



# Ovotransferrin: Structure, bioactivities, and preparation

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## ABSTRACT

Ovotransferrin, accounting for about 12–13% of total egg white proteins, is synthesized by the avian transferrin gene in the oviduct. It is made of a monomeric glycoprotein consisting of 686 amino acid residues. As a member of transferrin family, ovotransferrin folds into two globular lobes, each containing an iron-binding site located within the interdomain cleft of each lobe. In addition to providing antimicrobial activity, it also functions to transport iron to the developing embryo. The antimicrobial property of ovotransferrin is thought to be due to its ability to sequester the iron necessary for the growth of microorganisms, rendering then iron deprived. Recent evidence further suggests that its role as an essential component of the egg's antimicrobial defense system is very likely iron independent. This antimicrobial property implies its applications as an infant formula ingredient, a food additive, and an antimicrobial agent for improving animal health. A wide range of bioactivities such as antifungal, antiviral, anticancer, antioxidative, antihypertensive, and immunomodulatory activities have been reported recently for ovotransferrin or its derived peptides. In this review, the structure, bioactivity, and preparation of ovotransferrin are presented, and its potential as a nutraceutical and functional food ingredient is described.

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## 1. Introduction

Transferrins, presenting in different biological secretions, are a group of glycoproteins with well-known iron binding properties. The iron binding ability confers antimicrobial activity by rendering iron unavailable for microbial growth (Abdallah & Chahine, 1999; Mason & Macgillivray, 2002). Transferrins can be divided into four types according to their occurrence in nature as shown in Table 1 (Mason & Macgillivray, 2002): (1) serum transferrin (pI 7.4) is found in blood plasma where its main function is iron transfer; (2) lactoferrin (pI 8.8) is found in milk and other mammalian secretions; it binds iron more tightly over a larger pH range than other transferrin proteins and has a diverse range of biological activities including innate defense; (3) melanotransferrins (pI 6.8–7.1) identified from the surface of melanoma cells contain a single iron binding site anchored to the cell membrane surface; (4) ovotransferrin is an acidic glycoprotein (pI 6.0) that constitutes about 12–13% of avian egg white, providing an antimicrobial defense mechanism to the avian egg.

Hen egg white ovotransferrin was first characterized by Schade and Caroline (1944) who called it conalbumin. It was renamed ovotransferrin when it was recognized as an iron-binding protein and a member of the transferrin family (Williams, 1968). It was later confirmed that ovotransferrin has amino acid and gene sequences

identical to those of hen serum transferrin (Jeltsch & Chambon, 1982; Lee, MacKnight, & Palmiter, 1980; Thibodeau, Lee, & Palmiter, 1978; Williams, Elleman, Kingston, Wilkins, & Kuhn, 1982). Ovotransferrin shows 50% homology with mammalian transferrin and lactoferrin but differs from the other transferrin proteins in its isoelectric point and its glycosylation pattern, which consists of a single glycan chain composed of mannose and N-acetylglucosamine residues in the C-terminal domain (Huopalahti, López-Fandiño, Anton, & Schade, 2007). The ovotransferrin molecule comprises two homologous halves, each containing a single iron binding site; it is well known for a high affinity to iron and the associated antibacterial property (Ibrahim, 2000; Williams, Evans, & Moreton, 1978). Ovotransferrin is also responsible for the transfer of ferric ions from the hen oviduct to the developing embryo (Huopalahti et al., 2007). Compared to extensive studies on lactoferrin (see reviews: Actor, Hwang, & Kruzel, 2009; Legrand et al., 2008; Vorland, 1999), research of ovotransferrin is somewhat limited. In this review, we summarize the structure, bioactivity, and preparation of ovotransferrin and highlight its potential as a nutraceutical and functional food ingredient.

## 2. Biosynthesis in eggs

The avian transferrin gene expresses serum transferrin in the liver and ovotransferrin in the oviduct. Serum transferrin and ovotransferrin differ in glycosylation patterns: the glycan of ovotransferrin is composed of four residues of mannose and four residues of N-acetylglucosamine whereas serum transferrin is composed of two residues of mannose, two residues of galactose, three residues of N-acetylglucosamine, and one or two residues of sialic acid at the C-terminal lobe (Ibrahim, 2000;

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**Table 1**  
The transferrin family and its physicochemical properties.

| Properties          | Serum transferrin                                       | Lactoferrin                                                                                                                   | Melanotransferrins                                                        | Ovotransferrin                                                                                          |
|---------------------|---------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
| Source              | Blood serum                                             | Human milk, tears, saliva, seminal fluids                                                                                     | Melanoma cells from human tissues, liver, and intestinal epithelial cells | Albumen                                                                                                 |
| Amino acid residues | 678                                                     | 641                                                                                                                           | 719                                                                       | 686                                                                                                     |
| Molecular weight    | 80 kDa                                                  | 80 kDa                                                                                                                        | 80 kDa                                                                    | 78 kDa                                                                                                  |
| Isoelectric point   | 7.4                                                     | 8.8                                                                                                                           | 6.8–7.1                                                                   | 6.0                                                                                                     |
| Biofunctions        | -Myelination<br><br>-Used to deliver bound DNA to cells | -Antimicrobial activity<br><br>-Immunomodulation<br>-Regulation of cell growth<br>-Antiviral activity<br>-Antifungal activity | -Involved in chondrogenesis and angiogenesis processes                    | -Antimicrobial activity<br><br>-Antifungal activity<br>-Antiviral activity<br>-Immunomodulatory effects |

