Biologically active peptides derived from egg proteins

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Summary

Bioactive peptides are specific protein fragments that positively impact body's function or condition and ultimately may influence health. These peptides are inactive within the sequence of the parent protein and can be released during proteolysis or fermentation. These peptides may exert a number of different activities *in vivo*, affecting cardiovascular, endocrine, immune and nervous system in addition to nutrient utilization. Eggs have traditionally been recognized as an excellent source of protein, vitamins and minerals. Research in the past decade, however, has produced a substantial amount of evidence indicating that egg peptides may exert several diverse biological effects, above and beyond fulfilling basic nutritional requirements. Several biological activities have now been associated with egg proteins, including novel antimicrobial activities, immunomodulatory, anti-cancer, and anti-hypertensive activities, highlighting the importance of egg proteins in human health, and disease prevention and treatment. Continued research to identify new and existing biological functions of hen egg proteins will help to define new methods to further improve the value of eggs, as a source of numerous biologically active compounds with specific benefits for human and animal health, and secure their role in the therapy and prevention of chronic and infectious disease.

Introduction

Eggs consist of approximately 9.5% eggshell (including shell membrane), 63% albumen, and 27.5% yolk (Cotterill and Geiger, 1977). The main components are water (75%), proteins (12%), lipids (12%), as well as carbohydrates and minerals (Burley and Vadehra, 1989). The proteins are distributed throughout the egg, with the majority found in the egg yolk and egg white, and a small proportion in the eggshell and shell membrane (Sugino *et al.*, 1997). The lipids are found almost exclusively in the egg yolk, mainly in the form of lipoproteins (Burley and Vadehra, 1989). Several minerals have also been found in eggs, most of them in the eggshell. Carbohydrates are a minor egg component, present throughout the egg, both as free and conjugated forms, attached to proteins and lipids (Sugino *et al.*, 1997). Many diverse biological functions of egg proteins/peptides will be discussed here.

Egg white

The egg white, or albumen, makes up about 60% of the total egg weight of which water and protein are the major constituents (Sugino *et al.*, 1997). The egg white proteins include ovalbumin, which is the major protein, followed by ovotransferrin and ovomucoid. Other egg white proteins include ovomucin, which is responsible for the viscosity of the albumen, lysozyme, avidin, cystatin, ovoinhibitor, and ovomacroglobulin (ovostatin) (Sugino *et al.*, 1997).

ANTIMICROBIAL ACTIVITY

Egg white contains a number of proteins with demonstrated antimicrobial activities, which act as part of the natural defence system of the egg. Lysozyme, which exerts bacteriolytic activity by hydrolyzing the β(1-4) linkage between N-acetylmuraminic acid and N-acetylglucosamine of peptidoglycan, the structural component of bacterial cell walls, has been studied at length, and has been applied as a natural food preservative (Losso *et al.*, 2000). It is most effective against Gram positive bacteria such as *Bacillus stearothermophilus*, *Clostridium tyrobutyricum*, and *Clostridium thermosaccharolyticum*, but this spectrum of activity can be broadened to include other spoilage and pathogenic organisms, as well as Gram negative bacteria, when used in conjunction with compounds such as EDTA, organic acids, or nicin (Losso *et al.*, 2000), or by coupling it with a hydrophobic carrier or compounds lethal to

