Chapter 23
Relevance of Peptides Bioactivity in Foods

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23.1 Introduction

Numerous food components having physiological functions have been isolated, characterized, and applied to functional foods (Arihara 2012; Arihara and Ohata 2011; Saarela 2011). Food protein-derived peptides are a group of such functional components (Arihara 2006; Gobbetti et al. 2007; Kannan et al. 2012; Korhonen and Pihlanto 2007). Although the activities of these protein-derived peptides in the sequence of proteins are latent, they are released by proteolytic enzymes. Therefore, food proteins have possible bioactivities beyond a nutritional source of amino acids alone.

Mellander (1950) first reported bioactive peptides generated from food proteins. He suggested that milk casein-derived peptides enhanced bone calcification in infants. Since then, numerous bioactive peptides from food proteins have been studied (Hettiarachchy et al. 2012; Kannan et al. 2012; Owusu-Aspenten 2010). Enzymatic hydrolysis of food proteins such as milk caseins produces various peptides with physiological functions, such as antihypertensive, opioid, immunostimulating, antimicrobial, antithrombotic, hypocholesterolemic, and antioxidative activities.

This chapter includes a brief overview of bioactive peptides generated from food proteins. Also, utilization of modern nutrigenomics techniques for such peptides is discussed here. Nutrigenomics has been rapidly applied to the field of nutrition and health (Affolter et al. 2009; Bidlack and Rodriguez 2012; Thomson-Smith 2010). This powerful tool contains gene, protein, and metabolite profiling (transcriptomics, proteomics, and metabolomics). Although application of this attractive strategy for

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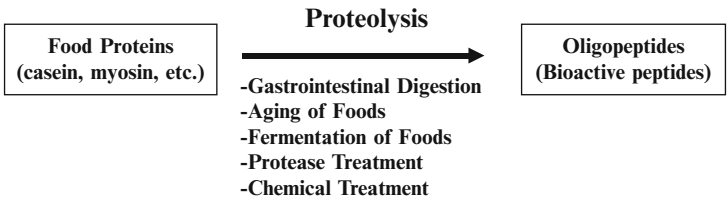

Fig. 23.1 Generation ways of peptides from food proteins

studying bioactive peptides generated from food proteins is still limited, the nutrigenomics approach offers a great possibility for understanding and utilizing bioactive peptides.

23.2 Generation of Peptides from Food Proteins

Most bioactive sequences of food proteins are inactive or incomplete within the parent proteins. Active peptide fragments are released from native proteins via proteolytic digestion. After such peptides are liberated from food proteins, they can act as regulatory (bioactive) compounds. Gastrointestinal proteolysis, aging, fermentation, enzymatic treatment, and other chemical treatments are the principal means for digestion of food proteins to generate bioactivities (Figure 23.1). During gastrointestinal proteolysis, bioactive peptides are liberated from food proteins (Pihlanto and Korhonen 2003). Upon ingestion, pepsin in the stomach digests food proteins into large peptides And once in the small intestine, trypsin and chymotrypsin cleave these peptides into small peptides and amino acids.

In addition to gastrointestinal digestion, there are several ways in which peptides are generated from food proteins. During aging or storage, food proteins are hydrolyzed by endogenous proteases. For example, meat proteins are hydrolyzed by muscle endogenous enzymes, such as calpains and cathepsins (Koohmaraie 1994). Such enzymatic hydrolysis contributes to the improvement of sensory properties (texture, taste, and flavor) of meat (Nishimura et al. 1988). Although there has been no report about the generation of bioactive peptides in meat during post-mortem aging, our preliminary study showed an increase in angiotensin I converting enzyme (ACE) inhibitory and antioxidative activity of beef and pork during storage at 4°C.