Mizutani et al., 2001). Expression of the transferrin gene in the oviduct can be regulated by levels of oestrogen and progesterone (McKnight & Palmiter, 1979). Oestrogen and progesterone levels do not affect the expression of the gene in the liver; this expression may be affected by iron levels, as some researchers have observed an increase in mRNA in chickens on iron deficient diets (Cochet et al., 1979; McKnight, Lee, Hemmaphardh, Finch, & Palmiter, 1980). Therefore, ovotransferrin is synthesized in the oviduct under steroid control by stimulation of adenohypophysis. When ovarian follicles are synthesized, the oestrogen released is transported to the oviduct via the blood and passes through the plasmalemma of cells in the endometrial glands. Once inside the cells, oestrogen is bound by a nuclear receptor protein, which binds to chromatin stimulating transcription followed by synthesis of the protein precursor (Palmer & Guillet, 1991). A 19 amino acid signal peptide is cleaved from the precursor, leaving the mature ovotransferrin molecule (Thibodeau et al., 1978). Ovotransferrin has three isoforms carrying 0, 1, or 2 iron atoms per protein molecule with three pI values for aferric, monoferric and diferric transferrin (Desert et al., 2001; Huang & Richards, 1997). Guerin-Dubiard et al. (2006) found five ovotransferrin protein spots using 2-DE but failed to characterize them. Three types of ovotransferrin – ovotransferrin BB (gi: 71274075), ovotransferrin CC (gi: 71274077), and ovotransferrin chain A (gi: 17942831) – were identified for the first time using two types of ligand libraries (D'Ambrosio et al., 2008); however, glycan structures of three types of ovotransferrin are not known.

Ovotransferrin is deposited in the egg albumin where it acts as a bactericidal agent and an iron transporter due to its capacity to bind to the red blood cells of the developing embryo (Ibrahim, 2000). The presence of ovotransferrin in egg shell was also reported; ovotransferrin provides a dual function to the egg shell as it acts alone or in combination with other proteins in the nucleation of outer egg shell membrane mineralization and also functions as a bacteriostatic filter against *Salmonella* and other bacteria (Gautron et al., 2001).

### 3. Structure of ovotransferrin

Ovotransferrin is a single glycopeptide chain containing 686 amino acids, with a molecular weight of 77.90 kDa and an isoelectric point of 6.0. It contains 15 disulfide bonds and no free sulfhydryl groups. This single chain is folded into two globular lobes (representing its N- and C-terminal halves) linked by an alpha helix of nine amino acid residues (residues 333–341) that can be released by tryptic digestion (Charter & Lagarde, 2004; Oe, Doi, & Hirose, 1988). The N and C lobes associate through noncovalent, mostly hydrophobic interactions (Charter & Lagarde, 2004; Oe et al., 1988); it has been demonstrated that isolated N and C lobes can reassociate in solution (Oe et al., 1988). Like other members of the transferrin family (Fig. 1), each lobe is further divided into two similarly sized subdomains (N1, N2 and C1, C2); the subdomains are linked by two antiparallel  $\beta$ -strands that allow them to open and close with a hinge-like mechanism (Kurokawa, Mikami, & Hirose, 1995). The N and C lobes share 37.4%

sequence homology; the main differences between the two lobes are in loop regions (Jeltsch, Hen, Maroteaux, Garnier, & Chambon, 1987; Williams et al., 1982). Although each lobe has the capability to reversibly bind one  $\text{Fe}^{3+}$  ion concomitantly with one bicarbonate anion, they have different iron-binding capacities; the iron-binding constant is  $1.5 \times 10^{18}$  for the C-terminal lobe and  $1.5 \times 10^{14}$  for the N-terminal lobe (Lin, Mason, Woodworth, & Brandts, 1994). The different iron-binding capacities might be explained by differences in the interdomain interactions in the two lobes; for instance, the C-terminal lobe contains an extra interdomain disulfide bond, Cys478–Cys671 (Williams, Moreton, & Goodearl, 1985).

