CHAPTER 25

Medicinal Effects of Peptides from Marine Microalgae

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Contents

Abstract

Nowadays, there are numerous commercial applications of microalgae, and they have been used to enhance the nutritional value of food and animal feed owing to their chemical composition. They are cultivated as a source of highly nutritional and valuable source. Recently, microalgae have been reported to use as a potent source for food additive, nutraceutical, or pharmaceuticals. According to the criteria of nutritional quality and cost, variety of marine organisms has been investigated for their suitability to be applied in the production of protein hydrolysates in functional foods. Recently, a great deal of interest has been expressed regarding marine-derived

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bioactive peptides because of their numerous health benefits. In addition, many studies have been reported that marine bioactive peptides can be used as functional foods, nutraceuticals, or pharmaceuticals due to their therapeutic potential in the treatment or prevention of various diseases. Hence, in this chapter, we discussed the importance of marine microalgae in relation to their medicinal value.

I. INTRODUCTION

Marine microalgae utilization by indigenous populations has occurred for centuries. However, the cultivation of microalgae is only a few decades old. Marine microalgae have been reported as valuable new sources with pharmacologically active compounds. Nowadays, there are numerous commercial applications of microalgae. For example, (1) microalgae can be used to enhance the nutritional value of food and animal feed owing to their chemical composition, (2) they play a crucial role in aquaculture, and (3) they can be incorporated into cosmetics (Borowitzka, 1995). Moreover, they are cultivated as a source of highly valuable molecules. However, their metabolites have not been studied extensively because of difficulties in the isolation and cultivation of microalgae (Brown *et al*., 1997). Microalgae perform a major part of primary production, being responsible for 46% of global productivity and supporting food webs in water from ponds to oceans (Kim *et al*., 2001). Dozens of microalgal species are produced commercially for single-cell proteins, polysaccharides, healthy food materials such as polyunsaturated fatty acids, and vitamins in the pharmaceutical and food industries (Spolaore *et al*., 2006).

Microalgae for human nutrition are nowadays marketed in different forms such as tablets, capsules, and liquids. They can also be incorporated into pastas, foods, candy bars or gums, and beverages. Owing to their diverse chemical properties, they can act as a nutritional supplement or represent a source of natural food colorants (Spolaore *et al*., 2006). The commercial applications are dominated by three strains: *Arthrospira, Chlorella,* and *Dunaliella salina*. *Arthrospira* (Fig. 25.1) is used in human nutrition because of its high protein content and excellent nutritive value. In addition, these microalgae have various possible health-promoting effects. *Chlorella* can also be used as a food additive owing to the taste and flavor-adjusting actions of its coloring agent. *D. salina* is exploited for its β-carotene content that can reach 14% of dry weight (Chisti, 2007). For human consumption, nutrition and health, the world's largest producer of this strain, offers *Dunaliella* powder as an ingredient of dietary supplements and functional foods. Also, microalgae are required for larval nutrition during a brief period, either for direct consumption in the case

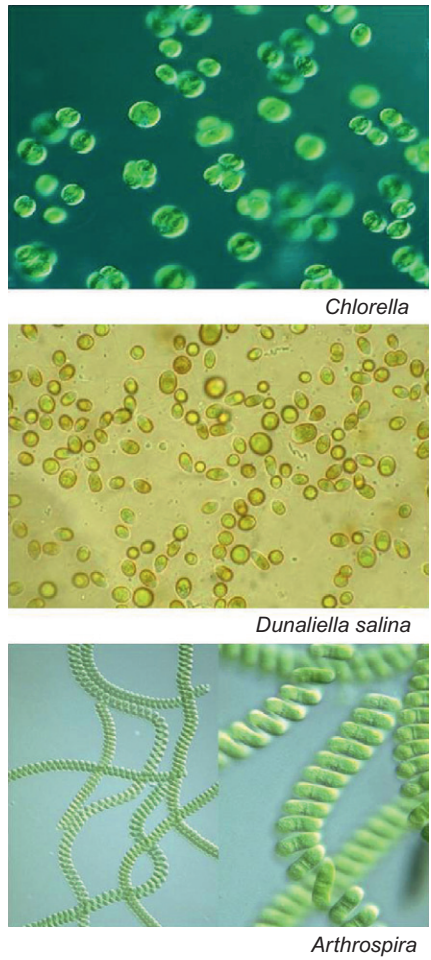

FIGURE 25.1 The commercial applications of microalgae species.

of mollusks and peneid shrimp or indirectly as food for the live prey fed to small fish larvae. The most frequently used species are *Chlorella, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema,* and *Thalassiosira* (Brown and Jeffreyi, 1995).

