to their inherent bioactive properties, peptides could be useful as therapeutic or

prophylactic agents. The production of peptide therapeutics from marine food proteins is therefore a promising approach exploitable by the pharmaceutical industry.

VI. CONCLUSIONS

Marine fish protein and peptides have recently attracted great attention due to their potential biological activities and natural abundance. Also they are underutilized natural resources that have potential to be used in various industries. Moreover, discarded wastes from seafood processing have been recognized as potential materials to derive bioactive peptides for various fields. They have been reported to have range of biological activities such as antioxidant, antihypertensive, anticoagulant, antibacterial, and calcium-absorbing abilities.

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