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Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins: A review

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ABSTRACT

Bioactive peptides are oligopeptides that consist of 2–20 amino acids that can exert beneficial effects on human health in addition to basic nutritional effects. Food derived protein hydrolysates or peptides with immunomodulatory and anticancer activities have been reported from a variety of food protein sources such as milk, egg, fish, rice, soybean, pea, chlorella, spirulina, oyster and mussel. *In vitro* hydrolysis of food proteins using commercial proteolytic enzymes is the most commonly employed process for the production of immunomodulatory and anticancer food protein hydrolysates. The immunomodulatory and anticancer activities of food derived protein hydrolysates or peptides are related to the amino acid composition, sequence and length. Most immunomodulatory and anticancer food protein hydrolysates or peptides were tested using cell culture and animal models, while a few involved clinical trials. This review provides a comprehensive overview of immunomodulatory and anticancer food derived protein hydrolysates or peptides, their production and mechanisms of action.

1. Introduction

Protein is an important macronutrient as a source of essential amino acids and energy. In addition to basic nutrition, some food proteins can provide extra health benefits through the release of bioactive peptides encrypted in their sequences. In recent years, research on protein hydrolysates or bioactive peptides for the production of value-added food ingredients has attracted great attention of food scientists worldwide. Generally, bioactive peptides are oligopeptides that are inactive within the sequence of the protein molecule but can be released by enzymatic hydrolysis, fermentation and gastrointestinal digestion (Bhat, Sunil Kumar, & Bhat, 2015; Chalamaiah, Dinesh Kumar, Hemalatha, & Jyothirmayi, 2012; Garcia, Puchalska, Esteve, & Marina, 2013; He, Cao, Pan, Yang, & Zhang, 2015). The low molecular weight (MW) peptides are easily digestible and more bioavailable than proteins for a variety of physiological functions of the human body. They generally exhibit greater bioactivity than the parent protein.

Protein hydrolysates (peptides) derived from several food proteins have been reported to possess a wide range of bioactivities including immunomodulatory, anticancer, antihypertensive, antioxidant, anti-inflammatory, mineral binding, opiate, antilipemic, osteoprotective, and antimicrobial effects (Bhat et al., 2015; Chalamaiah et al., 2012; Garcia et al., 2013). In addition to bioactivities, food derived protein hydrolysates (peptides) possess various physicochemical properties including solubility, lipid binding, foaming, and emulsification properties depending on their composition, sequence, and length (Cho et al., 2014; Pokora et al., 2013). Hence, food derived protein hydrolysates are promising ingredients for developing functional foods (Chalamaiah et al., 2012). Additionally, the peptide preparation could help the translation of underutilized food proteins and protein by-products into valuable products, which is of great interest to food companies.

Bioactive protein hydrolysates or peptides can be obtained from different food protein sources. The most investigated sources of bioactive peptides are milk, whey, egg, fish, marine species, soybean, rice,

Abbreviations: ACE, angiotensin converting enzyme; AO, acridine orange; BAD, Bcl-2-associated death promoter; BAX, BCL2-associated X protein; B cells, B lymphocytes; Bcl-2, B-cell lymphoma 2; CCL18, chemokine (CC motif) ligand 18; CD, cluster of differentiation; CDK2, cyclin-dependent kinase 2; CO_2, carbon dioxide; Con-A, concanavalin A; COX-2, cyclooxygenases 2; Da, dalton; DH, degree of hydrolysis; DMBA, 7,12-dimethylbenz[*a*]anthracene; DNA, deoxyribonucleic acid; EB, ethidium bromide; ELISA, enzyme linked immunosorbent assay; ERK, extracellular signal–regulated kinase; EW, whole egg white; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate; FPHs, food protein hydrolysates; IC_{50}, inhibitory concentration 50; IFN-γ, interferon-γ; Ig, immunoglobulin; IL, interleukin; iNOS, inducible nitric oxide synthase; kDa, kilodalton; LfcinB, lactoferricin; LPS, lipopolysaccharide; LYS, lysozyme; MAPKs, mitogen-activated protein kinases; MHC, major histocompatibility complex; MTT, 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MW, molecular weight; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK cells, natural killer cells; NO, nitric oxide; OM, ovomucoid; OVA, ovalbumin; PBMC, peripheral blood mononuclear cell; PGE2, prostaglandin E2; PI, propidium iodide; PS, phosphatidylserine; S-IgA, secretory-immunoglobulin A; SRBCs, sheep red blood cells; Tc cells, T cytotoxic cells; T cells, T lymphocytes; TGF-β, transforming growth factor beta; Th cells, T helper cells; TNF-α, tumor necrosis factor-alpha; Treg cells, regulatory T cells; WHO, world health organization