Proteolytic events often occur during fermentation of foods and various peptides are generated from food proteins (Hernández-Ledesma et al. 2004). For example, the proteolysis of raw sausages and dry-cured ham have been studied extensively, because components generated from meat proteins are critical for the development of sensory properties of fermented meat products (Hammes et al. 2003; Toldrá 2004). During fermentation of sausages, the content of peptides and amino acids reaches about 1% dry matter of products (Dainty and Blom 1995). We measured the

ACE inhibitory activities of extracts of several European fermented sausages and found that the activity level of all extracts was higher than those of extracts obtained from nonfermented pork products. Fermented dairy products (e.g., cheese and yogurt) contain bioactive peptides, such as ACE inhibitory peptides, generated by proteolytic activities of starter microorganisms (Bütikofer et al. 2008; Gagnaire et al. 2001; Gobbetti et al. 2000; Gomez-Ruiz et al. 2002; Saito et al. 2000).

Utilization of commercial proteases is the most practical approach for producing bioactive peptides from food proteins (Young and Mine 2009). Inasmuch as protease treatments of food proteins are an efficient method for hydrolyzing proteins, proteolytic enzymes are used for various processes in the food industry. Single proteinases from animal, plant, and microbial sources and combinations of them can be utilized for the digestion of food proteins for releasing bioactive peptides. Also, the production of peptides from food proteins can be achieved by the use of heat, acid, or base hydrolysis.

23.3 Representative Bioactive Peptides from Food Proteins

23.3.1 ACE Inhibitory Peptides

The most extensively studied bioactive peptides generated from food proteins are ACE inhibitory peptides (Aleixandre and Miguel 2012; Meisel et al. 2005). These peptides have attracted particular attention because of their ability to prevent hypertension and have been utilized for pharmaceuticals and physiologically functional foods. These peptides lower blood pressure through inhibition of ACE in the body. ACE is a dipeptidyl carboxypeptidase, which is widely distributed in mammalian bodies, predominantly as a membrane-bound ectoenzyme in vascular endothelial cells. ACE plays a critical role in the regulation of blood pressure in the renin angiotensin system (Figure 23.2). ACE converts the inactive decapeptide angiotensin I into the potent vasoconstricting octapeptide angiotensin II, resulting in increases in blood pressure. ACE also inactivates the antihypertensive vasodilator bradykinin. Therefore, by inhibiting the catalytic action of ACE, the elevation of blood pressure can be suppressed in the body.

ACE inhibitory peptides derived from food proteins were first found in the hydrolysate of gelatin by Oshima et al. (1979). Since then, ACE inhibitory peptides have been identified in the hydrolysates of various proteins from foods such as milk, fish, meat, eggs, soybean, corn, wheat, seaweed, and others (Aleixandre and Miguel 2012; Arihara 2006; Meisel et al. 2005). Some of these peptides have been reported to show antihypertensive effects by oral administration. As the examples of such peptides, two ACE inhibitory peptides (Met-Asn-Pro-Pro-Lys and Ile-Thr-Thr-Asn-Pro) were purified from thermolysin digest of porcine muscle myosin (Arihara et al. 2001). These ACE inhibitory peptides showed antihypertensive effects in spontaneously hypertensive rats (SHR) by oral administration (Nakashima et al. 2002). Also,

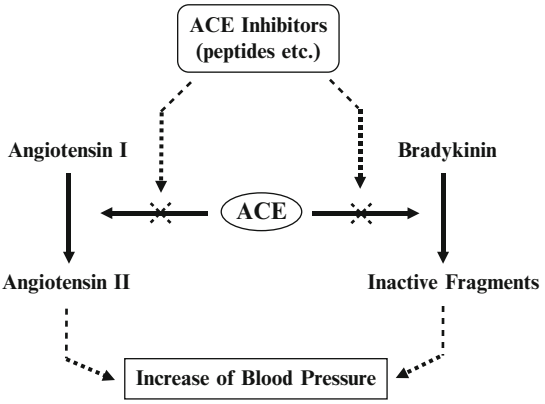

Fig. 23.2 Blood pressure regulation by Angiotensin I-Converting Enzyme (ACE)

six tripeptides that have parts of the sequences of the two peptides were orally administered to SHR. Among the six tripeptides, Met-Asn-Pro caused the most significant decrease in systolic blood pressure. Although many ACE inhibitory peptides have been found in various food protein hydrolysates, their structure–activity relationships have not been fully clarified (Li et al. 2004). However, it has been reported that many ACE inhibitory peptides have tryptophan, phenylalanine, tyrosine, or proline at their C-terminus and these amino acids would be critical for the ACE inhibitory activity of peptides.