The two iron-binding sites are located within the interdomain cleft of each lobe. Iron is bound by the same ligands in both lobes of ovotransferrin, in lactoferrin, and in serum transferrin (Baker & Baker, 2004). These ligands comprise two tyrosine residues (Tyr 92 and Tyr

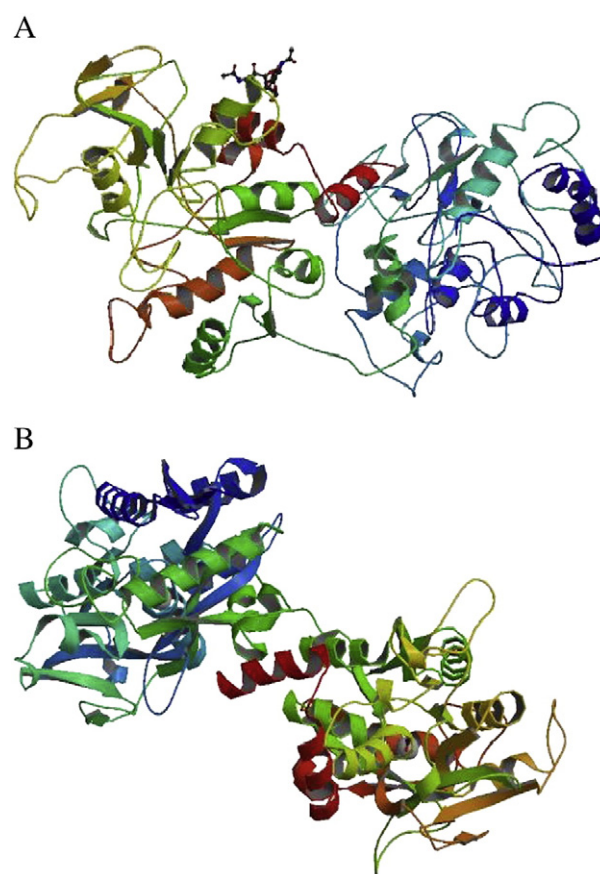


**Fig. 1.** Crystal structure of hen apo-ovotransferrin (A) (Kurokawa et al., 1999; <http://www.rcsb.org/pdb/explore.do?structureid=1AIV>) and diferric or holo-ovotransferrin (B) (Kurokawa et al., 1995; <http://www.rcsb.org/pdb/explore.do?structureid=1OVT>).

192), one aspartic acid (Asp60), and one histidine (His 253), together with two oxygens from the synergistically bound anion as shown in Fig. 2. This set of ligands is chemically and geometrically ideal for high affinity, reversible iron binding (Baker & Sykes, 1994). As shown in Fig. 2, both empty iron binding clefts are in an open conformation; this open structure exposes four iron-coordinating amino acid residues (Asp, Tyr, Tyr, His) to the solvent (Kurokawa, Dewan, Mikami, Sacchettini, & Hirose, 1999). Comparison of apo- and holo-structures demonstrates that iron binding or releasing in ovotransferrin proceeds via a mechanism that involves domain opening or closure. Current evidence suggests that human lactoferrin, transferrin, and ovotransferrin all share the same mechanism of iron binding or releasing (Baker & Baker, 2004). It was suggested by Baker and Baker (2004) that the carbonate ion ( $\text{CO}_3^{2-}$ ) binds first to neutralize the positive charge associated with an arginine residue (Arg121 in the N-lobe), and presents incoming iron with four ligands (Tyr192, Tyr92, and two  $\text{CO}_3^{2-}$  oxygens) clustered together on the inside surface of the N2 domain. The mechanism of iron ion release from such a high affinity protein is not fully understood. However, it was suggested that protonation of the carbonate ion and a subsequent protonation of the His ligand may weaken the iron binding as the ligands dissociate, the domains would then move apart to complete iron release (Baker & Baker, 2004). The rate-limiting step is the conformational change induced by the rapid removal of bound iron. It was recently reported that the protonation of iron-binding residues of ovotransferrin at acidic pH in the absence of anionic chelators is slow (Abdallah & Chahine, 1999). Studies suggested the release of iron ion from ferric transferrin requires the presence of a simple anion such as pyrophosphate, sulfate, or chloride; however, the rate of anion-mediated iron release is much slower for the C-lobe than for the N-lobe (Mizutani, Muralidhara, et al., 2001; Muralidhara & Hirose, 2000), as there are two anion binding sites in the N-lobe but only one anion-binding site in the C-lobe. Anion binding to the lobe is recognized as the key to iron ion release through multiple mechanisms.

Ovotransferrin contains carbohydrate moieties that are different from serum transferrin; galactose and sialic acid are absent in ovotransferrin (Williams, 1968). The absence of galactose and sialic acid was further confirmed by nuclear magnetic resonance spectroscopy (Dorland et al., 1979). Ovotransferrin has only one glycan bond to the Asn473 residue (Williams et al., 1982), containing 2.6% of glycan moiety. Using pronase digestion, Dorland et al. (1979) reported

two glycopeptides (GP-I and GP-II) that differed in arginine content only; both GP-I and GP-II contain the same carbohydrate moiety.

#### 4. Bioactivities of ovotransferrin

Ovotransferrin displays multiple bioactivities. Table 2 summarizes the biological properties of ovotransferrin from laying hen eggs.

