25 Marine-derived Bioactive Peptides: Their Cardioprotective Activities and Potential Applications

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25.1 INTRODUCTION

Marine biotechnology is the application of biotechnology tools to marine resources. It encompasses those efforts that involve the marine resources of the world, either as the source or the target of biotechnology applications. Biotechnology is the application of science and technology to living organisms, as well as parts, products and models thereof, in order to alter living or nonliving materials for the production of knowledge, goods and services. In the case of marine biotechnology, the living organisms derive from marine resources.

The ocean encompasses about 71% of the surface of our planet, but over 90% of the biosphere (since organisms are found throughout the water column), and represents the greatest extremes of temperature, light and pressure encountered by life. Adaptation to these harsh environments has led to a rich marine bio- and genetic diversity, with potential biotechnological applications related to drug discovery, environmental remediation, increase of seafood supply and safety and development of new resources and industrial processes (Mayekar *et al*., 2012).

Research into the pharmacological properties of marine natural products has led to the discovery of many compounds considered worthy of clinical application. There is great potential in bioprospecting from the sea, and marine natural-products research has just started to bloom. Today, marine sources have the highest probability of yielding natural products with unprecedented carbon skeletons and interesting biological activities. New habitats, such as deep ocean sample sand symbiotic systems, still have great possibilities for future research.

This chapter examines bioactive peptides which have properties relevant to cardiovascular health, including effects on blood pressure, atherosclerosis, oxidative stress, hemostasis and lipid metabolism.

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25.2 CARDIOVASCULAR DISEASES AND NUTRACEUTICALS

Acute myocardial infarction remains the leading cause of death in most Western and Asian countries. In Japan, several reports have demonstrated that the incidence of acute myocardial infarction has increased with the rapid progression of longevity and westernized lifestyle, despite recent substantial developments of appropriate diagnoses and therapeutics (Rumana *et al*., 2008). The underlying mechanisms of acute myocardial infarction include disruption of vulnerable plaques in the coronary arteries, subsequent formation of coronary thrombus and occlusion of the coronary arteries. Since atherosclerotic damage is present throughout the entire coronary artery system in patients with acute myocardial infarction (Asakura *et al*., 2001), the concept that there are vulnerable patients, not vulnerable plaque, is widely accepted (Naghavi *et al*., 2003). For these vulnerable patients, treatment with aspirin and statins achieves a reduction in mortality as well as in the occurrence of acute myocardial infarction.

If acute myocardial infarction does develop, acute-phase therapy of thrombolysis or precutaneous coronary intervention results in a marked improvement in prognosis, as has been well shown in the 20 years in which these interventions have been applied. As a consequence, the in-hospital death rate of patients with an acute myocardial infarction has decreased from approximately 20% to 5% during these 20 years. This better treatment of the acute phase of acute myocardial infarction has prevented patients with large infarct size in particular from cardiovascular death. However, as these patients easily develop post-infarction heart failure in the chronic phase, the prevention of post-infarction cardiovascular remodeling is mandatory. Several medications, such as angiotensin-converting enzyme (ACE) inhibitors (Pfeffer *et al*., 1992), aldosterone-receptor antagonists (Hayashi *et al*., 2001) and beta blockers (Dargie, 2001), have been used for the prevention of post-infarction heart failure in patients who have suffered from an acute myocardial infarction. However, these medications alone are unable to sufficiently prevent the progression and occurrence of post-infarction heart failure and left-ventricular remodeling, suggesting that the development of new therapies is required.

Recently, various cell therapies have been aggressively developed (Dimmeler *et al*., 2005), but we will have to wait several years to know their efficacy. One possibility concerns cell therapy or regenerative therapy using stem cells. Another is further adjunctive drug therapy in the acute phase in patients with myocardial infarction.

On the other hand, adjunctive drug therapies other than accepted medication have also been explored (Armstrong *et al*., 2007). Any treatment method that reduces infarct size following acute myocardial infarction is likely to be effective in suppressing the progression of post-infarction heart failure. However, no further beneficial adjunctive therapy in the acute phase of acute myocardial infarction besides ACE inhibitors, aldosterone-receptor antagonists and beta blockers has been established so far, though its developed is readily expected.

