Bioactive Proteins and Peptides as Functional Foods and Nutraceuticals
Part 1

Introduction
Chapter 1

Biologically Active Food Proteins and Peptides in Health: An Overview

Yoshinori Mine, Eunice C.Y. Li-Chan, and Bo Jiang

A growing body of scientific evidence in the past decade has revealed that many food proteins and peptides exhibit specific biological activities in addition to their established nutritional value (Mine and Shahidi 2006; Hartmann and Meisel 2007; Tripathi and Vashishtha 2006; Yalcin 2006; Möller et al. 2008). Bioactive peptides have been found in enzymatic protein hydrolysates and fermented dairy products, but they can also be released during gastrointestinal digestion of proteins (Meisel 2005; Korhonen and Pihlanto 2007a, 2007b; Gobbetti et al. 2007; Hartmann and Meisel 2007). Bioactive peptides may help reduce the worldwide epidemic of chronic diseases that account for 58 million premature deaths annually. Functional proteins and peptides are an important category within the nutraceuticals food sector currently valued at $75 billion/year. Nevertheless, several challenges should be addressed to allow sustained growth within this sector. Some earlier health benefits claimed for protein nutraceuticals were based on in vitro models or limited clinical trials leading to equivocal findings (Möller et al. 2008). Technological and fundamental problems remain in relation to large-scale production, compatibility with different food matrices, gastrointestinal stability, bioavailability, and long-term safety (Murray and FitzGerald 2007; Mine 2007). Research into consumer perception and legislation is also necessary. Nutritionists, biomedical scientists, food scientists, and technologists are working together to develop improved systems for discovery, testing, and validation of nutraceutical proteins and peptides with increased potency and therapeutic benefits. Bioactive peptides and proteins are being developed that positively impact on body function and human health by alleviating conditions such as coronary (ischemic) heart disease, stroke, hypertension, cancer, obesity, diabetes, and osteoporosis. Screening novel bioactive sources using high-throughput omics technologies, specific disease biomarkers, and comprehensive clinical trials will facilitate the development of nutraceutical proteins and peptides for a further range of health conditions (Gilani et al. 2008; Mine et al. 2009; Boelsma and Kloek 2009).

This book aims to compile current science-based advances on biologically active food proteins and peptides for health promotion and reduction of the risk of chronic diseases. The book is comprised of four parts: (1) Introduction, (2) Functions of Biologically Active Proteins and Peptides, (3) Examples of Food Proteins and Peptides with Biological Activity, and (4) Recent Advances in Bioactive Peptide Analysis for Food Application. Chapter 1 summarizes aims and scope as well as overall highlights of this book. Chapters 2 and 3 highlight antioxidative and anti-inflammatory proteins and peptides. Oxidative stress is a biological state that occurs when a cell’s antioxidant capacity is overwhelmed by reactive oxygen species (ROS), causing a redox imbalance. Oxidative stress and
inflammation are often related to chronic diseases involving the cardiovascular, neurological, and gastrointestinal systems. Proteins and peptides have been found not only to contribute to the body’s energy supply and growth but also to influence specific biological activities such as oxidative stress and inflammation. As our understanding of the efficacy and mechanism of action of antioxidative stress and anti-inflammatory proteins and peptides increases, so will the growing interest in their prophylactic, preventive, and therapeutic uses.

Recent advances in peptide research have led to the accumulation of mounting evidence that many endogenous peptides have the biological function to stabilize radicals and neutralize other nonradical oxidizing species. Furthermore, this biological function has been demonstrated by in vitro digests of proteins. With an improved understanding of the structure-function relationship, it is now possible to develop antioxidant peptides and peptide mixtures through enzymatic or microbial hydrolysis of common food proteins or by means of chemical synthesis. While the question of whether peptides can act as antioxidants no longer remains, it is still a big challenge to identify the fate of dietary antioxidant peptides and their exact biological activity once entering the circulation system and crossing the cell membrane. The production cost as well as the potential allergenicity of antioxidant peptides must also be assessed in the continuing effort to develop such novel antioxidants to complement the human body’s natural defense system, including antioxidant enzymes, vitamins, and nonprotein compounds.

