

Review

Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—A review

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ABSTRACT Egg white contains many functionally important proteins. Ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), ovomucin (3.5%), and lysozyme (3.5%) are among the major proteins that have high potentials for industrial applications if separated. The separation methods for these proteins from egg white have been developed since early 1900, but preparation methods of these proteins for commercial applications are still under development. Simplicity and scalability of the methods, use of nontoxic chemicals for the separation, and sequential separation for multiple proteins are very important criteria for the commercial production and application of these proteins. The separated proteins can be used in food and phar-

maceutical industry as is or after modifications with enzymes. Ovotransferrin is used as a metal transporter, antimicrobial, or anticancer agent, whereas lysozyme is mainly used as a food preservative. Ovalbumin is widely used as a nutrient supplement and ovomucin as a tumor suppression agent. Ovomucoid is the major egg allergen but can inhibit the growth of tumors, and thus can be used as an anticancer agent. Hydrolyzed peptides from these proteins showed very good angiotensin I converting enzyme inhibitory, anticancer, metal binding, and antioxidant activities. Therefore, separation of egg white proteins and the productions of bioactive peptides from egg white proteins are emerging areas with many new applications.

Key words: egg white protein, separation, industrial use, functional peptide

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INTRODUCTION

Eggs are one of the few foods that are used throughout the world regardless of religion and ethnic group (Stadelman and Cotterill, 2001). The chicken egg is one of the perfectly preserved biological items found in nature and is also considered as the best source of protein, lipids, vitamins, and minerals. However, total egg consumption in the developed countries has been declining over the past few decades because of its high cholesterol and fat content. Medical communities recognized eggs as an unhealthy and cholesterol-loaded food and discouraged people from consuming them, especially the elderly. However, the nutritional benefits of eggs are well recognized. Eggs also have many functional properties such as foaming, emulsifying, and a unique color and flavor, which are important in several food products.

Eggs consist of 3 main components: eggshell (9–12%), egg white (60%), and yolk (30–33%). Whole

egg is composed of water (75%), proteins (12%), lipids (12%), and carbohydrates and minerals (1%; Kovacs-Nolan et al., 2005). Proteins present in egg are distributed among the egg white and yolk, whereas lipids are mainly concentrated in the yolk. Yolk is covered with the vitelline membrane and mainly consists of water (50%), protein (15–17%), lipids (31–35%), and carbohydrates (1%). Protein present in egg yolk consists of lipovitellins (36%), livetins (38%), phosvitin (8%), and low-density lipoproteins (17%). Also, yolk contains 1% carotinoides, which makes it yellow in color (Stadelman and Cotterill, 2001).

CHEMICAL COMPOSITION OF EGG WHITE

Egg white mainly consists of water (88%) and protein (11%), with the remainder consisting of carbohydrates, ash, and trace amounts of lipids (1%). Ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3.5%), and ovomucin (3.5%) are considered as the main proteins and avidin (0.05%), cystatin (0.05%), ovomacroglobulin (0.5%), ovoflavoprotein (0.8%), ovoglycoprotein (1.0%), and ovoidinhibitor (1.5%) are the minor proteins found in egg white (Kovacs-Nolan et al., 2005). These proteins are recognized for their func-

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tional importance. Each protein has many functional properties, and the proteins have been separated from egg white using various approaches. Ovalbumin is the major egg white protein synthesized in the hen's oviduct and accounts for 54% of the total egg white proteins (Stadelman and Cotterill, 2001). The molecular weight of ovalbumin is 45 kDa with 386 amino acids. Ovalbumin does not have a classical N-terminal ladder sequence (Huntington and Stein, 2001), but has 3 sites of postsynthetic modification in addition to the N-terminal acetyl group. The amino acid composition of ovalbumin is unique compared with other proteins (Nisbet et al., 1981); the N-terminal amino acid is acetylated glycine and the C-terminal is proline. It is also known as a glycoprotein and contains a carbohydrate group attached to the N-terminal. Ovalbumin consists of 3 components, A1, A2, and A3, that contain 2, 1, and no phosphate group, respectively. The relative proportion of the subcomponents is 85:12:3 (Stadelman and Cotterill, 2001).

