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Transferrin in fishes: A review article

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ABSTRACT

Transferrin is a single monomeric glycoprotein of molecular weight of 80 kDa that transports iron involved in many metabolic processes amongst the sites of absorption, storage and utilization, hence considered as the major iron binding protein in the plasma of vertebrate species. In this study, transferrin structure, synthesis, receptor and the mechanism of cellular uptake of iron from transferrin have been reviewed. Besides, the major biological functions of transferrin and the different forms of it (polymorphisms) have been indicated.

1. Introduction

Most forms of life require iron for growth and survival, because it is involved in many cellular metabolic pathways and enzymatic reactions (oxygen transport, electron transport, DNA synthesis, *etc.*). Iron does not normally exist in a free form in animals, but excess iron can catalyze the conversion of hydrogen peroxide into free radicals, via the Fenton reaction[1,2]. This can damage the cellular structure and finally kill the cell[3,4]. The toxic effect of iron is avoided under normal circumstances through mechanisms that minimize free iron in the body. Any iron not incorporated as a functional moiety of proteins is bound to transport or storage proteins in a nontoxic ferric (Fe^{3+}) form[5]. The iron-complexing agents serve to solubilize iron in the media surrounding the living organism, transport it within the animal and deliver it to the cells. Iron exists as a heme complex in heme proteins (hemoglobin, myoglobin, cytochromes) or as a nonheme protein compounds (transferrin, ferritin and hemosiderin) to be transported as a redox-inactive form[1]. The nonheme ferritin and hemosiderin are mostly involved in iron storage, whereas transferrin is an iron transport protein that binds to iron atom, thus making it unavailable for catalysis of superoxide radical formation. Transferrin is a single monomeric glycoprotein of molecular weight of 80 kDa, approximately 700 amino acids in length that transports iron involved in many metabolic processes amongst the sites of absorption[6], storage and utilization, hence considered as the major iron binding protein in the plasma of vertebrate species. It is an iron-binding protein which reversibly binds iron and can create low-iron conditions and which restricts the growth of some pathogenic bacteria[7]. Members of transferrin group of proteins are evolutionarily related and include transferrin from serum (serotransferrin), ovotransferrin (conalbumin) from egg white, lactoferrin from milk, tears and leucocytes, and the membrane-bond, tumour-associated melanotransferrin[8].

2. Transferrin structure

Trasnferrin consists of a single polypeptide chain of about 700 amino acid residues organized into the C and N lobes, each consisting of two domains to which iron is coordinated[9-11]. The globular lobes are connected by a short helical section. Iron-free transferrin, or apotransferrin, reacts with iron to produce a complex. Each molecule of transferrin with its two specific iron binding sites is capable of binding two atoms of iron and two bicarbonate ions. The bicarbonate ions facilitate a stable interaction between Fe^{3+}

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and the specific iron binding sites of transferrin[12]. According to Welch[13], 42% of the amino acids in the N-terminal domain have identical counterparts in the C-terminal domain.

3. Transferrin synthesis

Transferrin is mainly synthesized in the liver and secreted into the plasma[8]. Significant expression of transferrin gene has also been found in other tissues including the brain, testes, ovary, spleen, mammary gland and kidney[14-16]. The synthesis of transferrin by some of these non-hepatic tissues may be important in those situations where cells are separated by blood barriers from the transferrin in plasma[13]. Under normal conditions, most of the iron in the blood plasma is bound to transferrin.

4. Transferrin receptor (transferrin R)

Cells take up iron bound to transferrin using transferrin R, thus, the biological function of the specific receptors is to bind transferrin on the cell surface and ingests it[11]. The transferrin R, which is expressed in all nucleated cells in the body, assists iron uptake into vertebrate cells through a cycle of endo and exocytosis of transferrin[17]. The transferrin R has been found in red blood cells, thyroid cells, hepatocytes, intestinal cells, monocytes, brain, the blood-brain barrier and also some insects and certain bacteria[18,19], and has a higher affinity to diferric transferrin than apotransferrin, and different transferrin R may have very different affinities to transferrin[20]. Two types of transferrin R, transferrin R1 and transferrin R2, are known with Transferrin 1 being the widely expressed and the best-characterized of the two receptors. Transferrin R1 is a homodimeric membrane glycoprotein of molecular mass ~190000 Da that binds two molecules of transferrin in a pH dependent manner and allows delivery of iron into cells[21]. According to Chen *et al*.[22], the zinc-transferrin complex interacts with the transferrin R and stimulates the proliferation of immature red blood cells in the head kidney of common carp (*Cyprinus carpio*). In malignant cells, there are elevated levels of transferrin R expression attributed to the requirement of high level of iron for their growth[23]. Besides, transferrin R is also reported to be involved in immune response against bacterial infection[24].

