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Review

Anion binding properties of the transferrins. Implications for function ☆

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ABSTRACT

Background: Since the transferrins have been defined by the highly cooperative binding of Fe^{3+} and a carbonate anion to form an Fe–CO_3–Tf ternary complex, the focus has been on synergistic anion binding. However, there are other types of anion binding with both apotransferrin and diferric transferrin that affect metal binding and release.
Scope of review: This review covers the binding of anions to the apoprotein, as well as the formation and structure of Fe–anion–transferrin ternary complexes. It also covers interactions between ferric transferrin and non-synergistic anions that appear to be important *in vivo*.
General significance: The interaction of anions with apotransferrin can alter the effective metal binding constants, which can affect the transport of metal ions in serum. These interactions also play a role in iron release under physiological conditions.
Major conclusions: Apotransferrin binds a variety of anions with no special selectivity for carbonate. The selectivity for carbonate as a synergistic anion is associated with the iron binding reaction. Conformational changes in the binding of the synergistic carbonate and competition from non-synergistic anions both play a role in intracellular iron release. Anion competition also occurs in serum and reduces the effective metal binding affinity of Tf. Lastly, anions bind to allosteric sites (KISAB sites) on diferric transferrin and alter the rates of iron release. The KISAB sites have not been well-characterized, but kinetic studies on iron release from mutant transferrins indicate that there are likely to be multiple KISAB sites for each lobe of transferrin. This article is part of a Special Issue entitled Transferrins: Molecular mechanisms of iron transport and disorders.

1. Introduction

In 1949 Schade et al. [1] reported that carbonate was tightly bound along with Fe^{3+} to the serum iron transport protein transferrin (Tf). Subsequent studies showed the same concomitant binding of a carbonate anion and ferric ion for lactoferrin (Lf), which is found in milk, tear and other secretions [2], and for ovotransferrin (ovoTf), which is found in avian egg white [3]. Both Lf and ovoTf appear to serve primarily as antibacterial agents by restricting the supply of free iron [4]. The lactoferrin in breast milk also has a role in iron absorption in the neonate [5]. A fourth protein, melanotransferrin, is a membrane-bound protein expressed by melanoma cells [5]. It binds only one iron and does not appear to be involved in iron metabolism [5]. Melanotransferrin is not included in this review, so hereafter, the term "transferrins" is used to refer to Tf, Lf, and ovoTf.

Following the discovery of serum transferrin, there was an extended controversy as to whether or not the protein was capable of binding iron in the absence of carbonate [6–8]. The difficulty was distinguishing between high-affinity, stoichiometric binding along with carbonate at a specific metal binding sites versus hydrolysis/non-specific binding of Fe^{3+}. Eventually Bates and coworkers established conclusively that carbonate, now referred to as the synergistic anion, was required for specific iron binding to transferrin [9,10].

Transferrin is a bilobal protein, as shown in Fig. 1. There is one high affinity Fe-binding site in each of the lobes, which are designated as C-terminal and N-terminal. The structure in Fig. 1 shows the location of the ferric ions and the carbonate anions within a deep cleft between two rigid domains in each of the lobes. The removal of the ferric ion results in a large scale conformational change that rotates the two domains away from one another by about 60° to produce the "open" form of the apoprotein [11,12]. The first coordination sphere of the ferric ion in the N-terminal lobe of human Tf is shown in Fig. 2. The

Abbreviations: Tf, serum transferrin; ovoTf, ovotransferrin; Lf, lactoferrin; Fe_C–Tf, monoferric transferrin in which the iron is located exclusively in the C-terminal lobe of the protein; Fe_N–Tf, monoferric transferrin in which the iron is located exclusively in the N-terminal lobe of the protein; Tf_{2N}, recombinant protein consisting of only the N-lobe of wild type transferrin; $ovoTf_{2N}$, recombinant protein consisting of only the N-terminal lobe of ovoTf; Lf_{2N}, recombinant protein consisting of only the N-terminal lobe of lactoferrin; KISAB site, kinetically significant anion binding site; SBP, sulfate binding protein; PBP, phosphate binding protein; NTA, nitrilotriacetic acid; EDTA, ethylenediaminetetraacetic acid; hepes, 4(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; MDP, methylenediphosphonic acid; EDP, ethylenediphosphonic acid; DPG, N,N-bis(phosphonomethyl)glycine; NTP, nitrilotris(methylenephosphonic acid); PAA, phosphonoacetic acid; EPR, electron paramagnetic resonance; TFR, full-length transferrin receptor; sTFR, soluble portion of transferrin receptor

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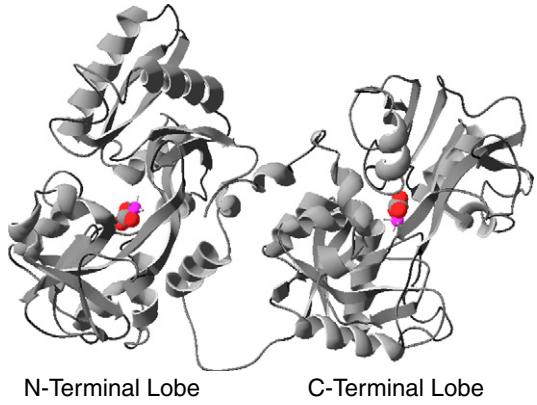

Fig. 1. Overall structure of diferric serum transferrin, showing the overall folding of the two lobes and the locations of the ferric ions and the synergistic carbonate anion. Based on coordinates for porcine transferrin (PDB ID: 1H76) from Ref. [13].

iron ligands are the same for both the C-terminal and N-terminal lobes of all the transferrins, consisting of the side chains of two tyrosines, one histidine, one aspartic acid. The synergistic carbonate binds as a bidentate ligand. There are additional carbonate–protein interactions which are discussed in detail in Section 3.1

The unique requirement for the synergistic carbonate anion to bind Fe^{3+} has been the defining feature of the transferrin family of proteins. The requirement for a synergistic carbonate to form a stable M–CO_3–Tf ternary complex applies to other metal ions as well [7]. The only exceptions appear to be the binding of vanadyl (VO^{2+}) [15] and vanadate ($H_2VO_4^-$ at physiological pH) [16], where one can argue that an oxo group on the vanadium may be acting as a replacement for the carbonate anion.