Ovotransferrin is a monomeric glycoprotein consisting of 686 amino acids with a molecular weight of 76 kDa (Stadelman and Cotterill, 2001). It has the same amino acid sequence as the transferrin in human serum and contains 15 disulfide bonds (Oe et al., 1988). Ovotransferrin accounts for 12% of the total egg white protein and was first characterized by Schade and Caroline (1944). It was called conalbumin, but was renamed as ovotransferrin after findings that it can bind iron (Williams, 1968). One molecule of ovotransferrin can bind 2 iron molecules and transports iron in the body. Ovotransferrin is folded into 2 lobes and 4 domains with each lobe composed of 2 distinct α - and β -domains. These 2 domains are linked with antiparallel β strands that open and close by a hinge (Huopalahti et al., 2007). Ovotransferrin is found in 2 main forms: apo- (iron free) and holo- (iron bound) forms. The chemical and physical properties of these 2 forms of ovotransferrin differ significantly (Azari and Baugh, 1967). The apo-form is colorless, whereas the holo-form has a salmon pink color. The holo-form is more resistant to chemical and physical conditions than the apo-form. Iron (Fe^{3+}) can be easily attached to ovotransferrin at $\text{pH} > 7.0$, but is released at $\text{pH} < 4.5$ (Ko and Ahn, 2008). The 2 iron-binding sites are situated within the interdormain cleft of each lobe. These ligands are comprised of tyrosine, aspartic acid, and histidine residues (Baker and Baker, 2004). Ovotransferrin has similar functions to lactoferrin found in milk, and both have iron scavenging and iron delivery functions (Abdallah and Chahine, 1999).

Lysozyme is another important protein found in egg white. Naturally, there are many forms of lysozyme found, but lysozyme found in the egg is the most soluble and stable among them. Lysozyme was first identified by Alderton et al. (1946). It is a ubiquitous enzyme that can hydrolyze the β -linkage between *N*-acetylneuraminic acid and *N*-acetylglucosamine in the bacterial cell wall (Stadelman and Cotterill, 2001; Huopalahti et al., 2007); it is called *N*-acetyl-muramic-hydrolase

and consists of lysine and leucine in the N- and C-terminal, respectively. The molecular weight of lysozyme is 14,400 Da and consists of a single polypeptide chain with 129 amino acids. In nature, this protein is found as a monomer but is occasionally present as a dimer with more thermal stability. It is considered as a strong basic protein present in egg white (Huopalahti et al., 2007). Lysozyme has 4 disulfide bridges leading to high thermal stability, and its isoelectric point is 10.7. It has a tendency of binding to negatively charged proteins such as ovomucin in egg white (Wan et al., 2006).

Ovomucin is another major egg white protein, which accounts for 3.5% of the total egg white protein (Stadelman and Cotterill, 2001). Ovomucin is composed of soluble and insoluble components: the soluble component consists of 8,300 Da and insoluble component ranges from 220 to 270 kDa (Omana et al., 2010). It is one of the large molecular weight proteins with a carbohydrate attached and is responsible for the gel-like structure of egg white (Hiidenhovi et al., 1999). Ovomucin is mainly consisted of 2 types of subunits and they are called α and β ; α -ovomucin is homogeneous, whereas β -ovomucin is heterogeneous. α -Ovomucin has 2 subunits called $\alpha 1$ and $\alpha 2$, which has less carbohydrate group than β -ovomucin, which is rich in carbohydrates. Ovomucin has more coiled regions at its extremities, like the structure of human mucin. Because it has long-coiled regions, a randomly coiled structure is observed. β -Ovomucin is mainly consisted of serine and threonine (Robinson and Monsey, 1975), and α -ovomucin is mainly composed of acidic amino acids such as aspartic acid and glutamic acid, but no difference in ovomucin from thick egg white and thin egg white was found (Omana et al., 2010). Previous studies have shown that at least 3 types of carbohydrate chains are found in ovomucin, which are composed of galactose, galactosamine, sialic acid, and sulfate with a molecular ratio of 1:1:1:1. On average, 33% of ovomucin is carbohydrates (Mine, 2008).