23.3.2 Opioid Peptides

Opioid peptides are defined as peptides that have an affinity for an opioid receptor as well as opiatelike effects (Pihlanto and Korhonen 2003). These peptides influence the nerve system and gastrointestinal functions. Typical opioid peptides (i.e., endorphins, enkephalin, and prodynorphin) have the same N-terminal sequence, Tyr-Gly-Gly-Phe. Opioid peptides bind to specific receptors of the intestinal target cell. These receptors participate in specific effects, such as emotional behavior, suppression of intestinal motility, and appetite. Brantl et al. (1979) identified the first group of opioid peptides (casomorphins) generated from milk casein. Proteolysis of alfa-lactoalbumin (a milk whey protein) generated a opioid peptide (Tyr-Gly-Leu-Phe) and was named α-lactorphin (Chiba and Yoshikawa 1986). Although administration of α-lactorphin lowered blood pressures in SHR, the antihypertensive mechanism of this peptide is not by ACE inhibition but rather appears to be due to interaction with opioid receptors (Nurminen et al. 2000). Opioid peptides were also identified in hydrolysates of wheat gluten and blood hemoglobin.

23.3.3 Antioxidative Peptides

The intake of antioxidants may decrease the risk of diseases, such as cardiovascular disease and certain types of cancer (Hertog 1996). Endogenous antioxidative peptides (e.g., glutathione, carnosine, and anserine) have been reported to play many physiological roles, such as prevention of diseases related to oxidative stress (Hipkiss and Brownson 2000). In addition to these endogenous nonprotein peptides, several antioxidative peptides have been identified from soybean, milk, eggs, and meat (Kim et al. 2012). For example, Saiga et al. (2003) reported that hydrolysates obtained from porcine myofibrillar proteins by protease treatment exhibited high antioxidant activity. Antioxidative peptides were isolated and sequenced as Asp-Ser-Gly-Val-Thr, Ile-Glu-Ala-Glu-Gly-Glu, Asp-Ala-Gln-Glu-Lys-Leu-Glu, Glu-Glu-Leu-Asp-Asn-Ala-Leu-Asn, and Val-Pro-Ser-Ile-Asp-Asp-Gln-Glu-Glu-Leu-Met. Acidic amino acids, Asp or Glu, were found in all five peptides. Although it has been reported that basic amino acids, such as His and Lys, show strong antioxidative activity (Chen et al. 1995), it was revealed that acidic peptides as well as basic peptides possess antioxidative activity.

23.3.4 Immunomodulating Peptides

Immunomodulatory peptides affect both the immune system and cell proliferation responses (Korhonen and Pihlanto 2007). It has been reported that several hydrolysates of milk caseins stimulate the immune system. Although peptides generated from milk caseins by pancreatin or trypsin inhibited the proliferative responses of murine splenic lymphocytes and Peyer's patch cells, digests generated by pepsin or chymotrypsin did not show such activity (Otani and Hata 1995). Opioid peptides generated from milk proteins also have a modulatory function in the immune system, because opioids can alter the characteristics of cellular components of the immune system (Webster 1998).

23.3.5 Other Bioactive Peptides

In addition to the above-mentioned peptides, it has been found that various bioactive peptides have been found in the hydrolysates of food proteins. Nagaoka et al. (2001) discovered a hypocholesterolemic peptide (Ile-Ile-Ala-Glu-Lys) from enzymatic hydrolysates of β-lactoglobulin. This peptide has a strong effect on serum cholesterol level, and the hypocholesterolemic activity of the peptide was greater than that of the drug β-sitosterol in rats. The peptide is speculated to reduce the micellar solubility of cholesterol and inhibit cholesterol absorption (Nagaoka et al. 2001).

Antimicrobial peptides have been isolated from milk and egg proteins. Antimicrobial peptides generated from milk lactoferrin have been studied most extensively (Tomita et al. 1994). A hen egg ovotransferrin-derived antimicrobial peptide that is active against *Staphylococcus aureus* and *Escherichia coli* has been isolated (Ibrahim et al. 2000).