##### 4.1. Antimicrobial activity

Antimicrobial activity was recognized when ovotransferrin was first purified (Schade & Caroline, 1944). As ovotransferrin is a member of an iron-binding protein family, this property is thought to be due to its ability to sequester iron, an activity that would deprive the iron necessary for the growth of microorganisms (Bullen, Roger, & Griffiths, 1978; Lock & Board, 1992; Tranter & Board, 1982; Valenti, Antonini, Visca, Orsi, & Antonini, 1983). This bacteriostatic (growth inhibition) property is reported to be effective against a wide spectrum of microorganisms (Abdallah & Chahine, 1999; Baron, Fauvel, & Gautier, 2000; Valenti et al., 1983). However, the sensitivity of bacteria to ovotransferrin antibacterial activity was found to vary widely among bacterial species: the most sensitive species were *Pseudomonas* sp., *E. coli* and *S. mutans*; the most resistant ones were *S. aureus*, *Proteus* sp., and *Klebsiella* (Valenti et al., 1983, 1980; Valenti, Antonini, Fanelli, Orsi, & Antonini, 1982). It was reported that the addition of bicarbonate increased the antibacterial effect of ovotransferrin toward transferrin-sensitive strains such as *S. epidermidis* and *S. saprophyticus* but not the resistant strains such as *S. aureus* (Valenti et al., 1981). Because bicarbonate is required for the initial binding of iron to ovotransferrin and is involved in the formation of the “protein-ferric-bicarbonate” complex, it is thought that some bacteria have difficulty obtaining the chelated iron for their own use. An antagonizing effect was observed when citrate was added to ovotransferrin in the presence of bicarbonate. However, adding EDTA (ethylenediaminetetraacetate) enhanced the antibacterial activity of ovotransferrin against *Escherichia coli* O157:H7 and *Listeria monocytogenes* (Ko, Mendonca, & Ahn, 2008a; Ko, Mendonca, Ismail, & Ahn, 2009).

The bactericidal (bacterial killing) property of ovotransferrin was also recognized in metal ion saturated ovotransferrin, suggesting a mechanism independent of iron deprivation of bacteria (Tranter &

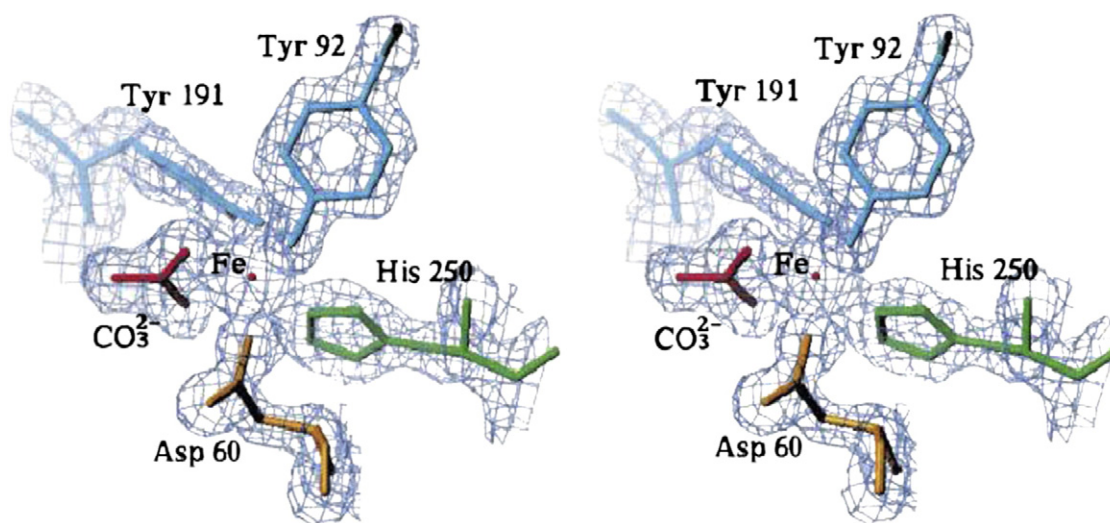


Fig. 2. Ovotransferrin with iron-binding site, calculated with electron density map ( $2\text{Fo} - \text{Fc}$ , contoured at 1 s) of the holo-N-lobe. The final model is superimposed in a stick presentation for the iron-coordinating amino acid residues (Tyr92 and Tyr191 in blue, His250 in green, Asp60 in yellow) and carbonate anion (in red). Iron atom is shown by a small sphere colored in orange (Mizutani, Mikami, & Hirose, 2001).