Ovomucoid is one of the most highly glycosylated proteins found in egg white (Kovacs-Nolan et al., 2000). The molecular weight of ovomucoid is 28 kDa, but the band in SDS-PAGE appears at 30 to 40 kDa. It is well known as trypsin inhibitor and is considered as the main food allergen present in egg white. Each ovomucoid molecule binds one molecule of trypsin, and its 3-dimensional structure is secured with the 3 disulfide bonds in it (Oliveira et al., 2009). Kovacs-Nolan et al. (2000) showed that peptides derived from ovomucoid using pepsin showed IgE binding activity and retains its trypsin inhibitory activities. Matsuda et al. (1985) reported that ovomucoid did not lose its immunoreactivity even after hydrolysis using pepsin, but the role of carbohydrates on the immunoreactivity of ovomucoid is not known. Nagata and Yoshida (1984) reported that ovomucoid can be used to control *Streptomyces erythraeus*. Even the protein is considered as a trypsin inhibitor, which is a negative property of the protein; it has the capability of controlling microorganisms.

Therefore, it can be used as an antimicrobial agent for foods.

SEPARATION OF MAJOR EGG WHITE PROTEINS

Ovalbumin is one of the first proteins that was isolated. It was first separated in the 1900s by using ammonium sulfate under acid conditions. A high level of saturated ammonium sulfate along with acetic acid was used to separate ovalbumin from the rest of egg white (Hopkins, 1900; Chick and Martin, 1913). Although it was separated, the purity of the protein was not reported and the amount of salt added was too high. Ammonium sulfate was used again to separate ovalbumin by Warner and Weber (1951) and Warner (1954). They also used high concentrations of ammonium sulfate, but the purity of the separated protein was low. This indicated that ammonium sulfate precipitation of ovalbumin is not a good approach to separate ovalbumin from egg white. Sodium dodecyl sulfate-PAGE and 2-dimensional electrophoresis were also used to separate ovalbumin from egg white (Desert et al., 2001). However, the protein is denatured during the separation process. Foam fractionation was also used to separate ovalbumin from egg white (Ward et al., 2007). It is been reported that air alone at a low flow rate with or without little amount of water can separate ovalbumin from egg white. Rhodes et al. (1960) were the first researchers who used a chromatographic method to separate ovalbumin from egg white. Since then, various chromatographic methods, including anion exchange chromatography, Q-Sepharose FF, carboxy methyl (CM)-Sepharose, CM-Sepharose, and CM-Sephadex, have been tested to separate ovalbumin from egg white (Croguennec et al., 2001; Sakakibara and Yanagisawa, 2007). However, the use of ultrafiltration to separate ovalbumin was difficult in large scale because of the foaming properties of the protein. Recently, Datta et al. (2009) used a low concentration of NaCl followed by ultrafiltration with 30 and 50 kDa of polyethersulfone membranes to separate ovalbumin. However, use of ultrafiltration to separate ovalbumin was difficult in large scale due to foaming properties of the protein. Also, controlling trans-membrane pressure, stirrer speed, solution pH, and feed dilution on the membrane were difficult to maintain. Therefore, a simple and economical way of separating ovalbumin is important to separate the protein in large scale and use it in the food industry.

Ovotransferrin was first separated from egg white using ammonium sulfate at low pH conditions (Fraenkel-Conrat and Feeney, 1950). However, the amount of ammonium sulfate used was very high, and thus scaling up was not easy. Various chromatographic methods including CM cellulose columns with ion exchange resins (Azari and Baugh, 1967), affinity chromatography

(Al-Mashikhi and Nakai, 1987), Cu-Sepharose 6B and DEAR affinity gel (Chung et al., 1991), Q-Sepharose fast-flow chromatography (Awade and Efstahiou, 1999; Vachier et al., 1995; Tankrathok et al., 2009), and electrophoretic method (Desert et al., 2001) were used for laboratory-scale separation of ovotransferrin. Recently, Ko and Ahn (2008) developed a method to separate ovotransferrin using ethanol: they used 43% ethanol to precipitate all the proteins in egg white except for ovotransferrin followed by 59% ethanol to precipitate ovotransferrin from the ovotransferrin-containing supernatant fraction. The yield of ovotransferrin was >95% and the purity was >80%. The method was suitable and effective for scale-up preparation of ovotransferrin from egg white, but the ovotransferrin produced was holo-form and needed to convert to apo-form if it was intended to be used as an antimicrobial agent. Abeyrathne et al. (2013) also developed a simple and scalable method to separate the apo-form of ovotransferrin using a low-level ammonium sulfate and citric acid combination. This method separated the apo-form of ovotransferrin from egg white, and the purity and yield of ovotransferrin was greater than >80 and >90%, respectively.

