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Review

Kinetics of iron release from transferrin bound to the transferrin receptor at endosomal pH☆

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ABSTRACT

Background: Human serum transferrin (hTF) is a bilobal glycoprotein that reversibly binds Fe^{3+} and delivers it to cells by the process of receptor-mediated endocytosis. Despite decades of research, the precise events resulting in iron release from each lobe of hTF within the endosome have not been fully delineated.
Scope of review: We provide an overview of the kinetics of iron release from hTF ± the transferrin receptor (TFR) at endosomal pH (5.6). A critical evaluation of the array of biophysical techniques used to determine accurate rate constants is provided.
General significance: Delivery of Fe^{3+} to actively dividing cells by hTF is essential; too much or too little Fe^{3+} directly impacts the well-being of an individual. Because the interaction of hTF with the TFR controls iron distribution in the body, an understanding of this process at the molecular level is essential.
Major conclusions: Not only does TFR direct the delivery of iron to the cell through the binding of hTF, kinetic data demonstrate that it also modulates iron release from the N- and C-lobes of hTF. Specifically, the TFR balances the rate of iron release from each lobe, resulting in efficient Fe^{3+} release within a physiologically relevant time frame. This article is part of a Special Issue entitled Molecular Mechanisms of Iron Transport and Disorders.

1. Introduction

1.1. Overview of iron and human serum transferrin (hTF)

The one electron transfer between ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron is accomplished with relative ease. Due to its inherent redox properties, iron is critical to a number of biological processes including oxygen and electron transport [1]. However, these same redox properties that provide versatility also make iron dangerous. In oxygen rich environments Fe^{3+} is extremely insoluble and Fe^{2+} can be toxic. Specifically, reduction of O_2 by Fe^{2+} generates superoxide, which ultimately can lead to the formation of the hydroxyl radical, a powerful oxidant known to damage DNA, proteins and lipids [2]. To avoid Fenton chemistry, iron must be carefully chaperoned through the body by human serum transferrin (hTF) where it is solubilized and stabilized as Fe^{3+} or stored in ferritin.

An ~80 kDa bilobal protein, hTF is divided into four subdomains (N1, N2, C1 and C2) which form two lobes (termed N- and C-lobes). Each lobe of hTF binds one Fe^{3+} ion tightly (~10^{22} M^{-1}), yet reversibly. In humans iron absorbed from diet passes through duodenyl crypt cells into the serum where it is acquired by hTF. In both hTF lobes the Fe^{3+} is coordinated by identical ligands: two tyrosine residues, one aspartic acid and one histidine residue (Tyr95, Tyr188, Asp63 and His249 in the N-lobe; Tyr426, Tyr517, Asp392 and His585 in the C-lobe). The distorted octahedral coordination of the iron is completed by a synergistic anion, identified as carbonate, which is anchored in place by a conserved arginine residue (Arg124 in the N-lobe and Arg456 in the C-lobe).

Given the ability to bind iron in either or both lobes, hTF circulates in the blood as four different species differing only in iron content. The ~25–50 μM hTF in the serum is unevenly distributed between diferric (Fe_2hTF, ~27%), monoferric N-lobe hTF (Fe_NhTF, ~23%), monoferric C-lobe hTF (Fe_ChTF, ~11%) and iron-free hTF (apohTF, ~40%) [3]. At the pH of the serum (~7.4) iron-bearing hTF (either Fe_2hTF, Fe_NhTF or Fe_ChTF) binds with nM affinity to the specific transferrin receptor (TFR), located on the cell surface of all iron-requiring cells (Fig. 1). The hTF/TFR complex is endocytosed in a clathrin-dependent manner.

Abbreviations: hTF, human serum transferrin; TFR, transferrin receptor; Fe_2hTF, diferric hTF; Fe_NhTF, hTF with iron bound only in the N-lobe; Fe_ChTF, hTF with iron bound only in the C-lobe; $Lock_N$hTF, Fe_2hTF that cannot release iron from the N-lobe; $Lock_C$hTF, Fe_2hTF that cannot release iron from the C-lobe; sTFR, soluble portion of the TFR; KISAB, kinetically significant anion binding; LMCT, ligand to metal charge transfer

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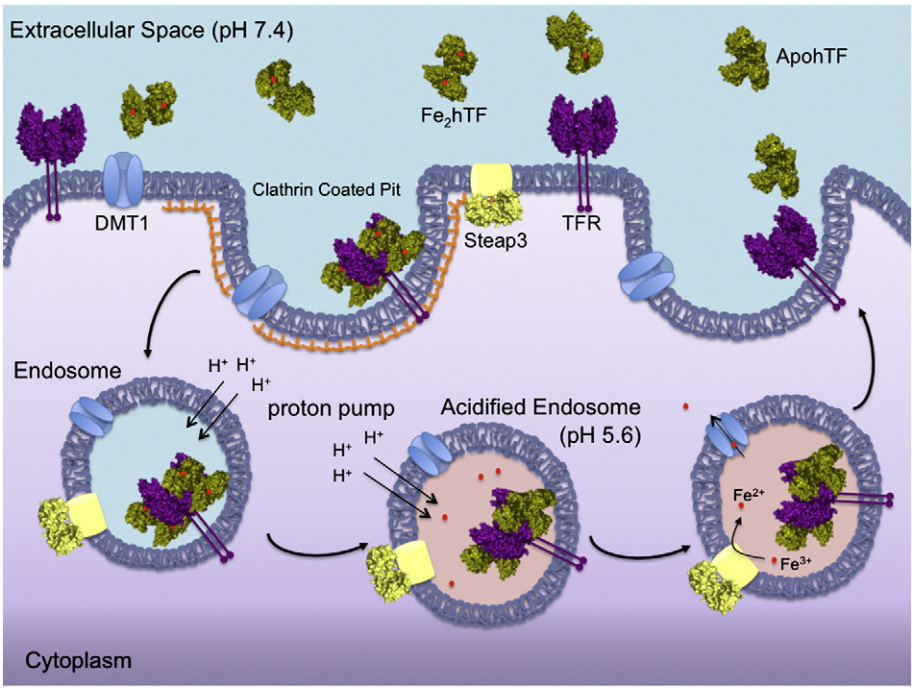