Recently, microalgae have been gained to more attention as nutraceutical and healthy food additive in markets. Extract of *Chlorella* sp. and *Spirulina* sp. is proposed to use with noodles, bread, green tea, beer, and candy (Liang *et al*., 2004). Protein content of *Navicula incerta* was found to be higher than lipid and carbohydrates (Kang *et al*., 2011). The percentage of carbohydrates in *N. incerta* was similar to that in *Skeletonema costatum* (4.9%), and the lipid content (6.54%) was very low compared with that in

Nitzschia closterium, *Cylindrotheca fusiformis*, and *S. castatum* (18–20%). However, the protein content (50.10%) of this species was very high compared with that of *N. closterium* and *C. fusiformis* (16–38%) (Brown and Jeffreyi, 1995; Kang *et al.*, 2011). Some microalgae with high protein content are used very commonly as can be seen in Table 25.1. Many microalgae species have similar amino acid compositions and were rich in the essential amino acids (Brown, 1991).

II. POTENTIAL FUNCTIONAL PEPTIDES FROM MICROALGAE

A. Bioactive peptides from microalgae

Bioactive peptides released by enzymatic proteolysis of various proteins that act as potential physiological modulators of metabolism during intestinal digestion have been reported in recent reports. These peptides usually contain 3–20 amino acid residues, and their activity depends on their amino acid composition and sequence (Pihlanto-Leppala, 2001). Based on their structural, compositional, and sequential properties, they may exhibit different kinds of bioactivities such as antioxidative (Jung *et al.*, 2005; Kim *et al.*, 2001), antihypertensive (Suetsuna *et al.*, 2004), and immunomodulatory effects (Chen *et al.*, 1995; Tsuruki *et al.*, 2003).

Arthrospira, *Chlorella*, and *D. salina* were used in human nutrition diets because of their high protein content and their excellent nutritive value. In addition, this microalga has various possible health-promoting effects: the alleviation of hyperlipidemia, suppression of hypertension, protection against renal failure, growth promotion of intestinal *Lactobacillus*, and suppression of elevated serum glucose level. A significant amount of

TABLE 25.1 General composition of different human food sources and microalgae (% of dry matter)

Commodity	Protein	Carbohydrate	Lipid
Milk	26	38	28
Rice	8	77	2
Meat	43	1	34
Baker's yeast	39	38	1
Chlorella vulgaris	51–58	12–17	14–22
Dunaliella salina	50–57	30–35	6–10
Porphyridium cruentum	28–39	40–57	9–14
Spirulina maxima	50–60	13–16	6–7
Navicula incerta	45–50	10–11	8–10
Chlamydomonas reinhardtii	40–48	15–17	11

Arthrospira production is realized in China and India (Kato and Suzuki, 1971; Morris *et al*., 2007). Therefore, new interest has been developed to search natural and safe bioactive peptides from natural sources. Furthermore, antioxidant peptides have been isolated from hydrolysates of various proteinaceous food materials and recently the possible roles of food-derived bioactive peptides in reducing the risk of diseases have been reported (Kim and Wijesekara, 2010). In addition, two peptides were identified from the enzyme hydrolysis of *N. incerta*. Table 25.2 shows some microalgae-derived peptides.

B. Antioxidant activity of the peptides from microalgae

Many synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate have been used to retard the oxidation process; however, the use of synthetic antioxidants are under strict regulation due to potential health hazards (Park *et al*., 2001). The search for natural antioxidants as alternatives is therefore of great interest among researchers.

Recently, bioactive peptides from enzymatic hydrolysis of various food proteins such as soy protein, casein, whey protein, gelatin, and wheat gluten have been shown to possess antioxidative activity (Elias *et al*., 2008). However, antioxidative peptides from marine food sources are gaining more attention as new antioxidative alternatives in the past few years (Mendis *et al*., 2005; Qian *et al*., 2007, 2008; Rajapakse *et al*., 2005). *Chlorella vulgaris* is a popular edible microalga in Japan and its safety is well established (Suetsuna and Chen, 2001). The commercial applications of microalgae as nutritional supplements, natural dyes, and skin care products are reported (Spolaore *et al*., 2006), but there are no studies reporting the antioxidative activity of microalgae protein-derived peptides.

