Biochimica et Biophysica Acta 1820 (2012) 437–451

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Insect transferrins: Multifunctional proteins ☆

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ARTICLE INFO

Article history:
Received 7 May 2011
Received in revised form 13 July 2011
Accepted 17 July 2011
Available online 23 July 2011

Keywords:
Development
Ferritin
Immunity
Insect
Oxidative stress
Transferrin

ABSTRACT

Background: Many studies have been done evaluating transferrin in insects. Genomic analyses indicate that insects could have more than one transferrin. However, the most commonly studied insect transferrin, Tsf1, shows greatest homology to mammalian blood transferrin.
Scope of review: Aspects of insect transferrin structure compared to mammalian transferrin and the roles transferrin serves in insects are discussed in this review.
Major conclusions: Insect transferrin can have one or two lobes, and can bind iron in one or both. The iron binding ligands identified for the lobes of mammalian blood transferrin are generally conserved in the lobes of insect transferrins that have an iron binding site. Available information supports that the form of dietary iron consumed influences the regulation of insect transferrin. Although message is expressed in several tissues in many insects, fat body is the likely source of hemolymph transferrin. Insect transferrin is a vitellogenic protein that is down-regulated by Juvenile Hormone. It serves a role in transporting iron to eggs in some insects, and transferrin found in eggs appears to be endowed from the female. In addition to the roles of transferrin in iron delivery, this protein also functions to reduce oxidative stress and to enhance survival of infection.
General significance: Future studies in Tsf1 as well as the other insect transferrins that bind iron are warranted because of the roles of transferrin in preventing oxidative stress, enhancing survival to infections and delivering iron to eggs for development. This article is part of a Special Issue entitled Transferrins: Molecular mechanisms of iron transport and disorders.

1. Introduction

1.1. Importance of iron to life

Iron is essential to the survival of almost all organisms because it plays an indispensible role in numerous metabolic processes. These include respiration, energy generation, oxygen transport, gene regulation, DNA biosynthesis and immunity [1–3]. In addition in insects, iron is involved in cuticle formation, tanning, melanization and wound healing [4]. Iron is an extremely attractive prosthetic biocatalyst or electron carrier because it can readily change oxidation and coordination states. Despite its requirement and these valuable features, excessive or misplaced iron is highly destructive because it can generate oxygen radicals by the Fenton–Haber Weiss reaction [5,6]. Iron-catalyzed oxidative stress can have many adverse consequences [7]. In humans, disorders of iron overload can lead to neurodegenerative and endocrine changes, cardiomyopathy, arthropathy, neoplasia and increased infections [7]. Excess iron also can propagate virus-mediated diseases by facilitating viral replication, and promoting tissue injury or secondary infections [8]. The toxic potential of iron has encouraged organisms to develop strategies that allow iron transport, but prevent the accumulation of redox-active iron in sensitive tissues [7]. Proteins of the transferrin superfamily serve these roles in many animals.

2. The mammalian transferrins

Transferrin is a glycoprotein found in multi-cellular animals. Transferrin and related superfamily members have been studied for many years in mammals and this has provided the basis for comparative analyses in insects. Mammalian blood transferrin (Tsf) and its closely-related superfamily members, lactoferrin (LF), melanotransferrin (MTsf) and ovotransferrin (OTsf), are characterized by the presence of a binding pocket with an extremely high affinity for

Abbreviations: 20E, 20-hydroxyecdysone; JH, Juvenile hormone; LF, Lactoferrin; MTsf, Melanotransferrin; ORF, Open reading frame; OTsf, Ovotransferrin; TfR, Transferrin receptor; Tsf, Transferrin

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doi:10.1016/j.bbagen.2011.07.011

iron [1,9]. Tsf consists of two lobes, one each in the N and C-termini, that bind ferric at $k_d \sim 10^{-23}$ [10], and this gives rise to the three isoforms found in human blood: apo-Tsf, monoferric-Tsf and diferric-Tsf [3]. In adults, 30–50% of Tsf is iron saturated [10]. Thus, under physiological conditions, the high affinity constant of Tsf for iron coupled with the unsaturated state of the protein virtually ensures a relative absence of free iron in blood [2,10].

MTsf, found at high levels on malignant melanoma cells, is believed to be the oldest of the transferrin superfamily. In contrast to Tsf, MTsf binds iron only in the N-terminal lobe [11–13]. It is hypothesized that the original transferrin ancestor may have had only a single lobe, where the first duplication event resulted in tandem genes that eventually fused to form an ancestral double-lobed transferrin before the avian-mammalian split (>580 million years ago) [13,14]; all subsequent gene duplication events are thought to have diverged from this ancient *Tsf* [14]. The gene duplication for *LF*, a milk protein, is believed to have occurred more recently than that of *Tsf* after the placental/marsupial split (<125 million years ago) [13,14]. Members of the transferrin superfamily are found in mammals, marsupials and fish, as well as in insects and other invertebrates [13]. Transferrins have been reported in over 34 invertebrate species that encompass more than 13 orders. An exhaustive comparative summary of the insect transferrins as well as some from other invertebrate species is given as a supplementary table (Table 1s), as much of the information provided by this table is beyond the scope of this review.