Chapter 4 reviews the updated antihypertensive mechanism as well as the development of antihypertensive food products. The efficacy of peptide intake for borderline hypertensives is evidentially developed on the basis of extensive intervention trials, but such an effective foods for specified health use (FOSHU) produce has led to the question of whether the antihypertensive effect of peptides is achieved only by angiotensin-converting enzyme (ACE) inhibition or suppression of the renin-angiotensin system, like therapeutic ACE inhibitory drugs. Peptide research on this topic has become one of the growing fields in preventative medicinal chemistry, since clinical evidence demonstrated the efficacy of peptide intake for improving the treatment of hypertension.

Chapter 5 presents food protein-derived bioactive peptides as inhibitors for calmodulin (CaM), a protein that plays important roles in maintaining physiological functions of cells and body organs. Current knowledge indicates that CaM-binding peptides can be produced through the enzymatic hydrolysis of food proteins followed by separation and purification of cationic peptides. Inhibition of CaM-dependent enzymes seems to be dependent mostly on the level of basic (positively charged) amino acid residues for short-chain (<7-mer) peptides, whereas long-chain (16- to 25-mer) peptides interact with CaM mostly through hydrophobic interactions. Structural changes occur during peptide binding, leading to an increased unfolding of CaM such that the protein loses its ability to effectively interact with and activate target metabolic enzymes. It is evident that food protein-derived CaM-binding peptides constitute a potential source of ingredients that may be used to formulate therapeutic diets against human chronic diseases. However, the ultimate utility of these cationic peptides will depend on their oral bioavailability and in vivo inhibition of target metabolic reactions.

Chapter 6 describes the potential for soy protein to address the problem of metabolic syndrome (MS). Diets with increased protein and reduced carbohydrates have been shown to improve body composition and lipid and lipoprotein profiles, reducing blood pressure and playing a part in regulation of glucose and insulin homeostasis, all of which are factors associated with treatment of metabolic syndrome and obesity. Soy protein is a promising dietary component that needs to be further evaluated in regards to its long-term effect on adipogenesis and potential reduction of blood pressure. This will allow determination of the recommended intake of soy protein to reduce the risk of metabolic syndrome in humans from different backgrounds and environments. Attention must also be paid to the technological processes in order to optimize the protein quality, its bioavailability, and the main protein components that will produce the most biologically active peptides.
Chapter 7 deals with amyloidogenic proteins and peptides. To date, many different naturally occurring proteins and polypeptides have been recognized to be amyloidogenic in humans. These precursor proteins, clearly differing in their biochemical function and in their three-dimensional structure, may become prone to undergoing an irreversible transition from their native conformation into highly ordered amyloid fibrils. The extracellular deposition of amyloid fibrils in tissues is the basis of a wide range of human diseases, including neurodegenerative disorders of the central nervous system and various forms of systemic and cutaneous amyloidosis. Under certain conditions, soluble proteins and protein fragments (peptides) self-assemble into \( \beta \)-sheet-rich structures (cross-\( \beta \) conformation) and form amyloids. These amyloids can cause serious disorders known as amyloidosis such as Alzheimer’s disease, hemodialysis-associated disease, and bovine spongiform encephalopathies, which are characterized by the transformation of soluble proteins/peptides into aggregated fibrils in different organs and tissues. Recent reliable discovery of amyloid-forming sequences in amyloidogenic proteins should have a great impact on the development of antiamyloid agents.

Food allergy is classically defined as an adverse reaction to food components resulting from an overt response mediated by the immune system. Its prevalence has been estimated at 2–4% of adults and 6–8% of children in Western countries. These figures would represent approximately twice those reported only a decade ago. Chapter 8 summarizes recent advances in peptide-based immunotherapy (PIT) for food allergy. Peptide-based immunotherapy has been praised as a promising strategy for food allergy by a number of reviews. While PIT investigations carried out with inhalant allergens have reached clinical settings with encouraging results, there is currently little information on the curative potential of PIT in food-induced allergies. However, recent PIT investigations using murine models of food allergy point the way toward promising avenues. Of the various strategies proposed, an immunotherapy based on the use of T-cell epitope-containing peptides has been, to date, the object of most investigations. Elucidation of the mechanisms underlying PIT has guided research into further understanding of allergic responses, which may lead the way toward the design of more efficient immunotherapeutic approaches.