the bacterial membrane (Ibrahim *et al.*, 2001). Studies have suggested that lysozyme may possess further antibacterial activity independent of its calalytic functions (Ibrahim *et al.*, 2001). Enzymatic hydrolysis of lysozyme has been found to enhance its activity, by exposing antibacterial portions of the protein (Pellegrini *et al.*, 1997; Ibrahim *et al.*, 2001), and producing peptides with antibacterial activity. Peptides corresponding to amino acid residues 98-112 (Pellegrini *et al.*, 2000), 98-108, and 15-21 (Mine et al, 2004) possessed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Lysozyme has also demonstrated antiviral activity. Oral and topical applications of lysozyme were found to be effective in preventing and controlling several viral skin infections, including herpes simplex and chicken pox (Sava, 1996), and Lee-Huang *et al.* (1999) found that chicken lysozyme also possessed activity against HIV type 1. Peptides produced by the enzymatic digestion of ovalbumin, one of the major proteins present in egg white, and their synthetic counterparts, were found to be strongly active against *Bacillus subtilus* and to a lesser extent against *Escherichia coli*, *Bordetella bronchiseptica*, *Pseudomonas aeruginosa*, and *Serratia marcescens*, as well as *Candida albicans* (Pellegrini *et al.*, 2004). Ovotransferrin, a member of the transferrin family, a group of iron-binding proteins widely distributed in various biological fluids, has the capacity to reversibly bind iron (Ibrahim, 2000). Its suggested function is as an iron scavenger, to prevent its use by microorganisms, and as an iron delivery agent (Abdallah *et al.*, 1999), and it has demonstrated antibacterial activity against a wide spectrum of bacteria, including *Pseudomonas* spp., *Escherichia coli*, *Streptococcus mutans* (Valenti *et al.*, 1983), *Staphylococcus aureus*, *Bacillus cereus* (Abdallah *et al.*, 1999) and *Salmonella enteritidis* (Baron *et al.*, 2000). Ovotransferrin has also been found to exert antibacterial activity by permeating bacterial outer membranes, reaching the inner membrane and causing the selective permeation of ions and dissipation of electrical potential (Aguilera *et al.*, 2003). A 92-amino acid ovotransferrin peptide, OTAP-92, was found to be capable killing Gram negative bacteria by crossing the bacterial outer membrane by self-promoted uptake, damaging the cytoplasmic membrane (Ibrahim *et al.*, 2000). It has also shown antiviral activity, against Marek's disease virus in chicken embryo fibroblasts (Giansanti *et al.*, 2002). Avidin, which possesses the unique ability to specifically bind the water soluble vitamin biotin, has been found to inhibit the growth of biotin-requiring bacteria and yeasts (Banks *et al.*, 1986). Avidin antimicrobial activity has also been attributed to its ability to bind to various Gram negative and Gram positive bacteria, including *Escherichia coli* K-12, *Klebsiella pneumoniae*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermis* (Korpela *et al.*, 1984). Ovomucin-derived peptides, have demonstrated antiviral activity against Newcastle disease virus, bovine rotavirus, and human influenza virus *in vitro* (Tsuge *et al.*, 1996a,b, 1997a,b; Watanabe *et al.*, 1998a).

ANTI ADHESIVE PROPERTIES

The adhesion of microorganisms to host tissues is the first step in the infection process, in many cases mediated by an interaction between components on the surface of the microorganism and carbohydrates on the mucosal surface of the host (Sharon and Ofek, 2002). It has been suggested, then, that oligosaccharides and glycoconjugates, such as analogues of carbohydrates on the mucosal surface would competitively inhibit microorganism-carbohydrate adhesion on intestinal cells, thereby preventing microbial infection (Kobayashi *et al.*, 2004). Kobayashi *et al.* (2004) found, however, that enzymatic digestion of the egg white protein ovomucin, which is highly glycosylated, produced ovomucin glycopeptides (OGP), which possessed Escherichia coli O157:H7-specific binding sites, consisting of sialic acid. Based on these findings, it was suggested that OGP may be protective against E. coli O157:H7 infection *in vivo*, as well as a potentially novel probe for E. coli O157:H7 detection.