Lysozyme is among the first egg white proteins that has been isolated and used by industry. Separation of lysozyme was done mainly using ion exchange chromatography, but many different ion exchange resins were used to separate the protein from egg white. The main reason for separating lysozyme using ion exchange chromatography is due to its high isoelectric point value (Price and Nairn, 2009). Strang (1984) used carboxyl methyl cellulose to separate lysozyme from egg white, but scale-up production of lysozyme was not easy because carboxyl methyl cellulose had fine granule sizes and very slow flow rate when used in column chromatography, and was difficult to handle when used in batch systems. Recently, a magnetic cation exchange chromatography with porous glass fiber membranes coated with monophenyl trimethoxysilane was used as the cation exchange resins to separate lysozyme from egg white (Chiu et al., 2007; Safarik et al., 2007). Affinity chromatography (Weaver et al., 1977; Muzzarelli et al., 1978; Yamada et al., 1985) and gel filtration (Islam et al., 2006) were also tested to separate lysozyme. But these chromatographic methods were not suitable for large-scale production due to slow flow rates, high resin costs, or small capacity. Ultrafiltration was used by Wan et al. (2006). They used 2 different membranes (Biomax 30 kDa and Ultracel Amicon 30 kDa) to separate lysozyme, but this was done only in laboratory scale and could not be scaled up due to the limitations in the equipment used. A polysulphone hollow fiber membrane (H1P30-20, MWCO 30 kDa) was used to separate lysozyme (Ghosh et al., 2000), but this method cannot be used in a scaled-up procedure due to its complexity even though it produced 80 to 90% pure lysozyme. Reductants such as β -mercaptoethanol were

used at low concentrations (0.4–1.0%) to separate the protein in a rapid method (Chang et al., 2000). Before separation, eggs were pickled in saturated NaCl solution for 35 d to have the NaCl level in egg white to 5 to 6%. However, the use of mercaptoethanol is prohibited in human foods. Therefore, developing a simple, effective, and scaled-up method for separating lysozyme is important if it is to be used widely in food and drug industry.

Isoelectric precipitation is the most common strategy used for separating ovomucin. Hiidenhovi et al. (2002) used isoelectric precipitation followed by gel filtration combination to separate ovomucin from egg white and observed 3 subunits (β , $\alpha 1$, and $\alpha 2$ subunits. Omana and Wu (2009b) used calcium chloride and potassium chloride in combination with isoelectric point precipitation, and then gel filtration. Use of potassium chloride produced ovomucin with high impurities, whereas CaCl_2 obtained high purity. However, this method cannot be used for scaled-up production of ovomucin because of low sample-handling capacity of gel filtration. Hiidenhovi et al. (1999) used dual-column gel filtration to separate ovomucin subunits, and observed 8 peaks, but the purity of this method was not recorded. Electrophoresis was also used to separate ovomucin from egg white (Desert et al., 2001). The major limitation of this method was scaling up and denaturation of the protein during separation. Rabouille et al. (1990) used a $10\times$ dilution method to separate ovomucin, but a 10-fold increase in volume causes an important practical issue for scaled-up production. High speed of centrifugation was used to separate ovomucin in several occasions. Robinson and Monsey (1975) used isoelectric precipitation of ovomucin in Tris-HCl buffer and a high-speed centrifugation ($35,000 \times g$), Guérin-Dubiard et al. (2005) used alkaline pH condition and centrifugation at $24,000 \times g$ for 30 min at 4°C ; Omana and Wu (2009a) used NaCl and 2 times centrifugation at 10,000 rpm for 10 min; and Omana et al. (2010) soaked egg overnight in 100 mM NaCl solution, and the resulting ovalbumin was centrifuged at $15,300 \times g$ for 10 min at 4°C . However, high-speed centrifugation can be impractical for large commercial-scale preparation of ovomucin. A 2-step separation of ovomucin using pH and NaCl was used to separate ovomucin (Wang and Wu, 2012), but this method produced products with low purity.