Gamma-aminobutyric acid (GABA), a four-carbon nonprotein amino acid, is a significant component of the free amino acid pool in most prokaryotic and eukaryotic cells. Chapter 9 highlights the recent updated applications of GABA for food and human health. GABA was discovered first in potatoes more than half a century ago and subsequently in rat brains. GABA has many biological functions such as neurotransmission and induction of hypotensive, diuretic, and tranquilizer effects. GABA production by various microorganisms has also been reported using bacteria, fungi, and yeast.

Food intake in man is determined by both physiological and psychological factors. All factors are centrally received, organized, and integrated balancing both short-term and long-term energy intake with energy expenditure. The regulation of energy intake is determined by both the energy content and the energy source (fat, carbohydrate, and protein) of the meal, each of them having unique effects on satiation and satiety regulatory mechanism. Chapter 10 describes food-derived peptides as regulators of satiety. This chapter centers on protein-induced satiety, in particular focussing on mechanisms and regulation of protein-induced satiety. The evidence derived from studies with different experimental models with animals and humans is given, emphasis on the underlying biochemical and neural mechanisms.

Chapter 11 deals with health-promoting proteins and peptides in colostrum and whey. The high nutritional value of bovine milk proteins is widely recognized. Also, the multiple functional properties of major milk proteins are well characterized and exploited by various industries. Over the last 2 decades, milk proteins have attracted growing scientific and commercial interest as a source of biologically active molecules having distinct characteristics. The best characterized bioactive bovine whey proteins include immunoglobulins (Igs), lactoferrin (Lf), lactoperoxidase (LP), and growth
Part 1 Introduction

That is expressed in most biological fluids and is a major component of the innate immune system of mammals. Its protective effect ranges from direct antimicrobial activities against a large panel of microorganisms including bacteria, viruses, fungi, and parasites to anti-inflammatory and anticancer activities.

Chapter 14 focuses on bioactive peptides and proteins from fish muscle and collagen. As marine fish supplies are threatened due to excessive fishing and poor management, the need to obtain fish protein from sustainable sources like aquaculture and improved processing has increased. Post-harvest losses, as discards at sea, deterioration during storage, and wastage during processing, are substantial. Therefore utilizing and upgrading waste from fish processing into valuable food products is a major goal. Like mammalian muscle proteins, fish myofibrillar proteins display excellent gelation and emulsifying properties. In the last decade, fish collagen from skin and bones has been used to produce gelatin. This chapter reviews hydrolysates and peptides from fish muscle and collagen and their ACE inhibitory and antioxidant activity that may lead to their applications as bioactive ingredients in functional foods, conventional foods, and nutraceuticals.

Muscle-based bioactive peptides have been shown to possess many health benefits and are therefore also promising candidates as nutraceutical or functional food ingredients. Since meat and meat products are important in the diet in most developed countries, functional meat products would contribute to human health. The biological activities of muscle-based peptides are summarized in Chapter 15. Further studies will be required to demonstrate the clear benefits of muscle-derived bioactive peptides and their potential nutraceutical applications to benefit human health.

Chapter 16 describes recent technology and value-added utilization of rice bran proteins (RBPs) and their peptides for human health promotion. Cereals are the most widely grown crops of the world and the kernel or caryopsis consists of bran, embryo, and endosperm. The bran is composed of fibers (pentosans, hemicelluloses, β-glucans, factors. They occur in colostrum in much higher concentrations than in milk, reflecting their importance to the health of the neonate. These native proteins are known to exert a wide range of physiological activities, for example, enhancement of nutrient absorption, stimulation of cell growth, enzymatic activity, inhibition of enzyme activity, modulation of the immune system, and defense against microbial infections. At present, milk proteins are considered the most important source of bioactive peptides. Over the last decade a great number of peptide sequences with different bioactivities have been identified in various milk proteins. The best-characterized milk peptides exhibit antihypertensive, antithrombotic, antimicrobial, antioxidative, immunomodulatory, and opioid activities.

Chapter 12 reviews antihypertensive peptides originating from fermented milk products and enzymatic hydrolysis of food proteins, such as milk casein whey proteins and fish meat. Most antihypertensive peptides with proven effects on spontaneously hypertensive rats have angiotensin I-converting enzyme inhibitory activities. Clinical experiences for these antihypertensive peptides were also reviewed to discuss the potential of antihypertensive peptides for high blood pressure. Studies reporting an in vivo mechanism, based mainly on animal studies, were also described. The benefits of antihypertensive peptides with ACE inhibitory activities generated from food proteins were introduced. Some of the peptides with antihypertensive effects in spontaneously hypertensive rats (SHR) demonstrated significant efficacies in humans. In Japan, some ACE inhibitory peptides with proven significant antihypertensive effects in humans have been approved as FOSHU products (functional food products) with health claims concerning high blood pressure. Taking into account the mild effects of ACE inhibitory peptides, which have no adverse effects, they may be considered to be ideal food-derived natural functional ingredients to keep blood pressure within the normal range.

