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Review

X-ray structures of transferrins and related proteins ☆

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ABSTRACT

Background: Transferrins are a group of iron-binding proteins including serum transferrin, lactoferrin and ovotransferrin.
Scope of review: The structures of transferrins are discussed.
General significance: The typical transferrin molecules are folded into two homologous lobes. X-ray crystallography revealed that each lobe is further divided into two similarly sized domains, and that an iron-binding site is contained within the inter-domain cleft. The six iron coordination sites are occupied by four residues and a bidentate carbonate anion.
Major conclusions: The structures of the apo- and holo-forms revealed that the transferrins undergo a large-scale conformational change upon the uptake and release of irons: domains rotate as rigid bodies around a screw axis passing through inter-domain contacts. The iron-release mechanism of transferrin N-lobe is also revealed by X-ray crystallography; two basic residues in two domains form an unusual hydrogen bond in neutral pH, and the bond should be broken and facilitate iron release at a low pH of the endosome. For ovotransferrin, the iron release kinetics of two lobes correspond well with the numbers of anion binding sites found in crystal structures. The structures of transferrins bound to other metals revealed that the flexibility of the transferrin structure allows the ability to bind to other metals. This article is part of a Special Issue entitled Transferrins: Molecular mechanisms of iron transport and disorders.

1. Introduction

Transferrins are a homologous group of iron-binding proteins that include serum transferrin, lactoferrin and ovotransferrin [1,2]. Although many transferrin family proteins are known in vertebrates and insects, no obvious transferrin family protein has been reported for lower eukaryotes and prokaryotes. Most transferrins, mammalian and avian serum transferrins, mammalian lactoferrin and avian ovotransferrin, are about 80-kDa single-chain bilobal proteins, and one iron-binding site is located in each lobe [1,2]. Mammalian and avian serum transferrins are known to deliver irons into target cells via transferrin receptor [3]. The genomes of fishes, amphibians and reptiles also have serum transferrin-like proteins and these proteins may act as iron-transporting proteins; however, protein-level studies of the organisms are insufficient to demonstrate functions of the proteins [4–7]. Lactoferrin is found in milk, saliva and tears, and it has multiple functions such as antimicrobial activity [8]. Ovotransferrin is contained in the egg white of birds and of some reptiles (such as turtles and crocodiles), and chicken ovotransferrin and chicken serum transferrins are known to be derived from the same gene [9–12]. In this review, we mainly introduce mammalian serum transferrins, mammalian lactoferrins and avian ovotransferrins whose structures and functions are well established.

2. Lobe and domain architectures of serum transferrins, lactoferrins and ovotransferrins

The transferrins reported so far are shown in Tables 1A and 1B. For serum transferrin, three mammalian (including human) transferrins and one avian transferrin have been reported. For ovotransferrin, two avian proteins are reported; since these proteins are made using the same gene of avian serum transferrin, they should share the same structural characteristics. For lactoferrins, many structures have been reported recently, and the structures of six mammalian lactoferrins are known. All three transferrins consist of two homologous lobes (N- and C-lobes) connected by short loop region, and have one iron-binding site in each lobe (Fig. 1). The two homologous lobes are further divided into two similarly sized domains, domain 1 and domain 2 (domains N1 and N2 in the N-lobe and domains C1 and C2 in the C-lobe), and the two iron-binding sites are located within the inter-domain clefts of each lobe (Fig. 1). Domain 2 (N2 and C2) consists of a contiguous polypeptide segment, but domain 1 is comprised of two polypeptide segments interrupted by domain 2. In ovotransferrin, domain N1 contains residues 1–91 and 247–332, and domain C1 contains residues 343–429 and 589–686, respectively (Fig. 1C) [13]. Domains N2 and C2 and the loop-region consist of residues 92–246, 430–588 and 333–342. The

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Table 1A
Reported structures (holo- and apo-forms).