Although ovomucin was first prepared in 1898, studies of ovomucin were difficult because of its insolubility and heterogeneity (Sleigh et al., 1973). Ovomucin is insoluble in neutral pH conditions if denaturing agents such as SDS or β -mercaptoethanol are not present (Robinson and Monsey, 1971; Hiidenhovi et al., 1999). Homogenization and sonication improved the solubility of ovomucin (Rabouille et al., 1990) to a certain degree by either cleaving the disulfide bonds or releasing the attached carbohydrate from the main protein chain (Omana et al., 2010). To dissolve the ovomucin, different chemicals were used; urea is one of the chemicals

that was used in the past to dissolve ovomucin (Huopalahti et al., 2007). Sodium dodecyl sulfate is an ionic detergent that has been used to solubilize ovomucin. Sato et al. (1976) dissolved insoluble ovomucin by storing it in alkaline conditions for a long time. Guanidinium chloride (6 M), along with 0.1 M sodium acetate buffer, was also used to dissolve ovomucin (Robinson and Monsey, 1975). The use of a high level of guanidinium chloride is not practical because of high cost, and it is not suitable for use in human foods. Combination of SDS and β -mercaptoethanol dissolved ovomucin well (Hiidenhovi et al., 1999), but β -mercaptoethanol cannot be used in human foods.

Ovomucoid was first separated using trichloroacetic acid and acetone by Lineweaver and Murray (1947) and trichloroacetic acid and ethanol combination by Fredericq and Deutsch (1949). Ovomucoid was also separated using higher level of ethanol (Fredericq and Deutsch, 1949), but the level of purity and yield was not reported. Tanabe et al. (2000) used lower levels of ethanol (25%, final concentration) to separate ovomucoid from egg white, but the purity of the protein was not reported and the recovery of the protein was around 70%. Yousif and Kan (2002) separated ovomucoid from egg white using SDS-PAGE with linear gradient (4–20%), but this method cannot be scaled up and the protein was denatured during separation due to the 2-mercaptoethanol used in the protocol. Davis et al. (1971) separated ovomucoid using 3-step chromatography using CM-cellulose, diethylaminoethyl-cellulose (Huopalahti et al., 2007), but it was not easy to separate them in large quantities. Therefore, developing a simple and effective way of separating the protein in large scale is important.

Most of the methods discussed above were for the separation of single protein from egg white and were in laboratory scale. Separation of more than one protein is done by a few research groups. Vachier et al. (1995) separated lysozyme, ovotransferrin, and ovalbumin in sequence using ion exchange chromatography, but the yield of lysozyme was as low as 60%. Other researchers (Shibusawa et al., 1998, 2001) also separated lysozyme, ovotransferrin, ovalbumin, and ovomucin in sequence from egg white using counter-current chromatography with cross-axis coil centrifuge method, but scale up to produce large amount of those proteins in a single sequence is not easy because of the complexity of the method used in the protocol. Tankrathok et al. (2009) separated ovalbumin, lysozyme, ovotransferrin, and ovomucoid using Q-Sepharose Fast Flow anion exchange chromatography in the first stage and then with CM-Toyopearl 650M cation exchange chromatography in the second step, but the yields were 54, 55, and 21%, respectively. Recently lysozyme, ovotransferrin, ovalbumin, and ovoflavoproteins were separated by using fast-flow anion exchange chromatography (Geng et al., 2012), but the scale-up was not easy due to the cost of the ion exchange resins used. Therefore, a simple proto-

col for separating lysozyme, ovomucin, ovalbumin, and ovotransferrin is important if they are to be used in food and drug industry.

POTENTIAL USE OF SEPARATED PROTEINS FROM EGG WHITE

Use in the Food Processing Industry

Lysozyme is one of the major bacteriolytic proteins found in egg white. Lysozyme has the capability of controlling foodborne pathogens such as *Listeria monocytogens* and *Clostridium botulinum* (Radziejewska et al., 2008), which are considered 2 major pathogens that cause problems in the food industry. Lysozyme effectively controls toxin formation by *Clostridium botulinum* in fish, poultry, and some vegetables. It is reported that modifications of lysozyme with chemical and thermal treatments increased its antimicrobial properties. Lysozyme not only has the capability to inhibit the microbial growth but also has antiviral, antiinflammatory, and therapeutic effects (Kovacs-Nolan et al., 2005). The World Health Organization and many countries allow the use of lysozyme in food as a preservative and it is currently used in kimuchi pickles, sushi, Chinese noodles, cheese, and wine production (Mine et al., 2004).