**Table 2**  
Biological properties of ovotransferrin from hen's egg white.

| Activity                  | Mechanism                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Reference                                                                                                                                                                                                                                   |
|---------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antimicrobial Activity    | Iron deprivation necessary for the growth of microorganisms.<br><br>Bicarbonate addition increases antibacterial activity by forming a "protein-ferric-bicarbonate" complex, making subtraction of chelated iron by bacteria difficult.<br>EDTA enhanced ovotransferrin antibacterial activity.<br>Bactericidal activity in holo- and apo-ovotransferrin against gram <sup>+</sup> and gram <sup>-</sup> bacteria.<br>Zn <sup>2+</sup> -ovotransferrin complex enhances antibacterial activity. | Bullen et al. (1978); Tranter and Board (1982); Valenti et al. (1983); Valenti et al. (1981)<br><br>Ko et al. (2009); Seol et al. (2009)<br>Ibrahim (1997); Beekman et al. (2007); Van Droogenbroeck et al. (2008)<br>Valenti et al. (1987) |
| Antifungal activity       | Against species belonging to genus <i>Candida</i> .<br>Iron saturation of ovotransferrin does not affect its antifungal activity.                                                                                                                                                                                                                                                                                                                                                               | Valenti et al. (1985)<br>Valenti et al. (1986)                                                                                                                                                                                              |
| Antiviral activity        | Antiviral activity against Marek's disease virus (MDV).                                                                                                                                                                                                                                                                                                                                                                                                                                         | Giansanti et al. (2005)                                                                                                                                                                                                                     |
| Immunomodulatory activity | Acute phase protein in chickens.<br><br>Immunomodulatory influence on macrophages and heterophils.<br>Biomarker of inflammatory diseases in chickens.                                                                                                                                                                                                                                                                                                                                           | Xie, Huff, Huff, Balog, Holt, et al. (2002); Xie, Newberry, Clark, Huff, Huff, et al. (2002)<br>Xie, Huff, Huff, Balog, and Rath (2002)<br>Rath et al. (2009)                                                                               |
| Anticancer activity       | Ovotransferrin can undergo thiol-linked autocleavage after reduction, which inhibits the proliferation of cancer cell lines.                                                                                                                                                                                                                                                                                                                                                                    | Ibrahim et al. (2006); Ibrahim and Kiyono (2009)                                                                                                                                                                                            |
| Antioxidative activity    | Ovotransferrin shows SOD-like activity.                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Ibrahim et al. (2007)                                                                                                                                                                                                                       |

Board, 1982; Valenti et al., 1983). Furthermore, ovotransferrin in egg white is largely free of iron and therefore its role in the egg's antimicrobial defense system is very likely iron independent. Many microorganisms are able to acquire iron bound to transferrins, including ovotransferrin (Law, Wilkie, Freeman, & Gould, 1992). Bactericidal activities against Gram-positive *Staphylococcus aureus* and *Bacillus cereus* and the Gram-negative *E. coli* were reported in both holo- and apo-ovotransferrin (Ibrahim, 1997). It was reported that Zn<sup>2+</sup>-saturated ovotransferrin was more active against bacteria than apo-ovotransferrin and other metal-ovotransferrin complexes; this effect was specific for the Zn-ovotransferrin complex, not for the Zn<sup>2+</sup> ions or the iron deprived ovotransferrin. Furthermore, direct contact between the Zn-ovotransferrin complex and the bacterial surface is required to achieve the bactericidal effect (Valenti, Visca, Antonini, Orsi, & Antonini, 1987). Ko, Mendonca, and Ahn (2008b) observed the same effect in combinations of ovotransferrin with sodium bicarbonate and zinc against *E. coli* and *L. monocytogenes*.

Because of its antimicrobial activity, ovotransferrin has been suggested as an infant formula ingredient for the treatment of acute diarrheas (Del Giacco, Leone, & Ferlazzo, 1985). *Chlamydophila psittaci* is an obligate intracellular Gram-negative bacterium causing respiratory disease in poultry, pet birds, and humans. Ovotransferrin was found to inactivate extracellular *C. psittaci* and significantly inhibit chlamydial entry in HD11 chicken macrophage by blocking actin polymerization (Beekman, VanDroogenbroeck, & DeCock, 2007). An antichlamydial effect of ovotransferrin was also observed recently in turkeys, suggesting the potential for the prevention of *C. psittaci* infection in turkey farms (Van Droogenbroeck, Beeckman, Harkinez-had, Cox, & Vanrompay, 2008). Ovotransferrin was recently suggested as a packaging ingredient to improve the shelf life of food products. Adding ovotransferrin in combination with EDTA into  $\kappa$ -carrageenan films showed improved inhibitory activity against *E. Coli* and less active against *Staphylococcus aureus* and *Salmonella typhimurium* in chicken breast (Seol, Lim, Jang, Jo, & Lee, 2009).

#### 4.2. Antifungal activity

Ovotransferrin has been found to possess antifungal activity against species belonging to the genus *Candida*; of over 100 strains tested, only *C. krusei* showed resistance to ovotransferrin (Valenti, Visca, Antonini, & Orsi, 1985). Although bicarbonate increased the antibacterial effect of ovotransferrin, addition of bicarbonate did not increase the antifungal activity of ovotransferrin against *Candida*. In

the presence of citrate, the results obtained with *Candida* were similar to those observed in bacteria that do not possess an iron transport system mediated by citrate. Lactoferrin and ovotransferrin showed similar effects, i.e., adsorption of transferrin to *C. albicans* and inhibition of *C. albicans* growth, but not to *C. krusei*. The group also reported that iron saturation of ovotransferrin did not abolish its antifungal activity. The difference in antifungal activity toward the two strains indicated that the antifungal activity is not due to iron sequestering (Valenti, Visca, Antonini, & Orsi, 1986). The authors proposed that the adsorption of ovotransferrin to the *C. albicans*' surface involves specific receptors, or is due to nonspecific interactions with the bacterial surface.