Fig. 1. Endocytic hTF/TFR cycle. Iron bound hTF (green) in the blood binds to the specific TFR (purple) with nM affinity at the cell surface (pH 7.4). The hTF/TFR complex is endocytosed in a clathrin-coated pit. Within the endosome, the pH is lowered to ~5.6 causing iron to be released from hTF to an, as yet, unidentified chelator. Fe^{3+} is reduced to Fe^{2+} by the ferrireductase Steap3 (yellow) within the endosome. The Fe^{2+} can then be transported out of the endosome via the divalent metal transporter DMT1 (blue) for use throughout the cell. The apohTF remains tightly bound to the TFR at pH 5.6 and is recycled back to the cell surface. Upon exposure to the slightly basic pH (7.4), apohTF is released or displaced from the TFR and free to bind more Fe^{3+}.

Through the action of ATP-dependent H^+ pumps, the pH within the endosome is lowered. The significantly lower pH (~5.6) in conjunction with salt and an unidentified chelator within the endosome initiates receptor stimulated iron release from hTF. Although the TFR influences the redox potential of Fe^{3+} bound to hTF [4], recent evidence suggests that the reduction of Fe^{3+} to Fe^{2+} may be accomplished by an endosomal ferrireductase (Steap3) [5], presumably following Fe^{3+} release from hTF. Critical to the hTF endocytic cycle, apohTF remains bound to the TFR with high affinity at endosomal pH, allowing it to return to the cell surface. ApohTF is released back into the serum, either through dissociation from the TFR or displacement by an iron-containing hTF [6], and free to bind more Fe^{3+}. The hTF/TFR cycle has become the classic example of clathrin-dependent receptor mediated endocytosis and often serves as a positive control for other systems.

Although the iron binding ligands in each lobe of hTF are completely conserved, the mechanism for iron release from each lobe differs. This is largely due to differences in "second-shell" residues (residues that do not directly coordinate the Fe^{3+} but form an intricate hydrogen bonding network with the primary iron ligands). In part because of differences in the composition of the second shell residues, the two lobes differ in their response to pH [7–9], anions [9,10], the TFR [11,12], and the conformation of the other lobe.

The crystal structure of the N-lobe of hen ovotransferrin (a closely related family member of hTF) provided the first description of a potential iron release mechanism from the N-lobe of hTF. Attributed to an unusually low pK_a value, two second shell lysine residues, Lys206 and Lys296, located in each subdomain on opposite sides of the cleft of the N-lobe are 3.0 Å apart, share a hydrogen bond and form what is referred to as the dilysine trigger [13]. When the pH is reduced, protonation of one of the lysine residues causes the positively charged lysines to repel each other (moving at least 9 Å apart in the apo N-lobe structure [14]) and literally triggers iron release. Studies have shown that mutation of either member of the dilysine trigger to a glutamate or alanine drastically slowed the rate of iron removal, validating the critical nature of the Lys206 and Lys296 interaction in the mechanism of iron release from the N-lobe of hTF [15].

The mechanism of iron release from the C-lobe differs from that of the N-lobe because the dilysine trigger is replaced by a triad of residues (Lys534, Arg632 and Asp634) [13]. Similar to the dilysine trigger, Lys534 and Arg632 in the C-lobe may share a hydrogen bond that is stabilized by Asp634, which upon protonation would trigger iron release from the lobe; however, in the structure of pig TF, the NZ and NE group of the homologous lysine (Lys543) and arginine (Arg641) are ~4.1 Å apart, too far to share a hydrogen bond [16]. Lacking a crystal structure of an iron-containing C-lobe of hTF, the precise mechanism by which the C-lobe triad triggers iron release remains unclear. However, it has been shown that mutation of Lys534 or Arg632 to an alanine severely retards iron release from that lobe, essentially locking iron in the C-lobe [17].

1.2. Anion binding to hTF

It is well documented that synergistic anion binding is an absolute requirement for high affinity Fe^{3+} binding by hTF [18]. Although carbonate is the physiologically relevant synergistic anion [19], other molecules (oxalate, glycolate, malonate, etc.) can substitute for carbonate to promote high affinity Fe^{3+} binding to hTF [20]. Synergistic anions appear to follow an interlocking site model [20] in which the anion contains a carboxylate group available to bind the anchoring arginine residue in each lobe of hTF, as well as a proximal electron donor group 1–2 carbon atoms away to complete the distorted octahedral coordination of the Fe^{3+}[21].

Along with synergistic anion binding, non-synergistic anions also bind to hTF. By definition non-synergistic anions do not facilitate Fe^{3+} binding to hTF, but bind to other sites. As detailed by Folajtar and Chasteen [22] the binding of non-synergistic anions to hTF follows the lyotropic series ($SCN^- > ClO_4^- > PP_i > ATP > Cl^- \gg BF_4^-$) and is suggested to induce structural changes that perturb the Fe^{3+} binding