Review

The Structural Mechanism for Iron Uptake and Release by Transferrins

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Transferrins are a group of iron-binding proteins that control the levels of iron in the body fluids of vertebrates by their ability to bind two Fe$^{3+}$ and two CO$^{2-}$ ions. The transferrin molecule, with a molecular mass of about 80 kDa, is folded into two similarly sized homologous N- and C-lobes that are stabilized by many intrachain disulfides. As observed by X-ray crystallography, each lobe is further divided into two similarly sized domains, domain 1 and domain 2, and an Fe$^{3+}$-binding site is within the interdomain cleft. Four of the six Fe$^{3+}$ coordination sites are occupied by protein ligands (2 Tyr residues, 1 Asp, and 1 His) and the other two by a bidentate CO$_2^2$$. Upon uptake and release of Fe$^{3+}$, transferrins undergo a large-scale conformational change depending on a common structural mechanism: domains 1 and 2 rotate as rigid bodies around a rotation axis that passes through the two antiparallel $\beta$-strands linking the domains. The extent of the rotation is, however, variable for different transferrin species and lobes. As a Fe$^{3+}$ release mechanisms at low pH from the N-lobes of serum transferrin and ovotransferrin, the structural evidence for ‘dilysine trigger mechanism’ is shown. A structural mechanism for the Fe$^{3+}$ release in presence of a non-synergistic anion is proposed on the basis of the sulfate-bound apo crystal structure of the ovotransferrin N-lobe. Domain-opened structures with the coordinated Fe$^{3+}$ by the two tyrosine residues are demonstrated in fragment and intact forms, and their functional implications as a possible intermediate for iron uptake and release are discussed.

Key words: transferrin; ovotransferrin; lactoferrin; structural mechanism; iron uptake and release

Transferrins are a group of iron-binding proteins that includes serum transferrin, ovotransferrin, and lactoferrin. These proteins serve to control the levels of iron in the body fluids of vertebrates by their ability to bind very tightly two Fe$^{3+}$ ions together with two CO$^{2-}$ ions. Serum transferrin acts as an Fe$^{3+}$ transporter to target cells. The transferrin-dependent Fe$^{3+}$ delivery to the target cells occurs in such a way that the dfferent transferrin first binds with the specific receptor that resides on the plasma membrane. The transferrin-receptor complex is then internalized into the cell and the complex releases Fe$^{3+}$ in the endosome at acidic pH. Ovotransferrin, a major egg white protein, should share the same structural characteristics as avian serum transferrin, which functions as an iron transporter, since these proteins are derived from the same gene and they differ only in their attached carbohydrate. Although an iron transport function for a developing chick embryo has not been proved, specific transferrin-receptor interactions have been demonstrated for hen egg white ovotransferrin. Aside from physiological function, ovotransferrin has implications also for the food functionalities of hen egg white, because of its low thermostability in the Fe$^{3+}$-free apo form. Lactoferrin exists in white blood cells and in secretary fluids including milk and saliva. Lactoferrin, with a non-iron-transporting nature, has a variety of physiological functions such as an antimicrobial activity independent of iron-binding and a sequence-specific DNA binding capacity.

Common properties of transferrins are the capacity to uptake Fe$^{3+}$ together with the physiological synergistic anion CO$_2$ with a very strong affinity at near neutral pH, and to release it at acidic pH. The requirement of the presence of a non-synergistic anion for Fe$^{3+}$ release is also a common characteristic of transferrins. The mechanism of the uptake and release of Fe$^{3+}$ have therefore been a central question for the understanding of the transferrin functions. Here I shortly review recent progress in the studies of the structural mechanism for Fe$^{3+}$ uptake and release by transferrins.

1. Overall Conformation and Disulfide Structures

The transferrin molecule consists of a single polypeptide chain with a molecular mass of about 80 kDa and folded into two similarly sized homologous N- and C-lobes. The two lobes are connected by a single bridge peptide segment. Each lobe is further divided into two similarly sized domains, domain 1 and domain 2 (domain N1 and N2 in the N-lobe; domain C1 and C2 in the C-lobe). An Fe$^{3+}$-binding site is located within the interdomain cleft of domain 1 and domain 2 of each lobe. The whole transferrin molecule has therefore the capacity to bind two Fe$^{3+}$ ions. As shown in Figure 1, domain 2 consists of a contiguous polypeptide segment, but domain 1 is comprised of two polypeptide segments interrupted by domain 2. These structural situations lead to the presence of two hinge segments that connect the two domains.
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A) Stereo Co plot of holo-ovotransferrin. N1 and N2 represent domains N1 and N2 and C1 and C2, domains C1 and C2, respectively. White balls are bound Fe$^{3+}$. The figure was produced using the crystallographic data with MOLSRIPT.