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peanut, chickpea, amaranth, corn, and algae. Among food-derived protein hydrolysates or peptides, those with antioxidant, anti-hypertensive, anti-inflammatory, antimicrobial, intestine-modulatory, opioid, hypocholesterolemic, and metal chelating activities have been extensively reviewed in literature (Chalamaiah et al., 2012; Erdmann, Cheung, & Schröder, 2008; Garcia et al., 2013; Stefanucci et al., 2017), while those with immunomodulatory and anticancer activities have not been reviewed to that extend. This encouraged the authors to review recent research on the production and mechanisms of action of immunomodulatory and anticancer protein hydrolysates or peptides derived from various food sources. Additionally, the assays for evaluation of immunomodulatory and anticancer activities of food protein hydrolysates are presented.

2. Immunomodulatory food protein hydrolysates

2.1. Brief overview of the immune system

The immune system is a network of cells, tissues, and organs that acts to eliminate potentially harmful substances such as bacteria, viruses, fungi, protozoans, and prevents the growth of cancer cells. The immune system is classified into two main functional categories namely innate and adaptive immunity. Innate immunity, also called natural immunity or native immunity, is non-specific and provides the first line of defense through anatomic (skin, mucous membranes), physiologic (low pH, temperature and chemical mediators), cellular (macrophages, polymorphonuclear leukocytes, dendritic cells and natural killer (NK) cells), or inflammatory components (cytokines, interferons, complement, defensins, leukotrienes, acute phase proteins and prostaglandins). The macrophages and neutrophils of the innate immune system play an important role in phagocytosis. NK cells play a vital role against tumor cells and virus-infected cells non-specifically.

Adaptive immunity, also called specific immunity or acquired immunity, is highly specific to potentially dangerous foreign antigens. The adaptive immunity is divided into two types, i.e. cell-mediated and antibody-mediated (humoral) immunity. T lymphocytes (T cells) and B lymphocytes (B cells) are the most important cells in adaptive immunity. Humoral immunity involves B lymphocytes that are responsible for the production of antibodies upon interaction with specific antigens. The antibodies can bind to antigens of invading pathogens and label them for destruction by macrophages.

Cell-mediated immunity consists of effector T lymphocytes which secrete immune regulatory factors, such as cytokines, and mediate a cellular immune response upon interaction with antigen presenting cells (APSs). T lymphocytes are divided into three sub-groups of cells namely helper T (Th cells) cells, cytotoxic T (Tc cells) cells and regulatory T (Treg cells) cells. Tc cells express a surface receptor, cluster of differentiation (CD) 8+, and recognize endogenous antigens associated with class I major histocompatibility complex (MHC) and kill cancer cells and cells infected with viruses. Whereas Th cells display a surface marker, CD4+ and recognize exogenous antigens complexed with MHC class II. Th cells secret cytokines such as interferon-γ (IFN-γ), interleukin (IL) 2, IL-4, IL-5, IL-6, IL-10, IL-13, and IL-25 and help activate B cells, T cells and other immune cells (e.g. macrophages) to participate in immune response (Nijkamp & Parnham, 2011). Treg cells are responsible for suppression of the immune responses of other T cells and prevent autoimmune diseases by maintaining self-tolerance.

2.2. Production of immunomodulatory protein hydrolysates or peptides from food proteins

An immunomodulator is any substance that can augment, decrease or modify the immune responses by altering any part of the immune system including both innate and adaptive functional categories of the immune system. The immune system is vital for our survival and provides defense against pathogens but can be influenced by many factors including stress, unhealthy lifestyle practices, pathogens, and antigens (Segerstrom & Miller, 2004). Several drugs such as cyclosporine, tacrolimus, glucocorticoids, phytol, aristolochic acid, plumbagin and levamisole have successfully been applied to modulate the immune response in humans (Gertsch, Viveros-paredes, & Taylor, 2011). However, the toxic side effects and high cost of the allopathic drugs have restricted their usage in patients and most immunomodulatory drugs are not appropriate for chronic or preventive uses (Wang et al., 2010). Modulation of immune function through the dietary components has been reported to be an effective and efficient strategy; meanwhile the discovery of novel immune modulating peptides from food proteins could provide further advantage to the dietary treatment (Chalamaiah et al., 2014).