2.1. Roles of transferrin superfamily members in mammals

2.1.1. Blood transferrin and iron metabolism

It is anticipated that other reports in this edition will discuss mammalian transferrins in depth. Here we provide a brief summary of mammalian iron metabolism and the role of transferrin in this process for comparative purposes. In mammals, the primary role of Tsf is iron transport [15]. Dietary iron is absorbed into intestinal enterocytes by several pathways. However, iron export from enterocytes into blood occurs primarily by way of a single membrane transport protein, ferroportin [16]. Ferroportin exports ferrous that is oxidized to ferric for transport by Tsf by multi-copper oxidases found in blood and on the surface of cells [17]. Tsf delivers dietary iron to the tissues [17] by binding to the cell surface receptors, transferrin receptor 1 (TfR1) and TfR2. TfR1 is the predominate pathway for iron delivery and is found on most cells [10]. Cells that are rapidly dividing or have specialized iron requirements, such as erythroid cells, express very high levels of TfR1 [10,13]. At blood pH, TfR1 binds diferric-Tsf with 10-fold higher affinity than monoferric-Tsf, and 2000-fold higher affinity than apo-Tsf [10]. The binding of holo-Tsf to the receptor initiates receptor mediated endocytosis and the formation of an endosome [15]. At a low pH inside the endosome, ferric is released from Tsf [10,15]. The low pH also increases the affinity of TfR1 for apo-Tsf, and the apo-Tsf-TfR1 complex [10] is recycled to the cell surface where apo-Tsf is released back into blood. A single round of transferrin-mediated iron delivery is completed in 5–20 min depending on the cell type [10]. Transferrin receptors have been identified by binding studies in marsupials, reptiles and amphibians indicating that Tsf serves an iron transport function in these animals [13]. However, despite considerable efforts, while transferrins are found in insects, no TfR1 has been identified suggesting that insect transferrin serves roles other than dietary iron delivery or that the transferrin receptor in these animals is very different from that of mammals [18–20].

The majority of mammalian Tsf originates from liver. Production is constitutive and regulated at transcription [10]. Tsf concentration varies over a limited range and is mildly increased in response to severe iron-deficiency. The half-life of human Tsf is eight days, and Tsf levels are considered indicative of recent protein intake [10]. Tsf is a negative acute phase reactant and increased by hypoxia and estrogen [10]. In addition to blood, it is found in cerebrospinal fluid and lymph [7,9]. Although it is expressed primarily in liver, expression also occurs in brain, testes, ovary, spleen, mammary gland and kidney [13]. Iron-bound Tsf is the most important dynamic iron pool [3] in the body. The majority of iron in the body is found in red blood cells. Iron released during red cell turnover is recycled by Tsf from macrophages in spleen and liver to bone tissues where it is incorporated into hemoglobin in newly formed erythroid cells [3,16]. Not only is this pathway dynamically important, it also is quantitatively the most important iron pool with a turnover of 23–24 mg iron/day [10,21]. Tsf is required for erythropoiesis [3], and its absence is characterized by chronic anemia. Iron absorbed from the diet contributes ~1 mg/day [10,21], and replaces losses of approximately the same level.

Iron levels in mammals are controlled by limiting dietary iron absorption. In humans, iron is stored primarily in the liver in cytoplasmic ferritin [2,7,8]. How the liver signals the intestine that adequate stores are in place is a complex process and has been the subject of recent intense investigation. Available information indicates that hepcidin is a key protein involved in limiting iron absorption [16]. Hepcidin is a 2.7 kDa peptide [8] secreted from the liver that binds to ferroportin expressed on cell surfaces; binding causes ferroportin internalization and degradation [16]. Since ferroportin functions as the primary protein for iron export from macrophages and hepatocytes as well as enterocytes, the presence of hepcidin limits export of dietary iron to blood and decreases iron recycling [2,8]. Thereby, hepcidin serves as a hepatic iron sensor that brings dietary iron absorption into synchrony with body iron needs [16].

Hepcidin expression is responsive to Tsf [16]. TfR2 is a low affinity Tsf receptor found on several cell types [9]. In liver, when holo-Tsf is bound to this receptor the expression of hepcidin is increased [16]. A second protein that represses hepcidin expression, hemojuvelin, is negatively regulated by iron-loaded Tsf [16]. Thus, when iron stores are adequate hepcidin expression is increased by both of these mechanisms [16]. More recent work indicates a relationship of Tsf saturation to hepcidin [22,23]. Interestingly, unsaturated Tsf inhibits bacterial growth [2] suggesting it has a role in immunity in addition to sequestering iron. Hepcidin is both an iron-regulated hormone and an antimicrobial peptide [2]. Its expression is increased during infection and inflammation by alternate pathways, and results in reduced iron recycling and blood iron levels [2,8]. This is thought to be part of an immune response aimed at sequestering iron in tissues to prevent its use by invading pathogens [2,8], and to reduce iron-generated oxidative stress in inflammatory states. Hepcidin has antifungal and antibacterial activity that closely resembles cysteine-rich antimicrobial peptides, such as the defensins and protegrins [2]. Although defensins are primary proteins involved in the immune response in insects [24–27], hepcidin has not been identified in these animals. Other antimicrobial, iron-related proteins have been identified in mammals, but are beyond the scope of this review [2,10].

Mammalian bacterial infection reduces blood iron concentration through various mechanisms including iron sequestering by Tsf, LF, and OTsf, suppression of iron efflux by hepcidin and increased synthesis of ferritin for iron storage [2,7,8]. Although decreased circulating iron brought about by these mechanisms contributes to an anemia observed in infection and chronic disease, it also provides a protective effect [21]. Sequestering iron from pathogens appears to be an ancient host defense mechanism [2,18]. In response, microbes have developed strategies to capture iron from the host. These strategies include the production of siderophores, expression and control of Tsf and LF receptors, proteolytic cleavage of iron binding glycoproteins, disruption of iron binding sites and reduction of ferric to ferrous [2,7,21]. Several examples of these behaviors are found in the literature. *Helicobacter pylori* uses heme as an iron source when invading the gastric wall, but obtains iron directly from LF when