5. Cellular uptake of iron from transferrin

The cellular uptake of transferrin-bound iron occurs by means of transferrin-specific cell membrane receptor protein. Iron-loaded transferrin binds to the transferrin R on the cell surface, and via coated pits and coated vesicles, the transferrin-TR complex becomes trapped within endosomes. Through the action of a proton-pumping ATPase of the endosomal membrane, the vesicle lumen is rapidly acidified (pH 5–5.5). Low pH of the endosome facilitates iron mobilization from transferrin, and the iron is transported across the endosomal membrane into the cytosol. At the pH of the endosomal lumen, the apotransferrin formed binds tightly to the transferrin R. The apotransferrin-transferrin R complex is sorted into exocytic vesicles, hence escaping lysosomal degradation. The exocytic vesicle fuses with the plasma membrane, and the apotransferrin-transferrin R complex is exposed to the extracellular PH. At this pH, the apotransferrin has a very low affinity for the transferrin R, hence apotransferrin dissociates from the receptor leaving it ready for another cycle of transferrin binding and endo-/exo-cytosis[8,25]. Generally, almost all serum iron are bound to transferrin. Iron-loaded transferrin (diferric transferrin) binds to the transferrin R on cell surface and the complexes are endocytosed through clathrin-dependent pathway. When pH decreases during the endosome maturation, the ferric iron dissociates from the Transferrin and the rest of the complex returns to plasma membrane. Once exposed to the neutral pH at cell surface, apotransferrin and transferrin R dissociate and are ready for another round of iron uptake[26]. In studying the functional plasticity of transferrins from four air-breathing channids (Genus, *Channa*: Channidae) and its relevance to their survival, Jabeen *et al*. concluded that transferrinin retains iron in exceptionally high amounts at acidic pH[27]. Securing free iron at low pH should be imperative if respiratory acidosis occurs, since under low oxygen free iron (as Fe^{3+}) precipitates even at physiological pH.

6. Biological functions of transferrin

6.1. Transferrin as transporter of iron and metal ions

The level of free iron in body fluids is controlled by transferrin, which can bind, sequester and transport Fe^{3+} ions in maintaining the availability of iron and preventing the deposition of insoluble ferric hydroxide aggregates. The main function of transferrin is to transport iron from the liver, intestine and reticuloendothelial cells to tissues requiring iron for normal growth and development. It is also involved in immune defense mainly via its ability to bind to iron (III) ions[28,29]. According to Sun *et al*.[20], transferrin is likely to be involved in transportation of a wide range of metal ions other than iron, such as therapeutic metal ions, radio diagnostic metal ions and some toxic metal ions. Since the metal binding sites of transferrin are occupied by iron only for approximately 30%, other metals can be bound without requiring the displacement of the more tightly bound iron in the absence of high concentrations of serum albumin. Transferrin has been postulated to play a significant role in transporting Ti^{4+}, VO^{2+} (V^{4+}), Cr^{3+}, Ru^{3+} and Bi^{3+}, all metal ions of potential therapeutic significance. Transferrin may possess a physiological role in the transport of manganese, as the trivalent ion. However, the protein may also play a role in carrying potentially toxic Al^{3+} and actinide ions, including Pu^{4+}, to the tissues[30]. De Smet *et al*. reported that transferrin of common carp has been recognized as the major protein for the transport of metals other than iron, such as cadmium[31]. In the same way, the increase of serum transferrin levels in Nile tilapia [*Oreochromis niloticus* (*O. niloticus*)] as a result of exposure periods to cadmium/zinc suggest the use of transferrin as biomarker parameter of heavy metal toxicity in fish[32]. In demonstrating the ability of a major carp seminal plasma protein-transferrin to bind cadmium ions and to neutralize the toxic effect of cadmium on carp sperm motility, Dietrich *et al*. reported that transferrin from carp seminal plasma can protect sperm motility from cadmium toxicity[33].