25.3 SOURCES OF MARINE PEPTIDES

Marine organisms are rich sources of structurally diverse bioactive compounds with various biological activities (Kim & Wijesekara, 2010). The importance of marine organisms as a source of novel bioactive substances is growing rapidly. With marine species

comprising approximately one-half of the total global biodiversity, the sea offers an enormous resource for novel compounds (Aneiros & Garateix, 2004; Barrow & Shahidi, 2008). Moreover, very different kinds of substance have been procured from marine organisms, because they live in very exigent, competitive and aggressive surroundings, different in many aspects from the terrestrial environment—a situation that demands the production of quite specific and potent active molecules. The marine environment serves as a source of functional materials, including polyunsaturated fatty acids (PUFAs), polysaccharides, minerals and vitamins, antioxidants, enzymes and bioactive peptides (Kim & Wijesekara, 2010; Kim *et al*., 2008; Pomponi, 1999).

There has been considerable activity in the area of marine natural products over the last 2 decades. To date, approximately 16 000 marine natural products have been isolated from marine organisms and reported in about 6800 publications. In addition, there are approximately another 9000 publications which covers syntheses, reviews, biological-activity studies, ecological studies and so forth on the subject of marine natural products. In the year 2003, over 656 marine natural products were isolated from marine organisms and reported in 243 research papers. Chemical investigation of different phyla of marine organisms has yielded different classes of compound (Ireland *et al*., 1987).

Recently, much attention has been paid to unraveling the structural, compositional and sequential properties of bioactive peptides (Kim & Wijesekara, 2010). Marine bioactive peptides may be produced by one of three methods: solvent extraction, enzymatic hydrolysis and microbial fermentation of food proteins. The enzymatic hydrolysis method is preferred, especially in the food and pharmaceutical industries, because of the lack of residual organic solvents and toxic chemicals in its products (Kim & Wijesekara, 2010). Bioactive peptides are inactive in the sequences of their parent proteins, but can be released by enzymatic hydrolysis (Kim *et al*., 1999; Lahl & Braun, 1994). They usually contain 3–20 amino acid residues and their activities are based on their amino acid composition and sequence (Pihlanto-Leppala, 2001). They have been detected in many different food sources (Dziuba *et al*., 1999; Pihlanto-Leppala *et al*., 1998). Depending on their amino acid sequence, they may possess various biological functions, including antihypertensive, immunomodulatory, antithrombotic, antioxidant, anticancer and antimicrobial activities, opioid agonism or antagonism and nutrient utilization (Clare & Swaisgood, 2000; Elias *et al*., 2008; Kim & Wijesekara, 2010). Some bioactive peptides have demonstrated multifunctional activities based on their structures and other factors, including hydrophobicity, charge and microelement-binding properties (Cho *et al*., 2008; Korhonen & Pihlanto-Leppala, 2003).

Marine bioactive peptides have been widely produced by enzymatic hydrolysis of marine organisms (Je *et al*., 2008; Slizyte *et al*., 2009). However, enzymatic hydrolysis is already carried out in the production of fermented marine food sauces, so bioactive peptides can be purified from them without further hydrolysis (Je *et al*., 2005a,b; Kim & Wijesekara, 2010). In addition, several bioactive peptides have been isolated from marine processing byproducts (Kim & Wijesekara, 2010; Kim *et al*., 2000). Marine-derived bioactive peptides have been shown to possess many physiological functions, including antihypertensive or ACE-inhibitory (Byun & Kim, 2001; Je *et al*., 2005b), antioxidant (Kim *et al*., 2007; Mendis *et al*., 2005), anticoagulant (Jo *et al*., 2008; Rajapakse *et al*., 2005a) and antimicrobial (Liu *et al*., 2008; Stensvag *et al*., 2008) activities. Moreover, some of these bioactive peptides may have potential for human health promotion and disease risk reduction (Kim & Wijesekara, 2010; Shahidi & Zhong, 2008). Thus possible

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role of food-derived bioactive peptides in reducing the risk of cardiovascular disease has been well demonstrated (Erdmann *et al*., 2008).

Increasing consumer knowledge about the link between diet and health has raised awareness and demand for functional-food ingredients and nutraceuticals (Kim & Wijesekara, 2010). Bioactive peptides derived from marine organisms and marine fish-processing byproducts have potential in the development of functional foods (Shahidi, 2007). Hence, marine-derived bioactive peptides can be used as versatile raw materials for the production of nutraceuticals and pharmaceuticals for human use.

