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Review

Physiological roles of ovotransferrin ☆

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ABSTRACT

Background: Ovotransferrin is an iron-binding glycoprotein, found in avian egg white and in avian serum, belonging to the family of transferrin iron-binding glycoproteins. All transferrins show high sequence homology. In mammals are presents two different soluble glycoproteins with different functions: i) serum transferrin that is present in plasma and committed to iron transport and iron delivery to cells and ii) lactoferrin that is present in extracellular fluids and in specific granules of polymorphonuclear lymphocytes and committed to the so-called natural immunity. To the contrary, in birds, ovotransferrin remained the only soluble glycoprotein of the transferrin family present both in plasma and egg white.
Scope of review: Substantial experimental evidences are summarized, illustrating the multiple physiological roles of ovotransferrin in an attempt to overcome the common belief that ovotransferrin is a protein dedicated only to iron transport and to iron withholding antibacterial activity.
Major conclusions: Similarly to the better known family member protein lactoferrin, ovotransferrin appears to be a multi-functional protein with a major role in avian natural immunity.
General significance: Biotechnological applications of ovotransferrin and ovotransferrin-related peptides could be considered in the near future, stimulating further research on this remarkable protein. This article is part of a Special Issue entitled Transferrins: Molecular mechanisms of iron transport and disorders.

1. Ovotransferrin at a glance

1.1. OTrf structure

The transferrin (Tf) family of proteins is divided into two branches: soluble glycoproteins and membrane melanotransferrins. Ovotransferrin, like the other 80-kDa Tfs glycoprotein family consists of two similarly sized and homologous N- and C-lobes, which are further divided into two similarly sized sub-domains (N1 and N2 in the N-lobe; C1 and C2 in the C-lobe), probably arising through ancient gene duplication/quadruplication events [1]. From multiple-sequence alignments and neighbor-joining trees using 71 transferrin family sequences from 51 different species, including several novel sequences found in the Takifugu and Ciona [2] genome databases, Lambert and coworkers have concluded that after the initial lobe duplication, a subsequent event occurred prior to the protosome/deuterostome split, at least 670 Mya [2]. Molecular analyses suggest that the ovotransferrin gene seems to have evolved around 310 Mya, following the mammalian-birds split [3]. Nevertheless ovotransferrin sequence continued to share high sequence homology with both human serum transferrin and lactoferrin [4,5].

Crystal structures of the diferric forms of ovotransferrin have been determined at 2.35 Å for duck [6] and at 2.4 Å for hen [7]. Crystal structures of the apo-ovotransferrin have been solved at 3.0 Å for hen [8] and at 4.0 Å for duck [9]. High resolution crystal structures are available for N-lobe of hen apo-ovotransferrin at 2.1 [10] and 1.9 Å [11], respectively, and for the N2 domain of iron-bound duck ovotransferrin at 2.3 Å [12,13]. Hen's OTrf (from *Gallus domesticus*) is a 77.7 kDa glycosilated protein of 686 amino acids [14]. The overall organization of the OTrf molecule is shown as a ribbon model in Fig. 1 (panel A). The polypeptide chain is folded into two lobes, each containing a single iron-binding site. The two lobes have very similar structures, as expected from their sequence identity of 37.4% [1,15]. The polypeptide chain includes amino acids 1–329 for the N lobe and 330–686 for the C lobe [14]. Interestingly, the two half molecules of ovotransferrin corresponding to the N-terminal and C-terminal lobes, obtained by a limited proteolysis procedure, have the ability to re-associate non-covalently in solution [16]. Most of the secondary structural elements are comparable between the two lobes. The main differences between the two lobes are in the loop regions, as expected by sequence insertions and deletions in the primary structure. Each lobe is comprised of two distinct, similar-sized α/β sub-domains (N-terminal lobe with N1 and N2 subdomains; C-terminal lobe with C1 and C2 subdomains). The two sub-domains are linked by two antiparallel β-strands that allow them to adopt either open or closed conformations. The iron-binding sites are located in the

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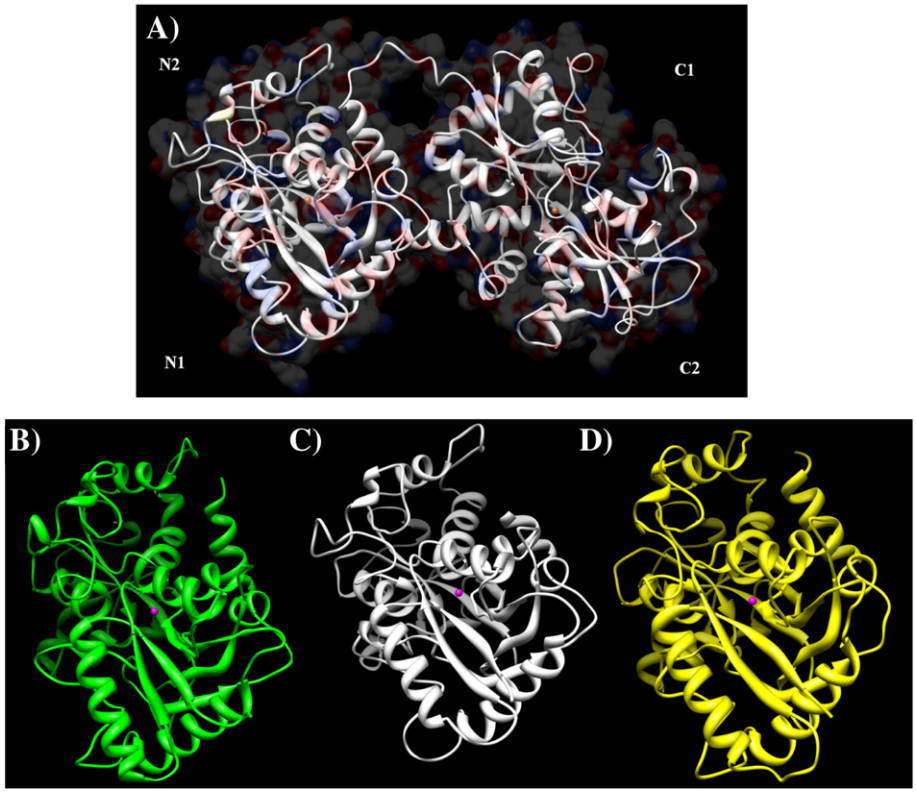