6.2. Transferrin as antimicrobial agent

By acting as a high affinity iron binding protein, transferrin has the potential of being an effective antimicrobial agent. The association of transferrin with the immune system derives from its ability to restrict serum free-iron levels, creating low-iron environments where

the infection capacity of pathogenic microorganisms is limited[34,35]. Humoral innate immunity is mediated by soluble factors that inhibit microorganism growth[36]. However, in inflammation conditions, transferrin is known to act mostly as a negative acute phase protein[37]. Ovotransferrin and lactoferrin may also have antimicrobial activity, which apparently depends on actual contact with the bacteria rather than simple iron deprivation[38]. Transferrin is a multi-function protein with a central role in iron metabolism and it is the function that is associated with a role in the innate immune system response. The clear link between transferrin and immune defense mechanism leads to propose transferrin as a candidate gene for disease resistance[39]. Liu *et al*. reported significantly up-regulated transferrin expression in cat fish (*Ictalurus punctatus*) after infection with *Edwardsiella ictaluri*, the causative agent of enteric septicemia[40]. Kovacevic *et al*. studied the expression of genes encoding the acute phase proteins during the course of *Trypanosoma carassii* infection in the goldfish (*Carassius auratus* L.) using quantitative PCR and concluded that transferrin was up-regulated throughout the acute course of infection in the liver, and in the kidney during the chronic phase of the infection[41]. Poochai *et al*. indicated that transferrin expression in tilapia experimentally infected with *Streptococcus agalactiae* was significantly upregulated and iron-deficiency in serum of bacterially infected fish was detected clearly indicating the function of transferrin in innate immunity[42]. Increased levels of transferrin expression were observed following bacterial infections in rainbow trout[37]. An increase in transferrin gene expression was seen in blood and spleen leukocytes of cod following an intra-peritoneal injection of heat-killed bacteria[43,44]. After infection with *Vibrio harveyi*, serum transferrin gene expression of the Chinese black sleeper (*Bostrichthys sinensis*) was found to increase mainly in the liver and stomach, acting as a positive acute protein, suggesting that serum transferrin is involved in the immune response[45]. In studying the transferrin gene expression in response to lipopolysaccharide challenge and heavy metal exposure in roughskin sculpin (*Trachidermus fasciatus*), Liu *et al*. reported that in the main immune organs (skin, blood and spleen), transferrin mRNA expression was up-regulated significantly suggesting that transferrin is involved in the innate immune response of roughskin sculpin[46]. Transferrin expression was up-regulated in the gill of orange-spotted grouper during exposure to *Cryptocaryon irritans* which suggested most of the expressed transferrin to be used by the host to produce a greater NO response that plays a major role in host resistance to parasite infection[47]. After a constitutive expression of transferrin in head kidney and spleen, an up-regulation following acute phase induction was demonstrated[48]. Ercan *et al*. investigated the transferrin gene expression of sea bass (*Dicentrarchus labrax*) during an experimental infection with *Vibrio anguillarum* and reported an increased transferrin gene expression during the first 2 days[49]. An up-regulation of transferrin gene expression has also been demonstrated in channel catfish and sea bass following bacterial infection[2,50]. Specific functional regions of the transferrin protein seem to be undergone positive natural selection in salmonids and goldfish (*Carassius auratus*), showing a possible relationship between transferrin and resistance to pathogens in fish[51,52].

6.3. Transferrin as fish macrophages activator

Macrophages are found across all vertebrate species, reside in virtually all animal tissues, and play critical roles in host protection and homeostasis. As resident cells in virtually all tissues, macrophages aid in maintaining homeostatic environments, and upon infection, are typically one of the first cell types to encounter intruding pathogens, where they orchestrate appropriate immune responses[53]. Transferrin plays a role as a primary activator of fish macrophages. The activation of fish macrophages by transferrin cleavage products may represent a primitive but highly conserved pathway for the induction of NO in lower vertebrates[54]. According to Stafford and Belosevic[55], in goldfish, transferrin cleavage products could act as a macrophage activator factor by stimulating macrophages to produce large amounts of NO. The same result was reported in carp blood transferrin, whereby immunostimulatory fragments of carp transferrin induce a NO response in carp macrophage. Activated macrophages were also indicated to be the source of necessary enzymes required for cleavage transferrin into immunostimulatory fragments[56]. Cysteine proteinases of *Trypanoplasma borreli* can also produce immunostimulatory fragments of carp transferrin[57]. Transferrin-derived synthetic peptide induces highly conserved pro-inflammatory responses of macrophages[58]. In coho salmon (*Oncorhynchus kisutch*) with the C allele of transferrin, increasing resistance against bacterial kidney disease has been reported[34]. Generally, as an acute-phase protein in fish, the concentration of transferrin indicates the condition of infection or stress, though its rise or fall varies with the infective microorganisms or injury in different tissues[2,40]. Transferrin is also involved in many biological functions, such as DNA synthesis, oxygen and electron transport, growth, differentiation and cytoprotection processes[8,13,55,59-61]. Relatively recent data uncovered an additional role of transferrin as an upstream regulator of hepcidin, a liver-derived peptide hormone that controls systemic iron traffic[62].