25.4 DEVELOPMENT OF MARINE BIOACTIVE PEPTIDES

A bioactive peptide generated from a particular protein is dependent on two factors: (1) the primary sequence of the protein substrate and (2) the specificity of the enzyme(s) used to generate it. Furthermore, different peptides can be generated by both acid and alkaline hydrolysis, although this approach is generally not compatible with food-ingredient manufacturing strategies. Although the structure–activity relationship of many bioactive peptides has not yet been fully established, several structural features have been identified which appear to influence their biological action. With ACE-inhibitory peptides, for example, binding of the peptide to ACE is strongly influenced by the presence of tyrosine, phenylalanine, tryptophan, proline, lysine, isoleucine, valine, leucine and arginine (Lopez-Fandino *et al*., 2006; Murray and FitzGerald, 2007). Positively charged residues are associated with the activity of antimicrobial peptides (Lopez Exposito & Recio, 2006). Amino acid residues such as histidine, leucine, tyrosine, methionine and cysteine are associated with radical-scavenging activity, while hydrophobic amino acids such as proline and hydroxyproline appear to play a role in the inhibition of lipid peroxidation (Mendis *et al*., 2005; Sarmadi & Ismail, 2010).

25.5 OXIDATIVE STRESS

The increased generation of reactive oxygen species (ROS) such as superoxide anion (O^{2-}) and hydroxyl (OH^{-}) radicals, in conjunction with the overpowering of endogenous antioxidant defense mechanisms (enzymatic and non-enzymatic), is another causative factor for the initiation of chronic diseases. These diseases include heart disease, stroke, arteriosclerosis, diabetes and cancer (Davalos *et al*., 2004). Furthermore, lipid peroxidation is a major cause of deterioration in the quality of foods (rancidity and 'off-flavors') (Di Bernardini *et al*., 2011). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate are added to food products to retard lipid oxidation. However, there are safety concerns over their use (Shahidi and Zhong, 2005). As a result, there has been increasing interest in the use of natural antioxidants, which have little or no side effects.

The exact mechanism by which peptides display antioxidant activity is not fully understood. However, research has shown that protein hydrolysates and peptides can act as radical scavengers, as well as transition-metal chelators, and exert antioxidant activities against enzymatic (lipoxygenase-mediated) and non-enzymatic peroxidation of lipids and fats (Erdmann *et al*., 2008; Sarmadi & Ismail, 2010). Furthermore, peptides have been shown to induce specific genes encoding for endogenous non-enzymatic

antioxidant components (heme oxygenase-1 and ferritin) and enzymatic systems in cell-culture models (Erdmann *et al*., 2006). Marine-derived protein hydrolysates and peptides produced from processing waste, mollusks and crustaceans have been shown to exert antioxidant activity *in vitro*.

While the exact structure–antioxidant activity relationship of peptides has not yet been established, the type, position and hydrophobicity of amino acids present in a peptide are thought to play an essential role. Amino acids such as histidine, leucine, tyrosine, methionine and cysteine are believed to enhance radical-scavenging activity by donating protons to electron-deficient radicals (Mendis *et al*., 2005; Sarmadi & Ismail, 2010). Several peptides with radical-scavenging activity derived from marine waste frame and skin, mollusks and crustaceans contain amino acids associated with proton donation (Je *et al*., 2005; Kim *et al*., 2007). Furthermore, lipid peroxidation-inhibition activities similar to the natural potent antioxidant α-tocopherol are seen with peptides derived from gelatin (Kim *et al*., 2001; Mendis *et al*., 2005). This potent lipid peroxidation-inhibition activity is thought to be associated with the presence of hydrophobic amino acids, which have high affinity for lipid systems. Oil-soluble radicals (i.e. hydrophobic peroxyl radicals) generated during oxidative attack of unsaturated fatty acids such as linoleic acid are believed to be neutralized by hydrophobic amino acid containing antioxidant peptides. Gelatin contains an abundance of hydrophobic amino acids such as glycine, valine, alanine, proline and hydroxyproline, and potentially could contain a range of peptides with potent lipid peroxidation-inhibiting activity (Kim & Mendis, 2006). The lipid peroxidation-inhibiting peptides isolated from gelatin include HGPLGPL and GE(− Hyp)GP(Hyp)GP(Hyp)GP(Hyp)GP(Hyp)G, both of which contain the characteristic repeating glycine–proline sequence associated with gelatin. Two peptides, NADFGLNGLEGLA and NGLEGLK, isolated from squid-muscle hydrolysates also contain this unusual di-amino acid (GP) repeating sequence. These peptides also inhibit the free radical-mediated oxidation of linoleic acid (Rajapakse *et al*., 2005).