#### 4.3. Antiviral activity

Lactoferrin has antiviral effects against a wide range of viruses through the interaction of lactoferrin with cell surfaces and/or viral particles. The antiviral activity of ovotransferrin was confirmed in a homologous cell system using cultures of chicken embryo fibroblast infected with Marek's disease virus (MDV) (Giansanti et al., 2005). The antiviral activity against MDV that affects domestic chicken, suggests a protective role in offspring defense (Giansanti et al., 2005).

#### 4.4. Immunomodulatory activity

Like other members of the transferrin family, ovotransferrin is a component of innate immunity (Giansanti et al., 2002; Giansanti, Giardi, Massucci, Botti, & Antonini, 2007). Ovotransferrin was characterized as an acute phase protein (APP) in chickens (Xie, Huff, Huff, Balog, Holt, et al., 2002), and its level was elevated during inflammation and disease (Xie, Newberry, Clark, Huff, Huff, et al., 2002). Ovotransferrin was reported to exert immunomodulatory influence on macrophages and heterophils Xie, Huff, Huff, Balog, and Rath (2002)). Ovotransferrin showed cytotoxicity for mouse spleen lymphocytes (Otani & Odashima, 1997); although the inhibitory mechanism is not clear, ovotransferrin inhibition of lipopolysaccharide (LPS)- and phytohemagglutinin (PHA)-induced proliferation of mouse spleen lymphocytes was not due to inactivation of mitogens or to blockage of mitogen receptors on lymphocyte surfaces. The possibility of using ovotransferrin as a biomarker of inflammatory diseases in chickens was studied by Rath et al. (2009), who suggested that the fluctuation level of ovotransferrin in chicken serum was related to inflammatory and microbial stress. Ovotransferrin also

inhibited proteoglycan synthesis by the sclera which reduces the rate of vitreous chamber elongation, facilitating the recovery of chick eyes from induced myopia (Rada, Huang, & Rada, 2001).

#### 4.5. Anticancer activity

Ibrahim, Haraguchi, and Aoki (2006) found that ovotransferrin can undergo thiol-linked autocleavage after reduction. This reduced, autocleaved ovotransferrin displays anticancer activity as it inhibits the proliferation of cancer cell lines by a cytotoxic mechanism that induces apoptosis (Ibrahim & Kiyono, 2009).

#### 4.6. Antioxidative activity

A recent study showed that ovotransferrin shows superoxide dismutase (SOD)-like activity promoted by metal binding; the N-terminal lobe of the ovotransferrin molecule was the most efficient scavenging domain (Ibrahim, Hoq, & Aoki, 2007). This activity implies that ovotransferrin may be involved in establishment of the cellular redox environment and thereby the biological status of the chicken egg during embryogenesis.

### 5. Ovotransferrin derived bioactive peptides

Bioactive peptides released during gastrointestinal digestion or food processing may have disease prevention and health promotion potential. Health effects of peptides derived from plant or animal proteins are receiving increased attention (Erdmann, Cheung, & Schröder, 2008). Several bioactive peptides from ovotransferrin were recently characterized (Table 3).

#### 5.1. Antimicrobial peptides

Peptides derived from lactoferrin, such as lactoferricin from the N-terminal region of lactoferrin, show more potent antimicrobial activity than the parent protein (Tomita, Takase, Bellamy, & Shimura, 1994). In a similar study, ovotransferrin did not release peptides with improved activity. The presence of antimicrobial domains is a unique feature of the transferrin family. Ibrahim, Iwamori, Sugimoto, and Aoki (1998) reported that almost all the bactericidal activity of ovotransferrin could be attributed to the N-lobe. An antimicrobial peptide (OTAP-92) – a 92-residue peptide (Leu109-Asp200) containing six cysteines engaged in the formation of three intrachain disulfide bonds – was produced using the partial-acid proteolysis method; maintaining the tertiary structure of OTAP-92 by the disulfide bonds is essential to the antimicrobial activity that is rendered through a membrane damage mechanism (Ibrahim, 2000). However, OTAP-92 does not bind iron (Ibrahim et al., 1998).

#### 5.2. ACE inhibitory peptides

Antihypertensive peptides have been widely studied for their potential to prevent high blood pressure (Hartmann & Meisel, 2007). The mechanism of action is due mainly to inhibition of the angiotensin converting enzyme (ACE), the key enzyme responsible for conversion

of angiotensin I to angiotensin II, a vasoconstrictor, and also inactivation of bradykinin, a vasodilator (Murray & FitzGerald, 2007). The peptide Lys-Val-Arg-Glu-Gly-Thr-Thr-Tyr was characterized from ovotransferrin as a pro-drug type ACE inhibitory peptide, as its hydrolyzed product by ACE, Lys-Val-Arg-Glu-Gly-Thr, has 11-fold more potent activity than the parent peptide (Lee, Cheng, Enomoto, & Nakano, 2006). In a systematic study of egg proteins as a source of ACE inhibitory peptides, we recently characterized three potent peptides – Ile-Arg-Trp, Leu-Lys-Pro, and Ile-Gln-Trp – from ovotransferrin, aided by an integrated bioinformatics and QSAR (quantitative structure and activity relationship) approach (Majumder & Wu, 2010). These peptides also showed anti-inflammatory activity in endothelial cells (Huang et al., 2010). Since inflammation is recognized as an important contributor to cardiovascular disease, this newly found peptide activity might provide new insight into *in vivo* mechanisms of ACE inhibitory peptides. Interestingly, Leu-Lys-Pro was previously reported from fish present in the prodrug type ACE inhibitory peptide Leu-Lys-Pro-Asn-Met; both peptides showed *in vivo* antihypertensive activity (Fujita & Yoshikawa, 1999).

