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Ovotransferrin: Structure, bioactivities, and preparation

Jianping Wu *, Alexandra Acero-Lopez

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

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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.

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 transferring (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;

* Corresponding author at: Department of Agricultural, Food and Nutritional Science, 4-10 Ag/For Building, University of Alberta, Edmonton, Alberta, Canada T6G 2P5. Tel.: +1 780 492 6885; fax: +1 780 492 4265.
E-mail address: jwu3@ualberta.ca (J. Wu).

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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	-Antimicrobial activity	-Involved in chondrogenesis and angiogenesis processes	-Antimicrobial activity
	-Used to deliver bound DNA to cells	-Immunomodulation -Regulation of cell growth -Antiviral activity -Antifungal activity		-Antifungal activity -Antiviral activity -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, Hemmaplardh, Finch, & Palmiter, 1980). Therefore, ovotransferrin is synthesized in the oviduct under steroid control by stimulation of adenohypophysis. When ovarian follicules 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 & Guillette, 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 *p*I values for aferric, monoferric and diferric transferring (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 ß-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 Fe^{3+} ion concomitantly with one bicarbonate anion, they have different iron-binding capacities; the iron-binding constant is 1.5×10^{18} for the C-terminal lobe and 1.5×10^{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

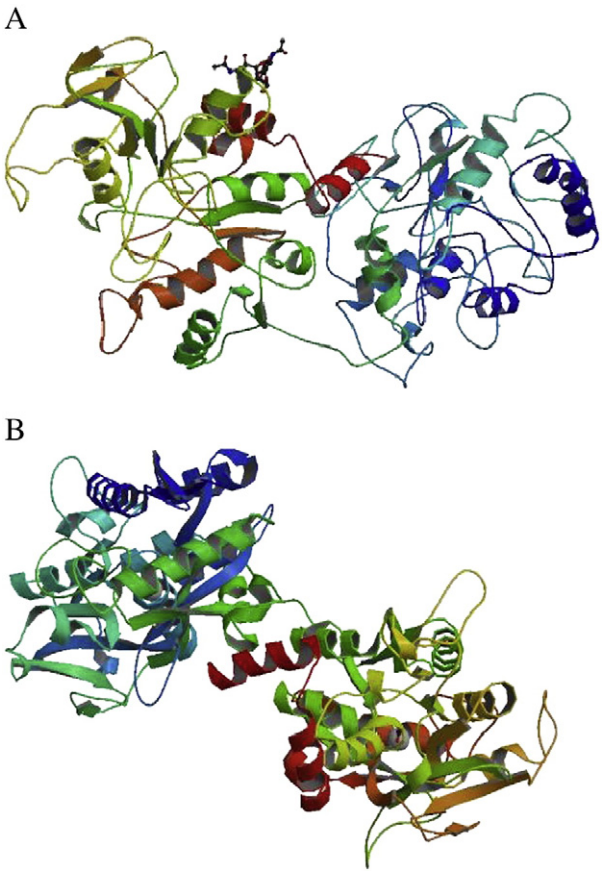

Fig. 1. Crystal structure of hen apo-ovotransferrin (A) (Kurokawa et al., 1999; http://www.rcsb.org/pdb/explore.do?structureId=1AIV) and diferric or holo-ovotransferrin (B) (Kurokawa et al., 1995; http://www.rcsb.org/pdb/explore.do?structureId=1OVT).