25.6 ANTIHYPERTENSIVE ACTIVITY

Blood pressure is controlled by a number of different interacting biochemical pathways and can be increased or decreased depending on which pathway predominates at any given time. Classically, blood-pressure control has been associated with the renin–angiotensin system, which plays an important role in regulating arterial pressure (Dostal & Baker, 1999). Renin converts angiotensinogen in the liver to the decapeptide angiotensin I, which in turn undergoes proteolytic cleavage to the biologically active octapeptide angiotensin II. The latter step is carried out by ACE, which is highly expressed on vascular endothelium, particularly in the lungs. ACE belongs to the class of zinc proteases that require zinc and chloride for their activation. It converts the biologically inactive angiotensin I to the potent vasoconstrictor and cardiovascular trophic factor angiotensin II (Dubay *et al*., 1993). Angiotensin II has many important actions, including increasing arterial pressure, increasing sodium and fluid retention, enhancing sympathetic adrenergic function and causing cardiac and vascular remodeling. For the most part, these actions are mediated by the plasma membrane AT1 and are generally opposed by the type-2 receptor (AT2) (Messerli *et al*., 1996). The ability of ACE inhibitors and AT1-receptor antagonists to influence cardiovascular status in hypertensive conditions is consistent with an important role for the renin–angiotensin system in physiological and pathophysiological states (Mazen

et al., 2005). Thus, inhibition of this enzyme or AT1 is believed to lower blood pressure. At present, the renin–angiotensin system is a key target for drugs combating hypertension.

Hypertension is a significant health problem worldwide. It is one of the major controllable risk factors associated with cardiovascular-disease events such as myocardial infarction, heart failure and end-stage diabetes (Dezsi, 2000). Various synthetic ACE inhibitors are widely used to treat cardiovascular disorders (Jaspard *et al*., 1992). Conventional antihypertensive drugs cause various adverse effects, so cheaper, safer alternatives are desirable. Some natural or synthesized peptides which act on the renin–angiotensin system have the ability to reduce blood pressure. At present, a higher dietary protein intake seems to have a favorable influence on blood pressure in hypertensive individuals. The Dietary Approaches to Stop Hypertension trial demonstrated that a diet rich in fruits, vegetables and low-fat dairy products will reduce blood pressure effectively (Allen *et al*., 2002). Increasing consumer knowledge of the link between diet and health has raised awareness and demand for functional-food ingredients and nutraceuticals. This is leading to a mindset of self-medication, often driven by the desire to avoid undesirable side effects associated with the consumption of organically synthesized chemical drugs and to avoid the increasing cost of drug therapy. It is well recognized that apart from their basic nutritional role, many food proteins contain encrypted within their primary structures peptide sequences capable of modulating specific physiological functions. The application of specific foods or food components to the prevention and/or treatment of disease is of particular relevance to the management of hypertension (Yoshiji *et al*., 2001). Although other mechanisms play a role, ACE inhibition by bioactive peptides released from food proteins may cause these antihypertensive effects. Indeed, peptides which are derived from food have certain advantages. Several studies in spontaneously hypertensive rats (SHR) suggest a significant suppression of the development of hypertension with a diet rich in ACE-inhibitory peptides (Moskowitz, 2002). A number of research reports have also demonstrated the antihypertensive effect of ACE-inhibitory peptides or foods containing these bioactive compounds in hypertensive patients (FitzGerald & Meisel, 2000). Overall, this points to the fact that ACE-inhibitory peptides, as part of a food product or as nutraceuticals, may be of functional interest in both the treatment and the prevention of hypertension. ACE-inhibitory peptides have lower ACE-inhibitory activity *in vitro* than the ACE-inhibitory drugs, but do not have their harmful side effects. They also lower the cost of health care (Michel, 2004). These peptides need to be considered hypotensive agents. Increasingly, research is exploring the relation between these peptides and their antihypertensive effects.