#### 5.3. Antioxidative peptides

Ovotransferrin was reported to retard oxidation in turkey meat (Frong, Ragan, & Niemann, 1986). A recent study also showed that ovotransferrin is an SOD mimic protein with potent superoxide anion scavenging activity under the natural xanthine/xanthine oxidase (X/XOD) coupling system (Ibrahim et al., 2007). The overall antioxidant activity of ovotransferrin was enhanced by disruption of its structure to increase the proximity of amino acid residues that scavenge free radicals and chelating pro-oxidative metals (Elias, Kellerby, & Decker, 2008). Our study showed that hydrolysis of ovotransferrin increased its antioxidant activity; 14 peptides were characterized from ovotransferrin (Shen, Chahal, Majumder, You, & Wu, 2010). Two tetrapeptides (Trp-Asn-Ile-Pro and Gly-Trp-Asn-Ile) showed the highest antioxidant activity, indicating that (Trp-Asn-Ile) might be the core motif responsible for their high antioxidant activity.

### 6. Ovotransferrin preparation: separation and purification

Ovotransferrin was first purified from egg white by Fraenkel-Conrat and Feeney in 1950 by precipitation of the protein at pH 3 in the presence of ammonium sulphate; the product had low purity and its electrophoretic behavior differed from that of the native protein (Fraenkel-Conrat & Feeney, 1950). Fractionation of ovotransferrin from egg white was approached by Warner and Weber (1951) using 50% ethanol at pH ranging from 6 to 9 but the product's purity was not improved. Chromatographic methods have been explored to improve the purity of ovotransferrin separated from egg white and to minimize its structural alteration during purification. Ion exchange chromatography is the most widely used chromatographic method since it is easy to use and practical to scale up (Azari & Baugh, 1967; Guerin & Brule, 1992; Rhodes, Azari, & Feeney, 1958). Rhodes et al. (1958) fractionated ovotransferrin on a CM cellulose column; however, the resultant ovotransferrin was contaminated by globulins. Later, Azari and Baugh (1967) improved the method by precipitating and

**Table 3**  
Ovotransferrin derived bioactive peptides and their activities.

| Activity               | Sequences                                                                  | Location         | References                                |
|------------------------|----------------------------------------------------------------------------|------------------|-------------------------------------------|
| Antimicrobial peptide  | Leu109-Asp200 (92 residues)                                                | N-Lobe           | Ibrahim (2000); Ibrahim et al. (1998)     |
| ACE inhibitory peptide | Lys-Val-Arg-Glu-Gly-Thr-Thr-Tyr<br>Ile-Arg-Trp; Leu-Lys-Pro<br>Ile-Gln-Trp | N-lobe<br>C-lobe | Lee et al. (2006); Majumder and Wu (2010) |
| Antioxidative peptides | Trp-Asn-Ile-Pro<br>Gly-Trp-Asn-Ile                                         | N-Lobe<br>C-lobe | Shen et al. (2010)                        |

**Table 4**  
Methods of hen egg white ovotransferrin purification.

| Purification method                                                                                                                   | Purity          | Solvent used                                                            | Reference                                        |
|---------------------------------------------------------------------------------------------------------------------------------------|-----------------|-------------------------------------------------------------------------|--------------------------------------------------|
| Protein precipitation at pH 3                                                                                                         | N/A             | Ammonium sulphate                                                       | Fraenkel-Conrat and Feeney (1950)                |
| Fractionation at pH 6–9; ovotransferrin in crystalline form                                                                           | N/A             | 50% ethanol in 0.02 M sodium chloride                                   | Warner and Weber (1951)                          |
| Chromatography CM cellulose column                                                                                                    | N/A             | 0.1 M ammonium acetate                                                  | Rhodes et al. (1958)                             |
| Chromatography CM cellulose column, followed by precipitation and crystallization of the elution                                      | N/A             | 0.3 M ammonium sulphate, pH 8.5                                         | Azari and Baugh (1967)                           |
| Immobilized-metal affinity chromatography (IMAC) Cu-Sepharose 6B column                                                               | 94–98%          | 0.05 M Tris-acetate/0.5 M NaCl, pH 8.2                                  | Al-Mashikhi and Nakai (1987)                     |
| DEAE affi-gel blue chromatography                                                                                                     | N/A             | 0.02 M potassium phosphate buffer, pH 7.5                               | Chung et al. (1991)                              |
| Duolite C-476                                                                                                                         | N/A             |                                                                         | Guerin and Brule (1992)                          |
| Anion exchange chromatography (Q-Sepharose Fast Flow)                                                                                 | 80%             | 0.05 M Tris-HCl with 0.02 M NaCl, pH 9                                  | Awadé et al. (1994)                              |
| Anion exchange chromatography (Q-Sepharose)                                                                                           | 75%             | 0.05 M Tris-HCl, pH 9                                                   | Vachier et al. (1995)                            |
| Anion exchange Chromatography (Q-Sepharose Fast Flow)                                                                                 | 97.5%           | Acetonitrile with 0.025% trifluoroacetic acid<br>50 mM Tris-HCl, pH 8.5 | Croguennec et al. (2001)                         |
| S Ceramin Hyper DF cation exchanger chromatography                                                                                    | 89%             | 0.5 M NaCl<br>10 mM citrate buffer, pH 5.2                              | Guérin-Dubiard et al. (2005)                     |
| Anion exchange chromatography (Q-Sepharose Fast Flow) followed by a second step of CM-Toyopearl 650 M cation exchange chromatography) | 80% (yield-21%) | 0.05 M Tris-HCl, pH 9                                                   | Tankrathok et al. (2009)                         |
| Anion-exchange chromatography (Q-Sepharose FF) followed by cation-exchange chromatography (SP-Sepharose FF)                           | 80%             | 0.14 M–0.5 M NaCl                                                       | Omana et al. (2010)                              |
| Counter-current chromatography                                                                                                        | N/A             | Potassium phosphate buffer, pH 6.8, 8.0, and 9.2                        | Shibusawa et al. (2001), Shibusawa et al. (1998) |
| Precipitation with ethanol of holo-ovotransferrin                                                                                     | 80%             | Ethanol 43%, pH 9.0                                                     | Ko and Ahn (2008)                                |