Several milk protein-derived peptides generated by enzymatic hydrolysis act as mineral trappers and result in enhancement of the absorption efficiency of minerals (Vegarud et al. 2000). Based on the knowledge that the mechanism of blood clotting is similar to that of milk clotting, milk protein-derived antithrombotic peptides have been studied (Fiat et al. 1993). Many studies have shown that hydrolysates of milk proteins exhibited stimulation of the growth of lactic acid bacteria and bifidobacteria (Brody 2000). The hydrolysate of porcine skeletal muscle actomyosin digested by papain also enhanced the growth of *Bifidobacterium* strains (Arihara et al. 2011a). Apart from bioactivities, food protein-derived peptides also contribute to the organoleptic properties of food, such as meat (Arihara 2006). Therefore, generation of peptides from food proteins has the potential to develop novel functional foods with favorable organoleptic properties.

23.4 Conventional Methods for Peptide Identification

After generation of bioactive peptides from food proteins, the next steps are the concentration, purification, and identification of peptides concerning bioactivities. In most hydrolyzed food protein digests, only a few peptides are responsible for objective activities. Also, these bioactive peptides are generated from food proteins in relatively low concentrations. Figure 23.3 shows a typical conventional procedure for the study of bioactive peptides derived from food proteins. After hydrolysis of food proteins, the hydrolysates can be fractionated and the peptides enriched by various methods, such as precipitation with solvents, membrane separation, ultrafiltration, and chromatography. Reversed-phase high-performance liquid chromatography (RP-HPLC) is a standard technique in the purification of peptides (Aguilar 2004). Also, a combination of HPLC and mass spectrometry (LC-MS) is a powerful tool not only for purification of peptides but also for structural identification of peptides (Shen and Noon 2004). Thus, in addition to the Edman degradation method, MS has become a standard tool for sequencing of peptides. LC-MS/MS-based peptide sequencing is also becoming a standard technique. For example, Ghassem et al. (2011) identified ACE inhibitory peptides in fish myofibrillar protein hydrolysates by utilizing HPLC coupled to electrospray ionization–time-of-flight mass spectrometry (ESI-TOF MS/MS). Two peptide sequences were identified as VPAAPPK ($IC_{50} = 0.45$ μM) at 791.155 *m/z* and NGTWFEPP ($IC_{50} = 0.63$ μM) at 1085.841 *m/z*, respectively. After purification and identification of peptides, the next step is characterization of peptides in vitro and in vivo. For these studies, synthesized peptides are prepared by a peptide synthesizer in most cases.

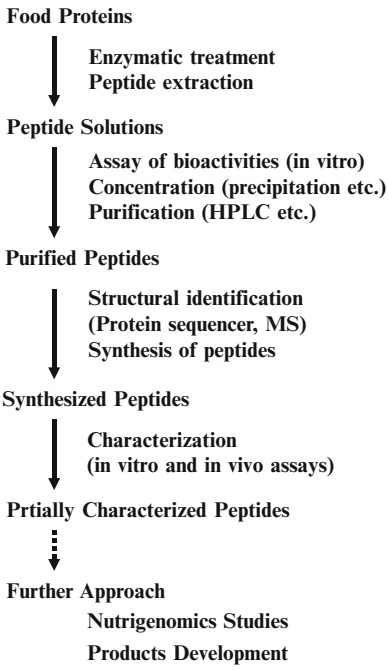

Fig. 23.3 Research strategy for food protein-derived bioactive peptides

Membrane-based separation techniques are utilized in the concentration of bioactive peptides, especially useful for commercial scale preparation (Pouliot et al. 2006). For industrial applications, efficiency of the process is critical, and the purification technique must be a balance between purity and efficiency. The large-scale fractionation of bioactive peptides was reviewed recently (Sato and Hashimoto 2012). Purification methods other than those described above are: chromatographic techniques (e.g., ion-exchange chromatography, hydrophobic interaction chromatography, size-exclusion chromatography, and affinity chromatography), electromembrane filtration, and isoelectric focusing.