Antioxidants have also been demonstrated to be effective in reducing the risk of carcinogenesis partially due to their antioxidative activity

TABLE 25.2 Some bioactive peptides from microalgae

Amino acid sequence of the peptide	Marine microalgae
Ile-Val-Val-Glu, Ala-Phe-Leu, Phe-Ala-Leu, Ala-Glu-Leu, and Val-Val-Pro-Pro-Ala	*Chlorella vulgaris*
Ile-Ala-Glu, Phe-Ala-Leu, Ala-Glu-Leu, Ile-Ala- Pro-Gly, and Val-Ala-Phe	*Spirulina platensis*
Pro-Gly-Trp-Asn-Gln-Trp-Phe-Leu, Val-Glu-Val-Leu-Pro-Pro-Ala-Glu-Leu	*Navicula incerta*

(Fazlul and Li, 2002; Yang *et al*., 2001). The peptide developed in the study had excellent antioxidant properties and might also be employed as an adjuvant to the conventional therapeutic modalities for gastric cancer potency. There are numerous reports on bioactive compounds in microalgae. Many studies have been done to evaluate the antioxidative potency of peptides from microalgae using different assays *in vitro* (Sheih *et al*., 2009; Suetsuna and Chen, 2001) and have shown significant activities. Therefore, the peptides from microalgae protein have the potential to be used as a good dietary supplement for the prevention of oxidative stress-related diseases such as atherosclerosis, coronary heart disease, and cancer.

C. Antihypertensive activity of peptides from microalgae

The angiotensin I-converting enzyme (ACE) participates in regulating blood pressure in the renin–angiotensin system. The inhibitors such as captopril (Suetsuna and Chen, 2001) and enalapril (Sawayama *et al*., 1990) have been used as antihypertensive drugs. The ACE-inhibitory activity of various source have studied, and it was found that some ACE-inhibitory peptides were produced by enzymatic digestion of various marine food proteins, including tuna muscle (Kohama *et al*., 1991; Ondetti, 1977), sardine muscle (Suetsuna *et al*., 1991), dried bonito (Yokoyama *et al*., 1992), dried-salted fish (Astawan *et al*., 1995), fish sauce (Okamoto *et al*., 1995), and fish water-soluble protein (Wako *et al*., 1999).

A lot of human and animal studies have revealed that ingestion of *Chlorella* results in decreased blood pressure, but the mechanism is still unknown. Murakami *et al*. (1987) and Miyakoshi *et al*. (1980) suggested that *Chlorella* decreases the blood pressure by regulating the renin–angiotensin system. Moreover, Inoue *et al*. (1995) also reported that ingestion of *Chlorella* decreases blood pressure in humans.

D. Anticancer activities of the peptide from microalgae

A variety of compounds such as flavonoids, phenolic acids, and carotenoids, derived from natural sources, have been shown to be beneficial for the inhibition of cancer. The mechanisms which suppress tumor genesis often involve inhibition of tumor cell-mediated protease activity (Yang *et al*., 2001), attenuation of tumor angiogenesis (Kandaswami *et al*., 2005), promotion of cell cycle arrest (Moosavi *et al*., 2005), induction of apoptosis (Lee *et al*., 2004), and immunostimulation (Tzianabos, 2000).

Anticancer peptides have attracted concern recently due to their characteristics features such as multifunction, high sensitivity, and stability. There are number of publications on anticancer peptides from food protein, such as fish sauce (Lee *et al*., 2004), soy protein (Kim *et al*., 2000),

mollusk protein (Leng *et al.*, 2005), milk protein, and beef protein (Jang *et al.*, 2008), but few studies have been reported about microalgae protein as a source for anticancer peptides. However, recent studies suggest that the microalgae-derived peptides could be potentially useful adjuncts in the treatment of gastric cancer (Sheih *et al.*, 2010). Hence, it will be potential protein source in future for industrial production of functional peptides.

E. Protective effects of peptide from microalgae on the liver

Alcohol is mostly metabolized in the liver and excessive alcohol use can lead to acute and chronic liver diseases including hepatitis, liver cirrhosis, fatty liver, and liver cancer (Dey and Cederbaum, 2006). Further, chronic alcohol abuse has become a major health problem that causes liver and pancreatic diseases and is known to impair hepatic alcohol dehydrogenase, myocardial infarction, pancreatitis, and disorders of the immune, endocrine, and reproductive systems (Lima *et al.*, 2006). Liver is the primary organ for metabolism, disposition, and toxicity of ingested ethanol. There is considerable interest in the role of oxidative stress and ethanol generation of reactive oxygen species (ROS) in the mechanism by which ethanol is hepatotoxic. The ethanol exposure can be extremely toxic to tissues due to heightened oxidative stress and it induces the production of ROS (Albano, 2006). Further, several systems likely contribute to the ability of ethanol to induce a state of oxidative stress. However, ethanol can also be metabolized by catalase and more selectively by cytochrome P-450 2E1 (CYP2E1) (Wu and Cederbaum, 1999). Induction of CYP2E1 by alcohol is proposed as a mechanism augmenting the formation of reactive paracetamol metabolites and its hepatotoxicity (Dilger *et al.*, 1997).