25.7 ANTICOAGULANT ACTIVITY

Blood coagulation is processed by coagulation factors in order to stop the flow of blood though the injured vessel wall whenever an abnormal vascular condition and exposure to non-endothelial surface at the site of vascular injury occurs. As endogenous or exogenous anticoagulants interfere with the coagulation factors, blood coagulation can be either prolonged or stopped (Jung *et al*., 2001). These anticoagulants are used for therapeutic purposes, for example as a cure for hemophilia.

Heparin has been identified and used for more than 50 years as a commercial anticoagulant and is widely used for the prevention of venous thromboembolic disorders. However, several side effects have been identified, such as the development of thrombocytopenia,

hemorrhagic effect and ineffectiveness in congenital or acquired antithrombin bound to fibrin (Pereira *et al*., 2002). Moreover, heparin is found only in very low concentrations in pig intestine and bovine lungs, from where it is primarily extracted. Therefore, the necessity of discovering alternative sources of anticoagulant has arisen.

The anticoagulant marine bioactive peptides have rarely been reported, but they have been isolated from marine organisms such as marine echiuroid worm (Jo *et al*., 2008), starfish (Koyama *et al*., 1998) and blue mussel (Jung & Kim, 2009). Moreover, marine anticoagulant proteins have been purified from blood ark shell (Jung *et al*., 2001) and yellow fin sole (Rajapakse *et al*., 2005a). The anticoagulant activity of these peptides has been determined by prolongation of activated partial-thromboplastin-time (APTT), prothrombin-time (PT) and thrombin-time (TP) assays and compared with that of heparin, the commercial anticoagulant. The anticoagulant peptide Gly-Glu-Leu-Thr-Pro-Glu-Ser-Gly-Pro-Asp-Leu-Phe-Val-His-Phe-Leu-Asp-Gly-Asn-Pro-Tyr-Ser-Leu-Tyr-Ala-Asp-Ala-Val-Pro-Arg, isolated from marine echiuroid worm, effectively prolonged the normal clotting time on APTT from 32.3 ± 0.9 to 192.2 ± 2.1 seconds in a dose-dependent manner, with IC_{50} 42.6 μg/ml (Jo *et al*., 2008). This peptide binds specifically with clotting factor FIXa, a major component of the intrinsic tense complex, and inhibits molecular interaction between FIXa and FX in a dose-dependent manner. Moreover, the anticoagulant peptide Glu-Ala-Asp-Ile-Asp-Gly-Asp-Gly-Gln-Val-Asn-Tyr-Glu-Phe-Val-Ala-Met-Met-Thr-Ser-Lys, derived from blue mussel, showed prolongation of clotting time to 321 ± 2.1 seconds on APTT (from 35.3 ± 0.5 seconds in control) and to 81.3 ± 0.8 seconds on TT (from 11.6 ± 0.4) (Jung & Kim, 2009). In addition, a protein derived from blood arch shell prolonged the APTT clotting time from 32 to 325 seconds, and 2.8 μg/ml of heparin prolonged clotting time on APTT, PT and TT to more than 300 seconds (Jung *et al*., 2001). These marine-derived anticoagulant peptides are also noncytotoxic.

25.8 CONCLUSION

Marine-derived bioactive peptides have potential for use as functional ingredients in nutraceuticals and pharmaceuticals due to their effectiveness in both prevention and treatment of cardiovascular diseases. Moreover, cost-effective and safe natural health products can be produced from marine bioactive peptides, though further studies and clinical trials are needed.

The marine world has become an important source of therapeutic agents with novel mechanisms of actions. Even though thousands of new molecules are discovered every year, only a small number of candidates are incorporated in clinical trials. The problem is creating a sustainable supply of these compounds from natural resources. Various strategies have been developed to tackle this issue, such as mariculture or aquaculture of source organisms, synthetic analogues of active compounds and fermentation of the microorganisms producing the compounds. Another possible solution is the use of genetic engineering to transfer the genes encoding the synthetic enzymes that produce the desired compound to microorganisms that can be grown in huge quantities. Development of these products and services, as well as the fundamental research from which they must be derived, will be enhanced by greater reliance on interdisciplinary sciences such as pharmacology, chemical ecology, molecular biology, genomics, metagenomics, computational and combinatorial chemistry and biology. In the future, marine plants, animals and microorganisms will be the basis of new technological products and services.

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Queries in Chapter 25

Q1. Please provide complete reference details for (Mendis et al., 2005 and Kim & Mendis, 2006) to be included in the references section.