23.5 Mechanisms of Bioactivities of Peptides

After oral ingestion of peptides, they are attacked by various enzymes in the stomach and the small intestine. Furthermore, cleaved small peptides can be digested by brush border membrane oligopeptidases and intracellular peptidases (Aito-Inoue et al. 2007). However, some orally ingested peptides can be transported intact into the bloodstream (Pihlanto and Korhonen 2003). After being transported into the bloodstream and once reaching their target sites, peptides induce their bioactivities via several mechanisms. According to the description of Young and Mine (2009),

bioactive peptides can be broadly categorized into two groups: peptides that exert their effects by direct physical interaction with another molecule and peptides that interfere with gene expression. As described above, ACE inhibitory peptides lower blood pressure through inhibition of ACE in the body. ACE inhibition involves direct interaction of the peptide with noncatalytic binding sites in the enzyme. However, the inhibitory potencies of ACE inhibitory peptides do not always correlate with their antihypertensive effects. Some peptides with potent ACE inhibitory activities in vitro are inactive with oral administration. Fujita et al. (2000) clarified the discrepancy of ACE inhibitory activity in vitro and antihypertensive effect in vivo. ACE inhibitory activity has been measured according to the method of Cushman and Cheung (1971) in most studies. This in vitro assay is based on the liberation of hippuric acid from Hip-His-Leu catalyzed by ACE. Prior to the assay, a sample solution of peptides is mixed with a solution containing Hip-His-Leu and NaCl and then pre-incubated. After the reaction initiated by the addition of ACE, the hippuric acid liberated by ACE is determined. The concentration of an ACE inhibitory peptide needed to inhibit 50% of ACE activity is defined as the IC50 value. Fujita et al. (2000) pre-incubated ACE peptides from several food proteins with ACE before measurement of ACE inhibitory activity and classified ACE inhibitory peptides into three groups. One group is inhibitor-type peptides, that is, peptides for which IC50 values are not affected by pre-incubation with ACE. Another group is pro-drug-type peptides, that is, peptides that are converted to true inhibitors by an ACE or other proteases. The third group is substrate-type peptides, that is, peptides that are hydrolyzed by an ACE to give peptides with weak activity. Both inhibitor-type and pro-drug-type peptides exert antihypertensive activities with oral administration.

Similarly to ACE inhibitory peptides, antimicrobial and mineral binding peptides directly interfere with other molecules in the body. On the other hand, peptides can produce their bioactivities by altering gene expression (Young and Mine 2009). Some bioactive peptides generated from food proteins can alter gene expression by (1) epigenetic modification of the proteins that attach to the DNA, (2) alteration of the cell's primary signaling ligand to influence transcription factor activity indirectly, and (3) interference with cell signaling and gene expression via the direct binding of peptide ligand to the receptor. Although studies of bioactive peptides classified into these categories are still very limited, the mechanisms of such activities will be revealed with new nutrigenomics and proteomics techniques. Some examples of such studies are described in the following section.

23.6 Nutrigenomics of Bioactive Peptides

Genomics is the study of all DNA sequences in the genome of an organism. Nutrigenomics seeks to discover the interaction between dietary factors and host genes and how genes and their products metabolize these constituents into health-promoting nutrients or disease-causing antinutrients, and bioactive compounds

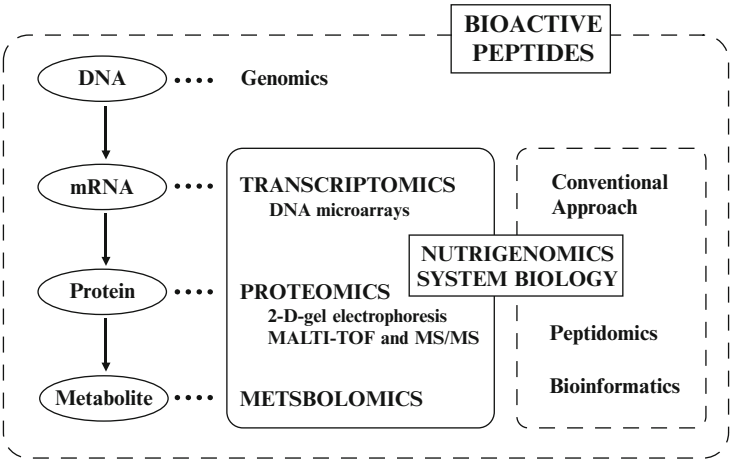

Fig. 23.4 Nutrigenomic approach for food protein-derived bioactive peptides

(Astley and Penn 2009; Kaput 2007; Thomson-Smith 2010). New omics technologies including transcriptomics, proteomics, and metabolomics offer exciting opportunities to address complex issues related to human health (Figure 23.4).