IMMUNOMODULATING ACTIVITY

The immune system responds to antigenic stimulation with a complex array of molecular events, involving antigen-presenting cells, B-cells, T-cells, and phagocytes. Cytokines play a significant role in regulating such immune responses (Wahn, 2003). Concominant is their involvement in the pathology of a wide range of diseases and their potential in replacement and immunomodulatory therapy. Several egg white proteins and peptides have demonstrated immunomodulating activity. Ovalbumin has been found to induce the release of tumour necrosis factor (TNF) alpha in a dose-dependant manner *in vitro*, when modified with dicarbonyl methylglyoxyl (Fan *et al.*, 2003), and immunogenic ovalbumin peptides have been used to enhance immune responses for cancer immunotherapy (Vidovic *et al.*, 2002; Goldberg *et al.*, 2003; He *et al.*, 2003). Tezuka and Yoshikawa (1995) found that the phagocytic activity of macrophages was increased by the addition of ovalbumin peptides, OA 77-

84 and OA 126-134, derived from peptic and chymotryptic digestions of ovalbumin, respectively. Likewise, ovomucin peptides have shown macrophage-stimulating activity *in vitro* (Tanizaki *et al.*, 1997). Synthetic ovomucoid peptides have also demonstrated immunomodulating activity, inducing T-cell secretion of cytokines interleukin- (IL) 4, IL-10, IL-13, interferon- (IFN) gamma, and IL-6 (Holen *et al.*, 2001). Lysozyme has been shown to act as an immune-modulating and immune-stimulating agent. It has been shown, when combined with immunotherapy, to be effective in improving chronic sinusitis (Asakura *et al.*, 1990), and to normalize humoral and cellular responses in patients with chronic bronchitis (Sava, 1996). It was also found to enhance antibody production in hybridomas and lymphocytes, being termed an immunoglobulin production stimulating factor (Sugahara *et al.*, 2000), and to regulate and restore the immune responses in immune-depressed patients undergoing anti-cancer treatments (Sava, 1996). Furthermore, it has been suggested that the antibacterial activity of lysozyme might also occur via stimulation of macrophage phagocytic function, and that the hydrolysis products of peptidoglycan may act as an adjuvant or immunomodulator (Li-Chan and Nakai, 1989). Ovotransferrin is an acute phase protein in chickens, serum levels of which increase in inflammation and infections (Xie *et al.*, 2002). Xie *et al.* (2002) demonstrated that ovotransferrin can act as an immunomodulator, modulating macrophage and heterophil functions *in vitro*. Further immunomodulating effects of ovotransferrin have also been shown, including the inhibition of proliferation of mouse spleen lymphocytes (Otani and Odashima, 1997) and the enhanced phagocytic response of peripheral blood mononuclear cells and polymorphonuclear cells in dogs (Hirota *et al.*, 1995). Finally, research has suggested that cystatins may also be involved in inflammation and immune responses through the cytokine network, through mechanisms unrelated to the known protease inhibitory regions of the molecule (Kato *et al.*, 2000). Verdot *et al.* (1996, 1999) found that cystatin induced the synthesis of TNF- and IL-10, resulting in an upregulation of nitric oxide *in vitro* using mouse peritoneal macrophages. It was also found to upregulate the production of IL-6 by human gingival fibroblast cells and murine splenocytes, and the IL-8 production of gingival fibroblasts (Kato *et al.*, 2000).

ANTI-CANCER ACTIVITY

Lysozyme has been studied extensively as an anticancer agent, and has been shown to inhibit tumour formation and growth, both *in vitro* and *in vivo* when administered orally, in a number of experimental tumours, including lung carcinoma (Sava *et al.*, 1991; Das *et al.*, 1992; Pacor *et al.*, 1999). It was also found to enhance the efficacy of chemotherapy treatments (Sava *et al.*, 1995), and have a preventative effect when administered to normal mice (Das *et al.*, 1992). Evidence suggests that the anticancer effects of orally administered lysozyme may rely heavily upon the host-mediated immune response, including activation of the spleen and macrophages (Sava *et al.*, 1991), however, more recent data indicates that lysozyme may also exert action on the tumour cells themselves (Pacor *et al.*, 1999). Pronase-prepared glycopeptides of ovomucin have also demonstrated anti-tumour effects in a double grafted tumour system in mice, suggested to be related to the anti-angiogenic activity of ovomucin, inhibiting tumour growth (Oguro *et al.*, 2001).