crystallizing ovotransferrin after elution, leading to a purer protein that had a different amino acid composition. Immobilized-metal affinity chromatography (IMAC), such as a Cu-Sepharose 6B column (Al-Mashikhi & Nakai, 1987) and DEAE affinity-gel blue chromatography (Chung, Chan, & Shimizu, 1991), significantly improved the purity of ovotransferrin (94–98%) as indicated by SDS-polyacrylamide gel electrophoresis (PAGE) and immunoelectrophoresis but is not practical for large scale preparation. Guerin and Brule (1992) developed a pilot scale method to purify ovotransferrin using lysozyme devoided egg white on a Duolite C-476 resin, but the process was time consuming. Awadé, Moreau, Mollé, Brulé, and Maubois (1994) developed a two-step method of purification: ovomucin and lysozyme were first purified from egg white by gel permeation chromatography, and in the second step, ovotransferrin was isolated by anion exchange chromatography. The purity of ovotransferrin was reported to be 80% determined by gel permeation HPLC. In a further study, the same group investigated the efficiency of a Q sepharose column to separate egg white proteins without prior treatment; a purity of 75% was obtained (Vachier, Piot, & Awadé, 1995). A purity of 97.5% was obtained after a second chromatography using the same Q Sepharose Fast Flow column (Croguennec, Nau, Pezennec, Piot, & Brulé, 2001).

Ovotransferrin can also be purified using cation exchange chromatography. A purity of 89% determined by RP-HPLC and SDS-PAGE was reported using a S Ceramin Hyper DF cation exchanger chromatography of mucin-free egg white (Guérin-Dubiard et al., 2005); it was found that this fraction contained the ovalbumin gene X, which had not been detected previously. A purity of 80% determined by HPLC was obtained using anion exchange chromatography on a Q Sepharose Fast Flow column followed by a second step of CM-Toyopearl 650 M cation exchange chromatography (Tankrathok, Daduang, Patramanon, Araki, & Thammasirirak, 2009); however, recovery of ovotransferrin by this method was low (21%). In a recent study, an 80% pure ovotransferrin was isolated from ovomucin-free egg white prepared by 100 mM NaCl; the ovomucin-free sample was passed through an anion-exchange column followed by cation-exchange chromatography (Omana, Wang, & Wu, 2010).

Using counter-current chromatography, ovotransferrin, ovalbumin, ovomucin, and lysozyme were isolated from egg white solution in a polymer phase system at pH 8.0 (Shibusawa, Iino, Shindo, & Ito, 2001; Shibusawa, Kihira, & Ito, 1998). Ko and Ahn (2008a) proposed a

purification method in which egg white was converted to its holo-form by addition of iron chloride in order to prevent denaturation, then holo-ovotransferrin was separated from other proteins by precipitation with ethanol; the holo-ovotransferrin was then converted into apo-ovotransferrin using an ion exchange resin; the resulting apo-ovotransferrin was 80% pure as determined by SDS-PAGE. A summary of various methods of ovotransferrin purification is listed in Table 4.

## 7. Conclusion

Ovotransferrin has multifunctional properties, but its iron binding capacity and the associated antimicrobial activity are of particular interest. This review summarized the structure, activities, and preparation of ovotransferrin and highlighted its antifungal, antiviral, anticancer, antioxidative, antihypertensive, and immunomodulatory activities. Further animal and clinical studies are needed to establish a role for ovotransferrin or its derived peptides as a functional food and nutraceutical ingredient. New properties and effects of this protein will be discovered as research progresses, opening up new application opportunities for ovotransferrin.

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