23.6.1 Transcriptomics of Bioactive Peptides

Transcriptomics analysis allows for a genomewide monitoring of expression for the simultaneous assessment of tens of thousands of genes and of their relative expression (Affolter et al. 2009). It measures the relative amounts of mRNAs in a given organism for determining patterns and levels of gene expression. The classical gene analysis approaches, such as Northern blotting and realtime RT-PCR, can only analyze gene expression for a limited number of candidate genes at a time. Microarray technology is a powerful high-throughput genomic tool (Mano et al. 2009). It can be used for profiling and monitoring the expression levels of numerous genes. It can also be used to determine the influence of food nutrients and factors maintaining homeostatic control of gene expression levels. Since transcriptomics is the most successful technology in nutrigenomics approaches, many nutrigenomic studies with microarray technology have investigated the relationship between genes and food intake and bioactive food components (Masotti et al. 2010).

A typical DNA microarray assay includes several procedures (Mano et al. 2009). A microarray is prepared by an arrayed series of many spots of DNA oligonucleotides of specific genes. This array is used to measure the mRNA abundance of a sequence in a sample relative to that in the control. mRNA is extracted from each sample (i.e., animal or cell) treated with food factors. The mRNA is reverse-transcribed to obtain complementary RNA (cRNA) and probes. The labeled mRNA

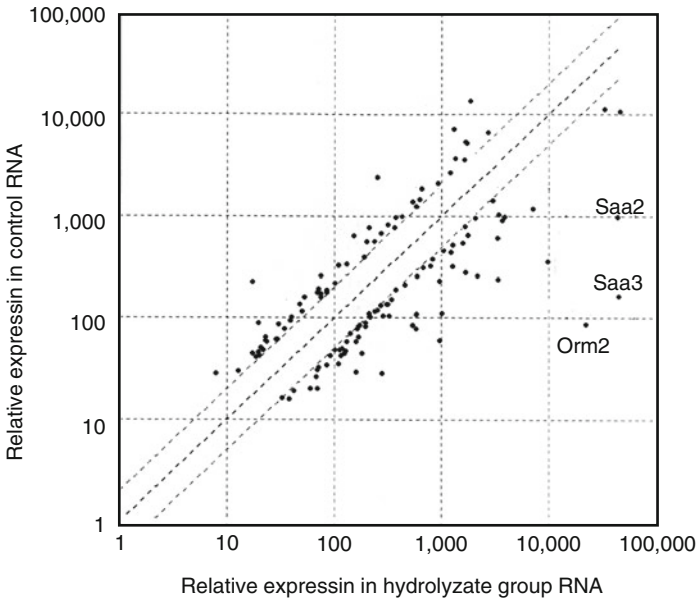

Fig. 23.5 The example result of the DNA microarray assay. This scatter plot represents the relative expression of transcripts in the control RNA sample (Y-axis) versus the relative expression of the transcript in the meat protein hydrolyzate treatment group RNA (X-axis). Each point represents the expression data of an individual transcript (significantly regulated genes)

of the sample functions as probes that hybridize only with the correct target sequence under high-stringency conditions. In microarray analysis, the probes covalently bind to a specific sequence of the bound-labeled probes, measured using microarray scanners. The expression data of tens of thousands of genes are visualized and statistically analyzed using specific bioinformatics tools.