Recently, a great deal of interest has been expressed regarding marine-derived bioactive peptides because of their numerous health beneficial effects. It has been reported the bioactivities from enzymatic hydrolysis of *N. incerta* (Kang *et al.*, 2011), but there are few publications on antihepatocyte peptides. Therefore, in our recent study, we investigated the protective effect of the peptides (Pro-Gly-Trp-Asn-Gln-Trp-Phe-Leu, Val-Glu-Val-Leu-Pro-Pro-Ala-Glu-Leu) from microalgae, *N. incerta*, on the liver disease (Table 25.2). Addition of peptides from *N. incerta* hydrolysate clearly reduced the amount of procollagen which is an important indicator for hepatic fibrolysis (Fig. 25.2). Moreover, many studies have reported that marine bioactive peptides can be used as antihypertensive, antioxidative, anticoagulant, and antimicrobial components in functional foods, nutraceuticals, or pharmaceuticals due to their therapeutic potential in the treatment or prevention of diseases.

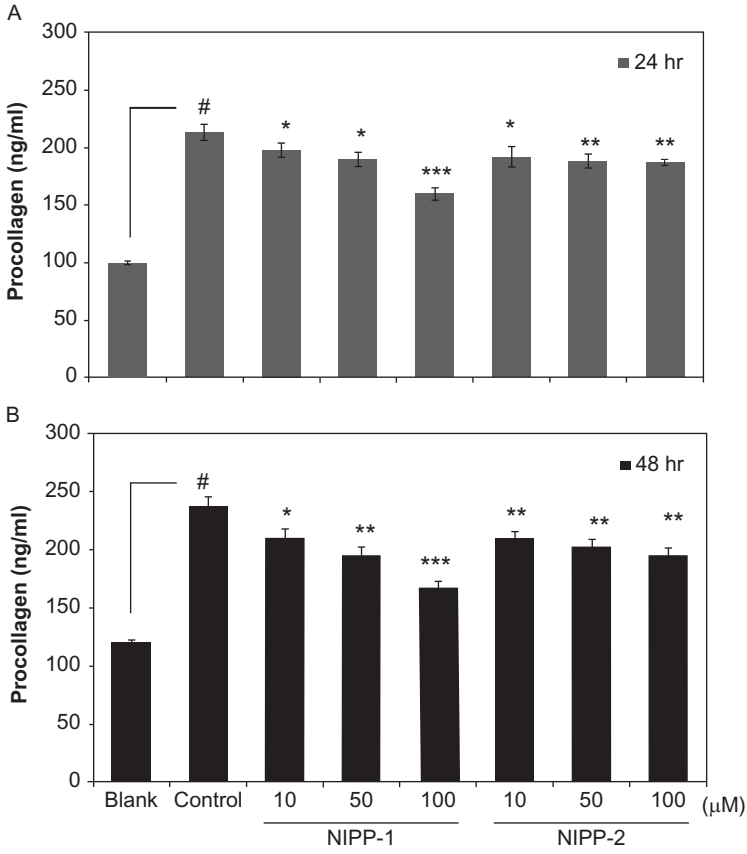

FIGURE 25.2 Effects of NIPP-1 and NIPP-2 on procollagen synthesis in LX-2 cells. Ten percent FBS was used for the tests to eliminate the interference of serum with the produced protein. Procollagen type IC-peptide assay was done after 24 h (A) and 48 h (B) of treatment. Each value was expressed as the mean ± SD of triplicate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ as compared with TGF-β1-treated cells. $^{\#}P < 0.001$ as compared with non-TGF-β1-treated cells.

III. CONCLUSIONS

Microalgae were studied as a potent source for food additive, nutraceutical, or pharmaceuticals. In fact, 30% of the current world algal production is used for animal feed where over 50% of the current world production of *Arthrospira* is used as feed supplement. It also needs to be of the correct size and shape to be ingested and to have high nutritional qualities and a digestible cell wall to make nutrients available. Protein content is a major factor determining the nutritional value of microalgae.

Recent studies have provided evidence that marine-derived bioactive peptides including the compound derived from microalgae play a vital role in human health and nutrition. In recent study, we have proved that two peptides isolated from *N. incerta* showed hepatoprotective activity. These evidences suggest that due to valuable biological functions with health beneficial effects, marine microalgae-derived bioactive peptides have potential as active ingredients for preparation of various functional foods or nutraceutical and pharmaceutical products.

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