Chapter 13 summarizes our knowledge of the structure and biological functions of lactoferrin and of peptides derived from it. Lactoferrin is an iron-binding glycoprotein of the transferrin family that is expressed in most biological fluids and is a major component of the innate immune system of mammals. Its protective effect ranges from direct antimicrobial activities against a large panel of microorganisms including bacteria, viruses, fungi, and parasites to anti-inflammatory and anticancer activities.
cellulose, lignin, and glucofructans), ash, enzymes, vitamins, and storage proteins. The various fractions of rice bran such as oil, carbohydrates, and dietary fibers (β-glucans, phytates, tannins), and RBPs have demonstrated their ability in prevention of diseases. Some of them are found to have potent anticancer and antioxidant activity. Some of the second-generation by-products of rice bran, that is, RBP concentrates and isolates, show tremendous potentials for future industrial utilization in view of their multifactorial functionality, including their medicinal value. The residual proteins being of high molecular weight and with considerable water-binding capacity may be used as hydrocolloids, which have demonstrated health benefits in improving lipid profile, controlling hyperglycemia, and lowering glycemic index of foods.

Chapter 17 summarizes recent advances of bioactive egg proteins and peptides. Nature forms an avian egg not for human food but to produce a chick. Birds invest in the reproductive process up front, transferring all the nutrients and energy needed to allow the embryo to reach the point of hatching. This concentration of highly available nutrients also makes the egg an ideal nutritional food source; eggs are one of the few foods that are consumed throughout the world. Recent studies have uncovered various roles of egg proteins that go well beyond their basic nutrient value. Indeed, egg proteins may gain increasing recognition in the future in human health, as well as in disease prevention and treatment. However, this value can only be captured through the development of industrially viable technologies that are essential for successfully harvesting valuable egg components for commercial use. Lysozyme and antibody technologies are two such examples of extracted components. Eggs contain more than 60 various types of proteins. The presence of biologically active proteins, especially minor egg proteins, has raised interest in developing novel agents from eggs against chronic diseases, such as cancer.

The potential for developing novel bioactive peptides deserves further study. Chapter 18 focuses on soy peptides as functional food materials. Soy protein is one of the vegetable proteins examined extensively for lipid-lowering effect in humans and in experimental animals. Although soy protein contains a certain amount of bioactive peptides that have distinct physiological activities in lipid metabolism, it is not clear that peptides are responsible for these effects. This chapter describes the nutritional benefits and the biochemical function of soy peptides. Soy peptides prepared from soy protein could well serve as an excellent nutritional supplement with improved absorption properties. The ingestion of soy peptides decreases the muscle damage induced after exercise and shows double effectiveness as compared with soy protein. On the other hand, it was revealed that soy peptides stimulate hypolipidemic events and accelerate antiobesity effects. It is important to conduct more research on active components in the future. Soy peptides may have the ability to prevent some lifestyle diseases.

Chapter 19 presents bioactivity of proteins and peptides from peas (*Pisum sativum*, *Vigna unguiculata*, and *Cicer arietinum L*). Legumes are important sources of dietary proteins. There has been increased interest in their beneficial effects in terms of several health claims that are professed. This chapter reviews recent studies on the nutraceutical/pharmaceutical/therapeutic biological activities of proteins and peptides from legumes commonly referred to as peas (chick peas, *Cicer arietinum*; garden/green peas, *Pisum sativum*; and cow peas, *Vigna unguiculata*).