We have studied bioactive peptides in enzymatic hydrolysates of porcine myofibrillar proteins (Arihara 2006). Hydrolysates and identified peptides exhibited ACE inhibitory and antioxidative activities. In addition to bioactivities in vitro, these hydrolysates and peptides have physiological activities in vivo. For example, they showed an antifatigue effect when orally administered to mice and rat in an experiment using a treadmill (Arihara et al. 2011b). Recently, we analyzed gene expression in mice liver by the DNA microarray method. The patterns of gene expression with or without oral administration of meat protein hydrolysates were analyzed. Microarray analysis revealed that the oral administration of the hydrolysate significantly regulated 91 genes (e.g., Saa2, Saa3, and Orm2) of 37,440 genes. Figure 23.5 shows the example result of the DNA microarray assay in our study.

Although studies for bioactive peptides generated from food proteins by DNA microarray are still limited, several examples of such studies are described here. The activity of milk whey proteins and their trypsin-hydrolyzed peptides to suppress the onset of inflammatory reactions was assayed by using the following mouse models:

LPS-induced sepsis and Concanavalin A-induced hepatis (Yamaji and Kume 2008). The patterns of gene expression before and after the onset of inflammation in these models were analyzed by means of DNA microarray methods and compared to those observed in mice fed milk whey proteins or their peptides. The analyses implied that whey protein and their peptides suppressed the onset of inflammation by influencing the gene expression of such factors as involved in signal transduction cascades for the production of pro-inflammatory cytokines.

Asn-Pro-Trp-Asp-Gln (107–111 of milk αs_2-casein) inhibits allergen permeation in Caco-2 cells as an in vitro human intestinal epithelial model. Yasumatsu and Tanabe (2010) have demonstrated that the mechanism underlying this inhibitory activity was examined in Caco-2 cells. Transepithelial resistance value increased in response to the addition of increasing Asn-PropTrp-Asp-Gln concentrations, which suggests that this peptide enhanced epithelial barrier function. Changes in mRNA expression by the addition of peptide were analyzed in Caco-2 cells using the microarray method. From the results of the microarray assay, it was suggested that Asn-Pro-Trp-Asp-Gln upregulated the expression of occludin in particular and enforced the tight junction barrier.

It has been reported that food-derived bioactive peptides express a variety of functions in vivo. Ile-Pro-Pro has been known as an ACE inhibitory and antihypertensive peptide derived from bovine milk protein (Nakamura et al. 1995). Huttunen et al. (2007) studied the in vitro effect of Ile-Pro-Pro on osteoblast proliferation and gene expression. They used UMR-106 osteosarcoma cells, human marrow-derived mesenchymal stem cells (hMSC), and osteoblasts differentiated from hMSC. Treatment with Ile-Pro-Pro increased UMR-106 cell and hMSC proliferation. The gene expression of hMSC-differentiated osteoblasts was analyzed by the DNA microarray method. Microarray analysis revealed that Ile-Pro-Pro upregulated 270 genes and downregulated 100 genes. Realtime PCR confirmed that Ile-Pro-Pro upregulated PTHrP, BMP-5 and CREB-5 and downregulated VDR and caspase-8. These results indicate that Ile-Pro-Pro possesses the potential to increase osteoblast proliferation, differentiation, and signaling.

Nagaoka (2012) studied lactostatin, a hypocholesteromic peptide derived from bovine milk beta-lactoglobulin, by the DNA microarray method. As they expected, the addition of lactostatin to HepG2 (human liver cells) increased the mRNA level of cholesterol 7α-hydroxylase, a key enzyme in cholesterol homeostasis. Also, genes of mitogen activated protein kinase (MAPK) cascades increased.

23.6.2 Proteomics

Although DNA microarray technology is a powerful tool for the study of gene–diet interactions, this technique has some problems or limitations (Zhang et al. 2010). One major problem is the nonreproducibility of gene expression profiles. Different conclusions could be drawn from the same experiment performed at different times or in different laboratories or platforms. Another major issue is the analysis of

datasets and their interpretation. Analyses only providing gene lists with significant *p*-values are insufficient to fully understand the underlying biological mechanisms. Also, the changes in mRNA concentration do not necessarily result in differences in the concentration and/or activity of the encoded protein. Therefore, other emerging functional genomics techniques should be considered (Bidlack and Rodriguez 2012; Bagchi et al. 2010; Mine et al. 2009). It is by combining information from nutrigenomics, genomics, proteomics, metabolomics, and appropriate bioinformatics that this will be a viable approach to understanding all aspects and implications of nutrition-modulated beneficial homeostasis. Although a large number of reports regarding gene expression profiling of food-derived bioactive compounds has been studied, the proteomic approach of these compounds has still been limited.

