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Comparative Biochemistry and Physiology, Part B 140 (2005) 11–25

CBP

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Review

Evolution of duplications in the transferrin family of proteins

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Received 9 July 2004; received in revised form 9 September 2004; accepted 10 September 2004

Abstract

The transferrin family is a group of proteins, defined by conserved amino acid motifs and putative function, found in both vertebrates and invertebrates. Included in this group are molecules known to bind iron, including serum transferrin, ovotransferrin, lactotransferrin, and melanotransferrin (MTF). Additional members of this family include inhibitor of carbonic anhydrase (ICA; mammals), major yolk protein (sea urchins), saxiphilin (frog), pacifastin (crayfish), and TTF-1 (algae). Most family members contain two lobes (N and C) of around 340 amino acids, the result of an ancient duplication event. In this article, we review the known functions of these proteins and speculate as to when the different homologs arose. 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* genome databases, we conclude that melanotransferrins are much older (>670 MY) and more pervasive than previously thought, and the serum transferrin/melanotransferrin split may have occurred not long after lobe duplication. All subsequent duplication events diverged from the serum transferrin gene. The creation of such a large multiple-sequence alignment provides important information and could, in the future, highlight the role of specific residues in protein function.

Keywords: Transferrin, Lactotransferrin; Melanotransferrin; Evolution; Molecular phylogeny; Gene duplication; Iron acquisition

Contents

1. Introduction

Transferrins are a superfamily of single-chain, glycosylated proteins that transport iron from plasma to cells or help regulate iron levels in biological fluids. Most

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1096-4959/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2004.09.012

members consist of two homologous lobes (N- and C-lobe) connected by a short hinge region. An N-terminal signal peptide region is removed once the protein is secreted from the cell. Each of the two lobes is able to reversibly bind a single ferric ion (Fe^{3+}), although the C-lobe binds iron more tightly and releases it more slowly (Aisen et al. 1978; Evans and Williams, 1978; Aisen 1998). A carbonate anion is also bound by each lobe in a synergistic relationship not seen in other groups of iron-binding molecules (Baker 1994). Human serum transferrin (hTF) is about 33.5 kb in length and 80 kDa in size, and the hTF gene is divided into 17 exons (Schaeffer et al. 1987). Serum transferrin (TF) is found predominantly in mammalian blood and appears to be the main iron regulatory/transfer molecule. Similar sequences have also been identified in fish (Kvingedal et al. 1993; Ford 2000) and amphibians (Moskaitis et al. 1990; Morabito and Moczydlowski, 1994).

The lack of human serum TF due to hereditary atransferrinemia is a disorder first described in 1961 (Heilmeyer et al. 1961), characterized by microcytic anemia and storage of excessive iron in tissues (Beutler et al. 2000). The condition is very rare and is inherited as an autosomal recessive. Hereditary hypotransferrinemia has also been reported in both humans (Asada-Senju et al. 2002) and mice (Bernstein 1987).

While a number of different molecules may have some evolutionary kinship with serum transferrin, three groups are most closely related: lactotransferrin, melanotransferrin (MTF), and inhibitor of carbonic anhydrase (ICA). All contain two globular lobes thought to have resulted from a gene duplication occurring around 850 Mya (Park et al. 1985). Some researchers also define a fourth group, ovotransferrin (OTF). This protein is the serum transferrin of birds but is found in both serum and egg whites, where it varies in time of expression and degree of glycosylation, as well as by location (Jeltsch and Chambon, 1982; Williams et al. 1982a; Jeltsch et al. 1987).

Transferrin family members have also been found in invertebrates. Among urochordates, sequences of two species have been found: *Ciona intestinalis* and *Halocynthia roretzi* (Abe et al. 2001). The predicted *Ciona* sequence (tag as genbank: GenBank accession no. AK113446) contains two lobes, while the *Halocynthia* sequence appears to be missing most of the C-lobe. Transferrin-like sequences occur in the major yolk proteins of five species of sea urchins (Brooks and Wessel, 2002), and, in the crayfish, *Pacifastacus leniusculus*, the heavy chain of pacifastin is a three-lobed transferrin relative (Liang et al. 1997). Transferrin sequences have also been identified in a number of insects (Huebers et al. 1988; Bartfeld and Law, 1990; Jamroz et al. 1993; Kurama et al. 1995; Yoshiga et al. 1997; Yun et al. 1999; Hirai et al. 2000; Thompson et al. 2003), although in some, the C-lobes have changed considerably and may no longer be able to bind iron. Recently, additional three-lobed transferrin-like sequences have been identified in marine algae (Fisher et al. 1997; Fisher et al. 1998; Brooks and Wessel, 2002; Schwarz et al. 2003), although these may represent a horizontal gene transfer event. Table 1 lists 71 full-length transferrin family members from 51 different species. Several are new sequences that are discussed further below. Fig. 1 illustrates a neighbor-joining tree resulting from the incorporation of all of these sequences.

2. Iron binding and acquisition

The ability to bind iron is common to most transferrins. The iron-binding properties of mammalian serum transferrin have been investigated extensively. Four ligand residues are conserved in both the N- and C-lobes, and site-directed mutagenesis has confirmed the importance of these amino acids for iron binding (reviewed by He and Mason, 2002). These four amino acids (in the N-lobe: Asp-63, Tyr-95, Tyr-188, and His-249) bind ferric iron with the assistance of a synergistic anion (usually carbonate). Protonation of the carbonate anion loosens the metal–protein bond, allowing the iron to be released. Other residues have also been reported to be involved in the binding and/or release of iron. These include the "dilysine trigger" (He et al. 1999) and other "second shell" residues (He et al. 1998). Table 2 summarizes these residues, their roles, and their locations.

Despite the strong sequence similarity between the N- and C-lobes, there is a marked difference in their iron-binding behaviors. The four iron-binding ligands are completely conserved between the lobes in all mammalian serum transferrins; therefore, other residues must also influence the rate of binding and release. Extensive work has been done on the mechanics of iron binding in the N-lobe (Muralidhara and Hirose, 2000; Adams et al. 2003; Baker et al. 2003). However, recombinant C-lobes have proven to be more difficult to produce and analyze in the laboratory, making comparisons difficult. An examination of the conservation of residues of interest in different species could help guide future mutational studies.

3. Lactotransferrin and inhibitor of carbonic anhydrase

Mammals have four distinct families of transferrin-like sequences: serum transferrin, melanotransferrin, lactotransferrin, and inhibitor of carbonic anhydrase. Of these four, lactotransferrin (LTF: also called lactoferrin) and inhibitor of carbonic anhydrase (ICA) appear to have arisen relatively recently. Lactotransferrin is found in mammalian milk, tears, and other secretions and has been shown to bind iron more tightly than serum transferrins does (Baker 1994); despite its affinity for iron, this protein is not required for iron homeostasis, as demonstrated by lactotransferrin knockout mice (Ward et al. 2003). Instead, this