7. Transferrin polymorphisms

Transferrin has a high degree of genetic poly morphism in all species[63]. A number of transferrin variants have been identified and characterized in many different species. Gene duplication, gene loss and horizontal transfer contributed to the diversification of transferrin family members[29].

Transferrin variants have been reported in different fish species. Yang *et al*. studied transferrin polymorphisms in goldfish (*Carassius auratus*) and identified three transferrin variants (A1, A2 and B1) in which substantial amount of amino acid variation was observed between variant A1 and B1[64]. By studying the resistance characteristic and cDNA sequence conservation of transferrin from crucian carp, *Carassius auratus*, Long and Yu demonstrated a polymorphism in which silver carps shared 1/3 transferrin alleles with crucian carps[65]. The transferrin of crucian carps had stronger Fe^{3+}-binding ability and transportation ability in plasma than that of sliver carps. Borges *et al*. studied plasma polymorphisms in scad (*Trachurus trachurus* L.) population of North East Atlantic and identified four allelic variants (transferrin 10, transferrin 105, transferrin 97 and transferrin 94)[66]. Four transferrin polymorphisms (alleles) have been identified in coho salmon (*Oncorhynchus kisutch*) and used to discriminate regional groupings of populations[67]. Natural populations of haddock (*Melanogrammus aeglefinus* L.) had been discriminated by the variation at the blood plasma transferrin molecule that revealed a series of 21 co-dominant alleles[68]. Using transferrin polymorphs as a biochemical marker, Nabi *et al*. screened the fresh water fish (*Channa punctatus*) in India and identified six transferrin variants (AA, BB, CC, AB, AC and BC) with the corresponding alleles of isoforms designated as transferrin Acp, transferrin Bcp and transferrin Ccp[69]. According to Jurecka *et al*.[56], 21 polymorphic sites were detected by comparing the cDNA of four different transferrin alleles (C, D, F and G) of European common carp (*Cyprinus carpio carpio* L.)[70], analysed sequence of transferrin gene in Nile tilapia (*O. niloticus*) and reported a single nucleotide polymorphism that are associated with salt water tolerance in Nile tilapia. Seven (A, B, D, E, F, H, G) different transferrin alleles were detected in Hungarian, European and Asian carp populations[71]. Transferrin cDNA sequence was used as the main tool for the study of the evolutionary relationship

and construction of phylogenetic trees in salmonids, where the transferrin cDNA sequence showed a duplicated structure and conserved anion-binding residues, iron-binding residues, and cystein residues for disulfide bridges[72]. In the same manner, phylogenetic trees based on the genetic distances between deduced amino acid sequences of transferrin from a variety of species have been constructed[2]. Two distinct Atlantic cod stocks at Faroe Plateau and Faroe Bank were reported based on the frequencies of five cod transferrin alleles[73]. Jaayid *et al*. investigated the existence of polymorphisms at transferrin locus in carp (*Cyprinus carpio*) and concluded that there existed seven transferrin genotypes consisting of 4 homozygote types (CC, DD, FF and GG) and two heterozygote types (CD, DG and FG)[74]. A relationship between sperm motility parameters and transferrin polymorphism of carp seminal plasma, which may affect sperm competitive ability, has been reported by Wojtczak *et al*.[75]. Jurecka *et al*. indicated the influence of transferrin polymorphism in the resistance of common carp to the blood parasite (*Trypanoplasma borreli*)[56]. According to Rengmark and Lingaas[70], a haplotype of Nile tilapia (*O. niloticus*) that is associated with salt water tolerance was identified in the complete genomic sequence of transferrin and functional studies.

Conflict of interest statement

I declare that I have no conflict of interest.

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