The role of anions in transferrin chemistry has expanded considerably beyond the role of carbonate to form M–CO_3–Tf ternary complexes. Other anions, particularly small carboxylic acids, can also function as synergistic anions in the absence of carbonate [10,17]. Other inorganic anions bind to apotransferrin [18–20], but do not act as synergistic anions to promote metal binding [10,21]. Lastly, anions appear to bind to allosteric sites on ferric transferrin and alter the rates of iron release from transferrin to different chelating agents [22–26]. The study of these interactions has been aided enormously by the availability of numerous crystal structures of transferrin complexes [27] and a large number of site-directed mutants of the recombinant N-lobe half-transferrin molecule [28].

This review is organized around the concept of three distinct classes of anion binding sites. The binding of anions to an "apoTf binding site" to form binary anion-Tf complexes is discussed in Section 2 of this paper. Although it appears that various anions bind in the same general area within the cleft of the protein, the details of the anion-protein interactions likely vary among anions, so it may be an oversimplification to refer to a single apoTf binding site. The discussion in Section 2 involves no metal ions and makes no distinction among synergistic and non-synergistic anions.

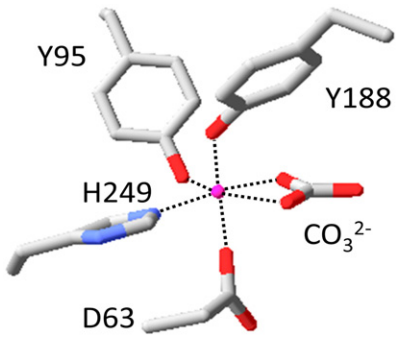

Fig. 2. Inner coordination sphere of ferric ion in the N-lobe binding site of human serum transferrin. Based on coordinates (PDB ID: 1A8F) from Ref. [14].

The connections between anion binding and metal binding are discussed in Section 3. This includes the formation of ferric-transferrin complexes with carbonate and other synergistic anions, and the structure of the synergistic anion binding site. Although the synergistic anion binding site is in the same area as the apoTf binding site, they are treated as two distinct entities. Since the synergistic anion is also bound to the iron, the model used here is that the synergistic anion binding site is created only when the iron binds.

Section 4 discusses the interrelationship between the anion-binding and metal-binding equilibria. This section also discusses the competition between the binding of metal ions and the binding of non-synergistic anions, and the role such interactions may play in intracellular iron release from Tf.

Section 5 discusses a third type of anion binding that involves an allosteric binding site on the diferric protein. Anion binding to this site affects the rate of iron release from the protein, presumably by altering the dynamics of the conformational change between the open and closed conformations Tf. This site has not been structurally characterized. Its existence has been inferred from the observed effects of different anions on reaction rates, and it is now commonly referred to the kinetically significant anion binding (KISAB) site. Section 5 will review the spectroscopic data on anion binding to ferric transferrin and kinetic studies on site-directed mutants that provide some indication of the possible location of the KISAB site(s).

2. Anion binding to apotransferrin

For many years, it was common to describe the binding of the metal ion and the carbonate anion as completely cooperative, i.e. neither the anion nor the metal ion would bind in the absence of the other [6,29]. However, data had appeared as early as 1970 that indicated that anions could bind in the absence of metal ions. Azari and Phillips [30] reported that the treatment of apo-ovoTf with periodate completely deactived iron binding by selectively oxidizing 3 to 5 tyrosine residues. Under similar conditions, no tyrosines of diferric ovoTf were oxidized. The implication was that periodate was binding as an anion near the tyrosine residues that were involved in metal binding. Hsuan [31] showed that the periodate inactivation could be inhibited by the addition of other anions that compete with iodate for the anion-binding site on the protein.

Zweier et al. [32] measured the ^{13}C NMR spectra of apotransferrin in the presence of a large excess of $H^{13}CO_3^-$, and detected two peaks that they assigned as protein-bound carbonate. Kojima and Bates [33] studied the rate of ferrous ion binding and oxidation to form ferric transferrin. They observed a hyperbolic relationship between the rate of iron binding and the bicarbonate concentration, which indicated that ferrous ion was binding to a binary carbonate-apoTf complex that was formed in a rapid pre-equilibrium between apoTf and free carbonate.

2.1. Difference UV studies of anion binding

Harris and coworkers discovered that the binding of inorganic anions such as phosphate and bicarbonate to apoTf could be easily and directly detected by difference UV spectroscopy [18,19]. Difference UV spectroscopy had already been used to detect metal-binding to apoTf [34–37], and had been developed into a method for measuring metal-protein binding constants [38,39]. In this method, the sample and reference cuvettes are filled with identical apoTf solutions and used to set a flat baseline. The metal ion is then added in small aliquots. Binding to the tyrosine residues of perturbs the UV spectrum of the aromatic ring, which produces peaks (positive and negative) in the difference spectrum. Metal binding produces a consistent pattern of a