Proteomics is the study of proteins expressed in a cells, tissue, or organism, including all protein isoforms and post-translational modifications (Bidlack and Rodriguez 2012; Bagchi et al. 2010; Mine et al. 2009). Proteomics is the large-scale analysis of a proteome expressed by a genome. A proteome is the entire complement of proteins synthesized in a biological system at a given time and under defined conditions, reflecting the expression of a set of specific genes in the situation pertaining to that time point. Proteomics allows for the high-throughput investigation of numerous proteins simultaneously in cells, tissues, or biological fluids. As an integral part of nutrigenomics, nutritional proteomics examines the effects of food components on protein expression and provides the potential to identify biomarkers sensitive to dietary interventions (Fuchs et al. 2005). The identification of biomarkers that reflect the outcome of peptide utilization will greatly benefit the field.

The numbers of reports on proteomic studies on bioactive peptides generated from food proteins is still very limited. In an attempt to search for novel biomarkers that could monitor the level of stress, we examined the influence of fatigue stress using a treadmill on the differential changes in the blood serum proteome in male SD rats using 2DE followed by peptide mass fingerprinting (PMF) analysis. Of numerous protein spots on 2-D gel maps of rat blood serum, a significantly upregulated protein spot was identified as creatine kinase by MALDI-TOF/MS (Akimoto et al. 2007). Research is now in progress by using this protein as a promising biomarker for antistress activities of a meat protein-derived peptides experiment.

23.6.3 Metabolomics

Metabolomics is a relatively new omics technology in nutritional approaches. It was originally defined as the quantitative measurement of time-related multiparametric metabolic responses of multicellular systems to pathophysiological stimuli or genetic modification (Whitfield and Kirwan 2010). The metabolome consists of the complete set of low molecular weight metabolites produced in a biological system, such as cells and tissues. Nutritional metabolomics has the potential to provide

insight into biochemical changes after dietary intervention and to affect food safety issues pertaining to genetically modified food. Although metabolomics has contributed significantly to the omics revolution, a global description of human metabolism is impossible at this point due to limitations in current technologies and diversity among individuals in terms of age, gender, diet, lifestyle, health status, and other internal and external factors. Currently, the extent to which food components in the human diet induce changes in nutritional metabolic profiles is poorly understood. However, with technological advances, the challenges of applying metabolomics in nutrition research of bioactive peptides can be overcome.

23.7 Conclusions and Perspectives

In past decades, much information has been accumulated regarding the bioactive peptides generated from food proteins. In addition to conventional research methods, the nutrigenomics approach promotes the understanding of bioactive peptides. With the advent of new proteomic and genomic techniques, the mechanisms underlying many of the biological properties of food protein-derived peptides will be revealed.

In proteomics, all proteins expressed in a cell or tissue are analyzed to identify the presence or absence of some key proteins. Although proteins are routinely separated by 2-D electrophoresis in proteomics, the physiologically interesting small bioactive peptides are neglected in most proteomic studies. Consequently, the concept of peptidomics was introduced (Boonen 2009; Mine 2009). The purpose of peptidomics is the identification of whole peptidome of a cell, tissue, or organism, because peptides (e.g., hormones, cytokines, and growth factors) play critical roles in many physiological processes. The field of peptidomics is relatively new and has the potential to progress in future with the advent of high-throughput MS-based technologies coupled with bioinformatics and genomic databases.

Bioinformatics involves the integration of computers, software tools, and databases in an effort to address biological questions in the omics, including genomics, proteomics, transcriptomics, metabolomics, and peptidomics. Systems biology involves the integration of genomics, proteomics, and bioinformatics information to create a whole-system view of a biological entity. By combining information from nutrigenomics, genomics, proteomics, metabolomics, peptidomics, and appropriate bioinformatics, it would be possible to understand all aspects and implications of bioactive peptides derived from food proteins.

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