Chapter 20 introduces wheat proteins and peptides. World wheat production accounts for about 30% of world cereal production, being about 587 million tons in 2006, according to the annual report of the United States Department of Agriculture (USDA) Foreign Agricultural Service. Wheat production and consumption are the highest in the European Union (EU) countries, followed by China and India. Wheat is a major crop that is consumed all over the world in the form of various products such as pasta, bread, cakes, and cookies. The quality of wheat products is affected by protein content because the viscoelastic property of gluten allows dough to expand during heating and provides firm texture to pasta after boiling. This characteristic of gluten is mainly attributed to the elastic property of
polymeric glutenins and the plasticizing property of gliadins. Glutelins and gliadins each account for about 40–46% of total protein, having unique amino acid sequences that are rich in glutamine and proline residues. Although the amount of albumin is not high, it contains amylase inhibitor that inhibits amylase activity and retards carbohydrate digestion, preventing the increase in postprandial hyperglycemia. However, amylase is resistant to proteolysis by digestive enzymes and may be a cause of allergies such as baker’s asthma. On the other hand, gliadins sometimes become a cause of wheat-dependent exercise-induced anaphylaxis (WDEIA) and coeliac disease. As epitopes in gliadins for WDEIA and coeliac disease are comprised of glutamine-rich tandem repeat motifs, the cleavage or modification of the motifs is effective to reduce their allergenicity. Some peptides, such as Ile-Ala-Pro from wheat ab-gliadin and Ile-Val-Tyr from wheat germ, prevent hypertension by inhibiting ACE. Wheat proteins have various functions. Science needs to be developed to maximize their favorable functions, such as prevention of diabetes and hypertension, and to minimize their adverse functions, such as allergenicity.

Peptidomics can be defined as the comprehensive multiplex analysis of endogenous peptides contained within a biological sample under defined conditions to describe the multitude of native peptides in a biological compartment. Peptidomics or “peptide proteomics” refers to the analysis of all the peptides of a cell or tissue (peptidome), while proteomics refers to the analysis of the proteins (proteome). Chapter 21 presents an overview of the advances in peptidomics and applications of peptidomics for discovering novel bioactive proteins and peptides in food and nutrition research.

Chapter 22 highlights in silico analysis of bioactive peptides. Research conducted by numerous scientific centers around the world has contributed to the identification and description of a vast number of biologically active peptides isolated from the body tissues and fluids of many organisms, including bacteria, fungi, plants, and animals. Biologically active peptides are the recommended ingredients of functional food, that is, food designed with the aim of generating the desired functional and biological properties. The emergence of new peptides from various sources necessitated the creation of a database and the establishment of additional criteria for assessing the value of proteins as precursors of bioactive peptides. The BIOPEP database of proteins and bioactive peptides was developed in 2003.

Bioinformatics methods applied in biotechnology and biochemistry are becoming increasingly popular due to short waiting times for results, low testing costs, the option of recording results in the form of text files, and, consequently, the recoverability of results, and they continue to be improved as new advances are made in the field of information technology. With the increasing demand for incorporating functional proteins and peptides into food ingredients, natural health products, and dietary supplements to enhance health, it is crucial to examine the flavor-active properties of these components that may influence their acceptance by consumers. Three of the five basic taste modalities that are currently recognized, namely sweet, salty, and umami, have historically been associated with foods that contain nutrients important for human health and well-being—energy containing carbohydrates (sweet), essential minerals (salty), and amino acids (umami). Chapter 23 explores the flavor-active properties of amino acids, peptides, and proteins that must be considered for successful incorporation and acceptance of components with biologically active properties as ingredients in natural health products, nutraceuticals, and functional foods.

The protein and peptide therapeutics have become an important class of drugs due to advancement in molecular biology and technology. However, an effective and convenient delivery of these drugs in the body remains a challenge. Over the last 10 years, extensive studies have been carried out to improve their delivery. Yet there are still a number of limitations due to their intrinsic physicochemical and biological properties, including poor permeation through biological membranes (due to large molecular size), short half-life, physical and chemical instability, enzymatic catalysis, aggregation, adsorption, bio-incompatibility, and immunogenicity. Chapter 24 presents the difficulties in delivery of proteins.
and peptides and discusses the liquid crystal–based controlled delivery systems that were produced and examined to improve delivery.

In summary, the collection of chapters in this book, written by an international team from industry and academia, provides a comprehensive overview of the fundamental concepts, mechanisms, and ongoing research needs, as well as current and prospective applications, of the major categories of biologically active proteins and peptides with potential for significant benefits to human health. It is hoped that the knowledge and insights gained from these chapters will pave the way to realizing the tremendous opportunities within this rapidly growing field of bioactive proteins and peptides from food sources as nutraceuticals and functional foods.

References