ANTI-HYPERTENSIVE ACTIVITY

A vasorelaxing peptide, ovokinin (OA 358-365), was isolated by the peptic digestion of ovalbumin (Fujita *et al.*, 1995a). Ovokinin (2-7), a peptide produced by chymotrypsin digestion, and corresponding to OA 359-364, was also found to possess vasorelaxing activity (Matoba *et al.*, 1999). Both peptides were found to significantly lower the systolic blood pressure in spontaneously hypertensive rats, in a dose-dependent manner, when administered orally (Fujita *et al.*, 1995b). The replacement of amino acids in the ovokinin (2-7) peptide has resulted in enhanced anti-hypertensive activity, with the most potent derivative resulting in 100-fold more potent anti-hypertensive activity (Matoba *et al.*, 2001; Yamada *et al.*, 2002). Two angiotensin I converting enzyme (ACE)-inhibitory peptides were also identified in ovalbumin by peptic (OA 183-184) and tryptic (OA 200-218) digestions (Yoshikawa and Fujita, 1994). Miguel *et al.* (2004) examined peptides with angiotensin-converting enzyme (ACE)-inhibitory properties produced by enzymatic hydrolysis of crude egg white, which were mainly derived from ovalbumin. Among these peptides, two novel peptides with potent ACE-inhibitory activity were found, with amino acid sequences Arg-Ala-Asp-His-Pro-Phe-Leu and Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu. Likewise, Davalos *et al.* (2004) reported that enzymatic hydrolysis of crude egg white proteins also resulted in the production of peptides with strong antioxidant activities, and suggested that their combined antioxidant and ACE-inhibitory properties would make a useful multifunctional preparation for the control of cardiovascular diseases, in particular, hypertension.

Ovomucin was also found to inhibit cholesterol uptake *in vitro* by Caco-2 cells, and reduce serum cholesterol in rats, displaying hypocholesterolemic action (Nagaoka *et al.*, 2002).

Egg yolk

The major constituents of egg yolk are proteins and lipids, present mainly in the form of lipoproteins, and can be separated into a granule fraction and plasma fraction (Sugino *et al.*, 1997). The granules contain α- and β-lipovitellins (high-density lipoproteins), phosvitin, and low-density lipoproteins (Burley and Cook, 1961). The plasma can be divided into the low-density lipoprotein fraction and the water-soluble fraction, which contains livetins, which are lipid-free globular proteins, among them γ-livetin, also referred to as immunoglobulin Y (Li-Chan *et al.*, 1995). Egg yolk also contains minerals, carbohydrates, most of it which are oligosaccharides bound to protein, as well as pigment (Sugino *et al.*, 1997).

ANTIMICROBIAL ACTIVITY

Although the egg white is the main line of defence against invading microorganisms, a number of egg yolk components have also demonstrated antimicrobial activity. One of the most extensively studied is immunoglobulin (Ig) Y, which has been reviewed in detail elsewhere (Kovacs-Nolan and Mine, 2004a, b). IgY has been produced against a number of bacteria and viruses, and has been shown to bind to and inhibit the infection and disease symptoms, *in vitro* and *in vivo*, of gastrointestinal pathogens (Kovacs-Nolan and Mine, 2004a, b). IgY against *Streptococcus mutans* have been shown to prevent adhesion of the bacteria *in vitro* and *in vivo*, and reduced dental caries development in an animal model (Hatta *et al.*, 1997; Smith *et al.*, 2001; Kruger *et al.*, 2004). In human studies, orally administered anti- *P. aeruginosa* IgY was found to prevent *P. aeruginosa* colonization in the lungs of cystic fibrosis patients, indicating its use as an alternative to antibiotic treatment (Carlander *et al.*, 2000; Kollberg *et al.*, 2003). And the suppression of *H. pylori* infection in humans was achieved/observed following the consumption of drinking yogurt fortified with IgY against *H. pylori* urease enzyme (Horie *et al.*, 2004). Brady *et al.* (2002) found that egg yolk lipoproteins were also important for lipid-mediated antimicrobial activity. The authors found that lipids and lipoproteins, as well as their component fatty acids, oleic and linoleic acid, extracted from egg yolk inhibited the growth of *S. mutans in vitro* (Brady *et al.*, 2003). Finally, egg yolk phosvitin, an iron-binding phosphoprotein, has also demonstrated antibacterial activity against *E. coli* under thermal stress, causing disruption of cells and DNA leakage, suggested to be a result of the synergistic effect of its high metal-chelating ability and high surface activity (Choi *et al.*, 2004).