Fig. 1. Panel A: Ribbon structure of OTrf complete of trasparence surface. N1, N2 and C1, C2 indicates a subdomains of each respective lobe. Panel B: N-Lobe of the hLf (Green). Panel C: N-Lobe of the OTrf (White). Panel D: N-Lobe of the hsTf (Yellow). In each panel the iron in its binding site is indicated as a red dot. Molecular graphics images were produced using the UCSF chimera package [134]. Hen's ovotransferrin (PDB ID:1N04) [14], human serum transferrins (PDB ID:1N84) [17], and human lactoferrin (PDB ID:1B0L) [18].

inter-sub-domain cleft of each lobe [7,8,10–14]. Fig. 1 compares the N-lobes of human lactoferrin, ovotransferrin and human serum transferrin [17,18] (Fig. 1, panels B, C and D, respectively). Strong similarities could be observed between the three proteins; despite few differences in amino acid sequences, the overall 3D structure is strictly conserved.

The amino acid sequence of avian serum transferrin and ovotransferrin is identical differing only in the nature of the carbohydrate groups linked to the proteins [19]. Contrary to the mammalian genome, the avian genome contains only one transferrin gene which is expressed both in liver and oviduct. The expression of this avian transferrin gene is modulated by iron level and steroid hormones and their respective products are known as avian serum transferrin and ovotransferrin, respectively [20]. There are two putative glycosylation sites in the polypeptide chain, both located in the C-terminal lobe (473–476 and 618–621). However, only a single carbohydrate chain, attached to Asn 473, was detected in both proteins [19,21]. In ovotransferrin (*i.e.* the protein synthesized in the oviduct), the oligosaccharide chain is composed of 4 residues of mannose and 8 residues of N-acetylglucosamine. To the contrary, in avian serum transferrin (*i.e.* the protein synthesized in the oviduct), most of the carbohydrate moiety is located in a single unit without any homogeneity with respect to its main glycan group, since the proteins appear in at least two electrophoretic variants [22,23], prevailing the glycan chain composed of two residues of mannose, two residues of galactose, three residues of N-acetylglucosamine, and either one or two residues of sialic acid [23].

1.2. Ovotransferrin iron binding site and releasing mechanisms

The crystal structure shows that OTrf iron-binding sites are very similar to those reported for human lactoferrin and for human serum transferrin [24]. Iron (III) ions bound to OTrf are hexacoordinated and the two iron binding sites show very similar coordination geometry.

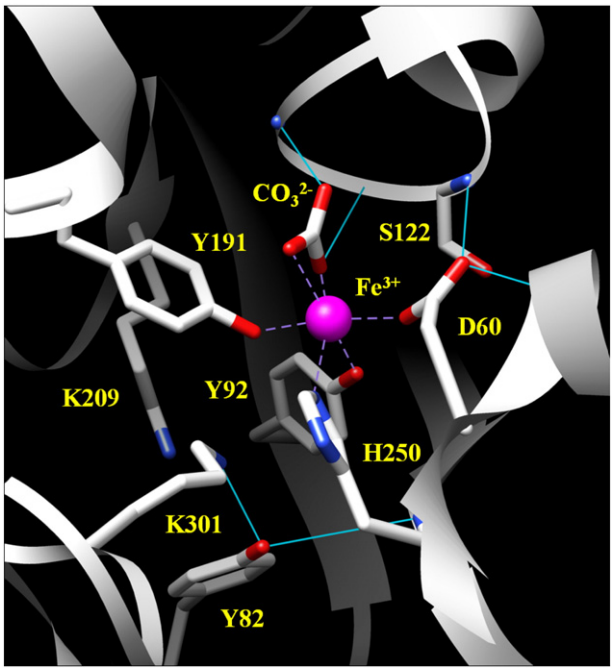

Fig. 2. N-lobe iron binding sites of hen's ovotransferrin. Hen's ovotransferrin (PDB ID:1N04) [14]. In figure the amino acids involved in the N-lobe iron binding (iron is represented as magenta sphere) are reported. Hydrogen bonds are shown by cyan lines, while the broken lines indicates coordination to iron. Molecular graphics images were produced using the UCSF chimera package [134].