Ovotransferrin is known to have a strong antimicrobial activity and, thus, can be used to improve the safety of foods. Babini and Livermore (2000) showed that ovotransferrin increased the activity of piperacillin-tazobactam against *E. coli* through its iron-chelating activity. Valenti et al. (1982) reported that ovotransferrin suppressed *Pseudomonas* sp., *Escherichia coli*, and *Streptococcus mutans*. Recently ovotransferrin was used in controlling *E. coli* O157:H7 and *Listeria monocytogens*, which is known to be problematic for foodborne pathogens (Ko et al., 2009). Ibrahim et al. (2000) showed that peptides derived from ovotransferrin (OTAP-92) have a capability of killing bacteria by damaging their cell membrane. Zhang et al. (2011) also reported that peptides derived from ovotransferrin had an ability to control microorganisms. So it is clear that both ovotransferrin and its peptides can be used as antimicrobial agents in foods. Wu and Acero-Lopez (2012) reported that ovotransferrin has an antioxidant effect on poultry meat by establishing the cellular redox environment.

Ovomucin showed good inhibitory activities against *E. coli*, *Bacillus* sp., and *Pseudomonas* sp. It is reported that ovomucin has a strong antimicrobial effect against food poisoning bacteria (Omana et al., 2010). Therefore, ovomucin can be used in food industry as a food preservative. Also, it has a good emulsifying and forming characteristics (Stadelman and Cotterill, 2001). Foaming and emulsifying are essential in the bakery industry. Adding ovomucin can enhance the nutritional level while giving a good texture in the product. Dávalos et al. (2004) reported that hydrolyzed peptides from crude

egg white proteins showed a strong antioxidant activity. Peptides containing Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu showed a strong free radical-scavenging activity (Kovacs-Nolan et al., 2005). Therefore, not only proteins in egg white but also their peptides can be used in the food industry as agents to reduce oxidation of lipids in foods.

Nutritional Values of Egg White Proteins

The egg as a whole is considered as a good source of protein and lipids, but egg white mainly consists of water (88%) and protein (11%) and it is lacking in lipids (Stadelman and Cotterill, 2001). Ovomucin is a highly glycosylated protein and approximately 33% of ovomucin is made up of carbohydrates (Omana et al., 2010). Therefore, ovomucin can be considered a good source of nutrients that can supply 2 vital nutrients, protein and carbohydrates. Ovalbumin is the major egg white protein, has well-balanced amino acid composition, and thus can be used as an excellent protein source for many food items. The rest of the egg white proteins also are considered to be good sources of essential amino acids.

Pharmaceutical Use of Egg White Proteins

Ovotransferrin can bind with iron and easily releases the bound iron at pH <4.5 (Ko and Ahn, 2008). Therefore, it can be an excellent source of iron supplementation for humans (Abdallah and Chahine, 1999). Ibrahim and Kiyono (2009) reported that ovotransferrin underwent thiol-linked auto-cleavage after reduction, and produced partially hydrolyzed products with very strong anticancer effects against colon and breast cancer cells. Lysozyme acts as an immune-modulating and stimulating agent and has a capability of suppressing tumor cells (Kovacs-Nolan et al., 2005). Therefore, lysozyme can be used as an anticancer agent. Ovomucin also is reported to have antitumor activity and antiviral effects (Oguro et al., 2000; Omana et al., 2010). Therefore, not only ovotransferrin but also ovomucin can be used to control tumor growth.

Human blood albumin is reported as an excellent drug carrier, indicating that ovalbumin also has potential to be used as a drug carrier (Kratz, 2008). Ovalbumin was also reported to have tumor necrosis releasing factors, which can apply in tumor suppression (Kovacs-Nolan et al., 2000). Ovomucoid has strong allergenic effects to some human populations. So, ovomucoid was subjected to immunochemical studies, but it is still not clear whether the carbohydrates attached to the protein or the disulfide bonds within the protein causes the immunoreactivity (Matsuda et al., 1985). It was reported that ovomucoid has a biospecific ligand, which can be used as a drug delivery agent (Kovacs-Nolan et al., 2005). Ovomucin is reported to enhance the surface migration of primordial germ cells, which helped the expression of PGCs from E3 to E7 in male embryo cells (Halfter et al., 1996).