ANTI ADHESIVE PROPERTIES

Several studies have demonstrated that oligosaccharides in foods such as milk and eggs have an inhibitory effect on the binding of bacteria and viruses to host cells (Sugita-Konishi *et al.*, 2004). The use of natural antimicrobials is a particularly attractive approach, due to the increasing prevalence of microorganisms resistant to, or untreatable by, traditional antibiotic therapy (Kobayashi *et al.*, 2004). Koketsu *et al.* (1995) examined the effect anti-adhesive effect of egg yolk sialyloligosaccharides on viral infection. They found that the oligosaccharide-enriched fraction of egg yolk and sialyloligosaccharide fraction inhibited rotavirus infection *in vitro* in a dose-dependent manner, suggesting that sialic acid plays an important role in binding and inhibition of the virus. They also found that egg yolk sialyloligosaccharides significantly decreased the incidence of rotavirus diarrhoea *in vivo*, in mice (Koketsu *et al.*, 1995) and in rats (Koketsu *et al.*, 1996). More recently, the effects of egg yolk and its components on bacterial adhesion have also been studied. Sugita-Konishi *et al.* (2002) examined the effects of egg yolk sialyloligosaccharides and their derivatives, asialo-yolk-derived sialyloligosaccharides, and sialylglycopeptide, on the binding of *Salmonella enteritidis* and *Escherichia coli*. These compounds inhibited the binding of both organisms to human intestinal cells *in vitro*, and prevented Salmonella infection when orally administered to mice. Non-immunized egg yolk powder was also found to eliminate and prevent the intestinal colonization of *S. enteritidis in vitro*, in laying hens when administered at a concentration of 5% in normal feed (Kassaify and Mine, 2004a), and eliminated or significantly reduced colonization of *S. typhimurium*, *Campylobacter jejuni*, and *E. coli* O157:H7, at concentrations of 7.5 and 10% (Kassaify and Mine, 2004b). Furthermore, these pathogens were absent or significantly reduced in internal organs, indicating that the egg yolk

supplement inhibited colonization and invasion, suggested that the observed effects were due to the presence a yet unidentified/novel anti adhesive factor of egg yolk (Kassaify and Mine, 2004a).

ANTIOXIDANT PROPERTIES

Phosvitin has also been recognized as an egg yolk antioxidant protein, acting as an antioxidant by chelating iron ions (Lu and Baker, 1986). Recently, phosvitin and its enzymatic digests were found to protect against iron-catalyzed hydroxyl radical formation, and protect DNA against oxidative damage induced by Fe(II) and peroxide, suggesting that phosvitiin may be useful for the prevention of iron-mediated oxidative stress related diseases, such as colorectal cancer (Ishikawa *et al.*, 2004).

NUTRIENT BIOAVAILABILITY

In general, phosvitin has been considered to have no real nutritional value, due to low iron bioavailability and resistance to proteolytic enzymes. However, phosvitin phosphopeptides, derived by tryptic hydrolysis following partial alkaline dephosphorylation have been found to enhance calcium-binding capacity and inhibit the formation of insoluble calcium phosphates (Jiang and Mine, 2000, 2001). Furthermore, phosvitin peptides demonstrated better calcium solubilizing activity than commercial casein phosphopeptides (Jiang and Mine, 2000; Choi *et al.*, 2004).

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