Organism	Holo	Apo	Domain structure of apo-form	
			N-lobe	C-lobe
Serum transferrins				
Homo sapiens (Human)				
		W (2HAV, 2.70 Å) [53]	Open (59°[a])	Open (49.5°[a])
	N (1A8E 1.60 Å) [19]	N (1BP5, 2.20 Å) [54]	Open (63°)	–
Sus scrofa (Pig)				
	W (1H76, 2.15 Å) [55]			
Oryctolagus cuniculus (Rabbit)				
	W (1JNF 2.60 Å) [55]			
	N (1TFD, 2.30 Å) [56]			
Gallus gallus (Chicken)				
	W (1NO4, 2.80 Å) [57]	W (1RYX, 3.50 Å) [58]	Open (53°)	Open (35°)
Ovotransferrins				
Gallus gallus (Chicken)				
	W (1OVT, 2.40 Å) [13]	W (1AIV, 3.0 Å) [15]	Open (53°)	Open (35°)
	N (1IEJ, 1.65 Å) [16]	N (1TFA, 1.90 Å) [29]	Open (50°)	
		C (1IQ7, 2.30 Å) [28]	–	Open (36°[b])
Anas platyrhynchos (Domestic duck)				
	W (1DOT, 2.35 Å) [59]	W (1AOV, 4.00 Å) [60]	Open (52°)	Open (50°)
Lactoferrins				
Homo sapiens (Human)				
	W (1LFG, 2.00 Å) [61]	W (1CB6, 2.00 Å) [14]	Open (54°)	Closed
	N (1LCT, 2.00 Å) [62]	N (1L5T, 3.00 Å) [63]	Open (52°)	
Bos taurus (Bovine)				
	W (1BLF, 2.80 Å) [64]			
	C (1NKX, 1.90 Å) [65]			
Bubalus bubalis (Domestic water buffalo)				
	W (1CE2, 2.50 Å) [66]			
Equus caballus (Horse)				
	W (1B1X, 2.62 Å) [67]	W (1I6B, 3.20 Å) [68]	Closed	Closed
Camulus dromedarius (Arabian camel)				
		W (1DTZ, 2.65 Å) [69]	Open (55°[c])	Open (56°[c])
Capra hircus (Goat)				
	W (1JW1, 4.00 Å) [70]			

W, N and C indicate whole molecule (both-lobes), N-half molecule (N-lobe) and C-half molecule (C-lobe). For open structures, extents of opening are shown in parentheses.
[a] Compared with pig transferrin.
[b] Compared with whole hen ovotransferrin.
[c] Compared with human lactoferrin).

lobe and domain architectures were well conserved in all of the reported structures of transferrins (Tables 1A and 1B and Fig. 1).

Reported structures of apo (iron-free) and holo (iron-saturated) forms are summarized in Table 1A. Only chicken ovotransferrin and human lactoferrin have reported structures of both apo- and holo-forms of a whole molecule (a molecule that has both N- and C-lobes) at reasonable resolution (better than 3 Å resolution). For the N-lobe, high-resolution crystal structures of both apo- and holo-forms have been reported for human serum transferrin, chicken ovotransferrin and human lactoferrin. Therefore, the combination of heterologous transferrin structures (such as apo human serum transferrin and holo rabbit serum transferrin) or employment of only the N-lobe is required to compare the apo- and holo-forms of many transferrins including mammal serum transferrins. In usual commercial crystal screening kits, metals that can bind to transferrins (discussed in Section 7) and low pH buffers that facilitate iron release (Section 6) are contained. The metals and low pH buffers might disturb crystallization of the apo- or holo-form of transferrins, respectively. The flexibility of inter-lobe loops in whole molecule, domain movement (Section 3) in the apo-form and glycosylation of native proteins might also prevent the crystallization of transferrins.

All of the transferrins bind carbonate with iron, and some anion and/or metal substituted structures have been reported besides apo- and holo-forms (Table 1B, Sections 6 and 7).

3. Domain movements of transferrins

The holo-form of transferrin has a closed domain structure as shown in Fig. 2, and the apo-form can have an open structure (Fig. 1C). The crystal structure of the apo-form of human lactoferrin (Fig. 1B) revealed that the N-lobe of the protein has an open-domain structure [14], and the crystal structure of the apo-form of ovotransferrin revealed (Fig. 1C) that both lobes of the protein have an open-domain structure (Fig. 1C) [15]. As a possible mechanism for the closed structure of C-lobe in lactoferrin, it has been postulated that equilibrium exists between the open and closed forms of an apo-form in solution, and that the observed closed structure is selected by crystal packing. Domains 1 and 2 rotate as rigid bodies with a small translation (around 2 Å) [16]. The values of the rotation are 53°, 54° in the N-lobes of ovotransferrin and lactoferrin, and the extent of the rotation is only 35° in the C-lobe of ovotransferrin. For reported open structures, extents of opening are summarized in Table 1A. The screw-axis of the rotation passes through the two inter-domain β-strands, and it also passes through inter-domain van der Waals contacts in the N-lobe or a disulfide bond in the C-lobe that exists at the corresponding position of the van der Waals contacts. The inter-domain van der Waals contacts in the ovotransferrin N-lobe consist of Trp125, Ile127, Ile129 and Trp140 in domain N1 and Tyr324 and Met331 in domain N2, and the disulfide bond in C-lobe consist of Cys478 in domain C1 and Cys671 in domain C2. The van der Waals contacts and the disulfide bond are significantly distant from the inter-domain β-strands (about 15 Å), and the van der Waals contacts in the N-lobe (or a disulfide bond in the C-lobe) help the precise hinge motion of domains around the β-strands as an alternative hinge (Fig. 3). The disulfide bond in the C-lobe is well conserved in all of the serum transferrins, ovotransferrins and lactoferrins, and N-lobe of human and rabbit serum transferrins also have a disulfide bond at the corresponding position.