Immunomodulatory substances in food play significant role as health-benefiting factors that protect the body from invading pathogens. Recently, immunomodulatory protein hydrolysates or peptides have been produced from a wide range of food protein sources. These include milk (Biziulevicius, Kislukhina, Kazlauskaite, & Zukaite, 2006; Kazlauskaite et al., 2005; Masotti, Buckley, Champagne, & Green-Johnson, 2011; Pan, Wu, Liu, Cao, & Zeng, 2013), whey (Javier, Ayoa, Francisco, & Ana, 2014; Ma et al., 2014; Saint-Sauveur, Gauthier, Boutin, & Montoni, 2008; Saint-Sauveur, Gauthier, Boutin, Montoni, & Fliss, 2009), egg (Lee et al., 2009; Lozano-Ojalvo, Molina, & Lopez-Fandino, 2016; Marie et al., 2009; Nelson, Katayama, Mine, Duarte, & Matar, 2007), fish (Karnjanapratum, O'Callaghan, Benjakul, & O'Brien, 2016; Chalamaiah, Hemalatha et al., 2015, 2014; Ahn, Cho, & Je, 2015; Mallet et al., 2014; Hou, Fan, Li, Xue, & Yu, 2012), shellfish (Kim, Kim, Hwang, Kang et al., 2013), sheep (Hua, Yu-hua, Li-zhen, & Bao-hua, 2009), frog (Huang et al., 2014), Oyster (Wang et al., 2010; Cai, Pan, Wu, Wan, & Sun, 2013), clam (He, Cao, Pan, Yang, & Zhang, 2015; Lee et al., 2012), rice (Takahashi, Moriguchi, Yoshikawa, & Sasaki, 1994), wheat (Horiguchi, Horiguchi, & Suzuki, 2005), soybean (Egusa & Otani, 2009; Kong, Guo, Hua, Cao, & Zhang, 2008; Masotti et al., 2011; Vernaza, Dia, Mejia, & Chang, 2012; Yimit, Hoxur, Amat, Uchikawa, & Yamaguchi, 2012), yellow pea (Ndiaye, Vuong, Duarte, Aluko, & Matar, 2012), lupin (Millan-Linares, Bermudez, Yust, Millan, & Pedroche, 2014), flaxseed (Udenigwe, Lu, Han, Hou, & Aluko, 2009), amaranth (Montoya-Rodriguez, Gonzalez de Mejía, Dia, Reyes-Moreno, & Milan-Carrillo, 2014) and microalgae (Vo, Ryu, & Kim, 2013; Senevirathne, Ahn, & Je, 2010; Morris et al., 2007).

The various steps involved in the production of food derived immunomodulatory protein hydrolysates or peptides are shown in Fig. 1. Various recent studies have shown that protein hydrolysates or peptides with immunomodulatory activities can be produced from a variety of food protein sources by using various methods such as *in vitro* enzymatic hydrolysis, autolytic process using endogenous enzymes and microbial fermentation (Chalamaiah, Hemalatha et al., 2015; Duarte, Vinderola, Ritz, Perdigon, & Matar, 2006; Karnjanapratum, O'Callaghan, Benjakul, & O'Brien, 2016; Lozano-Ojalvo et al., 2016; Ma et al., 2014; Masotti et al., 2011; Ndiaye et al., 2012; Nesse, Nagalakshmi, Marimuthu, & Mamta Singh, 2011). Among these methods, *in vitro* hydrolysis of food proteins using commercial proteolytic enzymes is the most commonly employed process, in which peptide bond cleavage allows the release of active peptides capable of exhibiting immunomodulatory activities. Selection of a suitable proteolytic enzyme is a vital factor, due to different cleavage specificity of enzymes, that can affect the release of immunomodulatory peptides from food proteins (Chalamaiah et al., 2014; Chalamaiah, Hemalatha et al., 2015; Lozano-Ojalvo et al., 2016). Several proteolytic enzymes from plant, animal and microbial sources have been successfully used to produce immunomodulatory protein hydrolysates or peptides. These include trypsin, Alcalase, pepsin, papain, pancreatin, chymotrypsin, thermolysin, Flavourzyme, Protamex, and Neutrase (Biziulevicius et al., 2006; Chalamaiah, Hemalatha et al., 2015; Kim, Kim, Hwang, Kang et al., 2013; Kong et al., 2008; Lee et al., 2012; Lozano-Ojalvo et al., 2016; Mallet et al., 2014; Morris et al., 2007; Ndiaye et al., 2012; Saint-