Modifications of Egg White Proteins and Their Potential Use

Not only egg white proteins as is, but also their hydrolyzed products, have many functional properties. Egg white proteins can be hydrolyzed using various enzymes under different conditions. Over the past few decades, numerous functional peptides that have beneficial health effects have been developed and marked. These functionally active peptides are produced with enzymes such as pepsin, trypsin, and α -chymotrypsin. Some researchers used egg white itself as a substrate for enzymatic hydrolysis and produced bioactive peptides; Chiang et al. (2006) used thermolysin to hydrolyze egg white and produced bioactive peptides that can inhibit the activity of ACE. Miguel and Aleixandre (2006) also produced peptides by hydrolyzing egg white with pepsin. Among the peptides with amino acid sequences of Tyr-Arg-Glu-Glu-Arg-Tyr-Pro-Ile-Leu, Arg-Ala-Asp-His-Pro-Phe-Leu, and Ile-Val-Phe showed strong ACE inhibitory activities. Feeding these peptides to spontaneously hypertensive rats reduced blood pressure to the rats.

Hydrolyzed ovalbumin showed a strong ACE-inhibitory activity (Miguel et al., 2007). Fujita et al. (2000) hydrolyzed ovalbumin with pepsin, trypsin, and α -chymotrypsin and produced 7 ACE-inhibitory peptides, which includes LKA, LKP, LAP, IKW, FQK-PKR, FKGRYP, and IVGRPRHQG. Some of the peptides produced from ovalbumin not only showed strong ACE-inhibitory effects but also lowered blood lipid content (Manso et al., 2008). Vasodilation effect (Miguel et al., 2007) was observed from the peptides derived from egg white as well as from ovalbumin. Yu et al. (2012) reported that ovokinin and ovokinin 2 to 7 showed the most prominent ACE-inhibitory activities and among the peptides produced.

Not only ovalbumin but also ovotransferrin has been used to produce valuable bioactive peptides. Hydrolysates of ovotransferrin showed a strong antimicrobial activity. Ibrahim et al. (2000) reported the peptides derived from ovotransferrin showed very good antimicrobial activity. However, ovotransferrin lost its ability to bind iron after hydrolysis (Wu and Acero-Lopez, 2012). Peptides derived from ovotransferrin also showed strong antioxidant activity. Ovotransferrin hydrolyzed with enzymes such as protamex, alkase, trypsin, and α -chymotrypsin showed protective effects against oxidative stress including DNA damage in human leukocytes (Moon et al., 2013). Also, the hydrolysates produced from ovotransferrin, which has the peptide sequence of Lys-Val-Arg-Glu-Gly-Thr, had a strong ACE-inhibitory activity as well as a vasodilatory activity (Wu and Acero-Lopez, 2012). Peptides derived from ovomucoid showed an immunomodulating activity against T-cells and those from ovomucin showed macrophage-stimulating activities in vitro (Kovacs-Nolan et al., 2005), indicating that they also can be good candidates for pharmaceutical use in humans.

SUMMARY AND FUTURE RESEARCH ON EGG WHITE PROTEINS

Egg contains many functional proteins, and their functional properties are very well known. However, the practical use of egg proteins by industry is highly limited. Separation of egg white proteins were done for many years but still new, simple, economical, and sequential methods with better yield and purity are emerging. If the separated proteins are mainly targeted for use in food and pharmaceutical industries to increase their values, however, the separation protocol should not use toxic chemicals. Among the egg white proteins, lysozyme is currently used as antimicrobial agents in the food industry, and others proteins such as ovalbumin has a strong potential as a drug carrier, ovotransferrin as an antimicrobial agent or iron carrier, and ovomucin and ovomucoid as antimicrobial and immunomodulating agents. Peptides derived from ovotransferrin, ovalbumin, ovomucoid, and ovomucin showed cytotoxic, anticancer, immunomodulating, ACE-inhibitory, antimicrobial, and antioxidant activities, and have high potentials to be used in the pharmaceutical, nutraceutical, and food industries. The industrial applications of egg white proteins as well as their enzyme hydrolysates are in their infant stage even though some research on the use of egg proteins has been published in recent years. Suggested future research directions on egg white proteins include developing simple, economical, and scalable methods that can separate multiple egg components, production of functional peptides from the separated components, physicochemical characterization of the peptides with specific functions, and testing the functional efficacy of egg white proteins as well as their peptides using animal and food systems. Separation of functional proteins from egg white and production of functional peptides from the separated proteins, and using as antimicrobial, nutraceutical, pharmaceutical, or nutrient-supplementing agents will increase the value and use of egg, which will improve the sustainability of the egg industry.

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