B) The figure represent the case for ovotransferrin that contains only the consensus disulfides of transferrins either in N- or C-lobe. The numbers represent the disulfide bonds: 1. Cys10-Cys45 (N-lobe) and Cys348-Cys380 (C-lobe); 2. Cys20-Cys36 and Cys358-Cys371; 3. Cys115-Cys197 and Cys454-Cys530; 4. Cys160-Cys174 and Cys488-Cys502; 5. Cys171-Cys182 and Cys495-Cys513; 6. Cys228-Cys242 and Cys570-Cys584; 7. Cys478-Cys502; 8. Cys421-Cys643; 9. Cys405-Cys680. Iron is coordinated by the side chain residues: D, Asp60 and Asp395; Y, Tyr92, Tyr191, Tyr431 and Tyr524; H, His250 and His592. The C-terminus of N-lobe and the N-terminus of C-lobe are connected by a bridge peptide segment (not shown). The regions shaded heavy and light represent, respectively, domain I and domain 2. The open regions correspond to hinges that connect the two domains.

The folded structure is stabilized by intrachain disulfide bonds. The distribution of disulfide bonds is conserved well for the three transferrins. The transferrins contain six consensus disulfide bonds in N-lobe: two (SS-1 and SS-2) in domain N1 and four (SS-3 to SS-6) in domain N2. In C-lobe, three consensus disulfides (SS-7, SS-8, and SS-9) exist in addition to the six disulfide motif; SS-7 is the only interdomain crosslink, and SS-8 and SS-9 crosslink the two non-contiguous subdomains of domain C1. Holo-ovotransferrin contains only the consensus disulfides in either N- or C-lobe (Fig. 1). Likewise, the N-lobe of human lactotransferrin consists of the six consensus disulfide only, but its C-lobe contains one additional disulfide in the C-terminal subdomain of domain C1. In the N-lobe of human serum transferrin, two disulfides are present along with the six consensus disulfides; one is the interdomain SS-7 and the other locates in close proximity to SS-4. The C-lobe of this transferrin contains two disulfide in addition to the nine consensus disulfides. On the basis of the disulfide distribution along with sequence homology, it has been proposed that transferrin lobes are proteins evolved after gene duplication from a common ancestor resembled the N-lobe of present-day ovotransferrin.

About the structural and functional roles of the disulfide bonds, only a limited amount of data is available. When all the six disulfides of ovotransferrin N-lobe are reduced in strong reducing conditions, the protein assumes a partially folded non-native conformation. Under less drastic disulfide-reducing conditions, the apo form of ovotransferrin N-lobe is first transformed into a four-disulfide intermediate in which SS-4 and SS-5 are selectively reduced. This four-disulfide intermediate retains
essentially the same Fe$^{3+}$-binding function as the disulfide-intact protein. Further reduction yields a three-disulfide form (SS-3 reduced) and then a two-disulfide form (SS-6 reduced); both the forms have a decreased native conformation and no iron binding capacity. These data strongly suggest that the four consensus disulfide bonds (SS-1, SS-2, SS-3, and SS-6) other than SS-4 and SS-5 are essential for the iron binding function of transferrin.

When the Fe$^{3+}$-loaded whole molecule of ovotransferrin is reduced by dithiothreitol, SS-7 (Cys478 and Cys671) in the C-lobe is selectively cleaved. The reduced protein shows lower iron-binding stability than the disulfide-intact protein, suggesting the involvement of the interdomain disulfide for iron-binding stability.

2. Apo and Holo Structures and Domain Movements

Crystal structures of the fully iron-loaded diferric forms and the monoferic N-lobes of several transferrins show that the two domains of each lobe are closed over an Fe$^{3+}$ ion (Fig. 1). Four of the six Fe$^{3+}$ coordination sites are occupied by protein ligands (2 Tyr residues, 1 Asp and 1 His) and the other two by a bidentate carbonate anion. Because of the closed domain structure of the holo forms, it has been generally believed that the two domains of each transferrin lobe must open to allow entry or release of Fe$^{3+}$. Indeed, X-ray solution scattering studies have shown iron-induced conformational changes in both lobes of several transferrins. The crystal structure of the apo form of transferrin has been first solved for human lactoferrin at 2.8 Å resolution and recently refined at 2.0 Å resolution. In that structure the iron-binding cleft in the N-lobe is wide open when compared to the closed holo-structure. As schematically shown in Fig. 2, upon uptake or removal of iron, domains N1 and N2 rotate 54° as rigid bodies around a rotation axis that passes through the two antiparallel β-strands linking the domains. This mode of the domain movement is a typical example of "double-hinged β-sheet" for the domain motion mechanism of proteins. As summarized in Table 1, the domain movements of serum transferrin and ovotransferrin occur by essentially the same mechanism except for differential extents of domain opening.

The C-lobe of human lactoferrin is however found in the closed conformation in both the holo and apo forms. This unexpected lack of conformational change may be related to the presence of an interdomain disulfide bridge SS-7 (Cys483-Cys677) in the C-lobe of lactoferrin. Recent crystal structure data of apo ovotransferrin, however, demonstrates an open conformation for both the N- and C-lobes. The interdomain crosslink SS-7 is located in an equivalent structural site in hen ovotransferrin (Cys478-Cys671) and in the lactoferrin C-lobe. The domain opening observed in the ovotransferrin C-lobe makes it unlikely that the interdomain disulfide is the major force preventing domain opening in the lactoferrin C-lobe. This conclusion is also supported by the apo crystal structure with an open conformation of the N-terminal half-molecule of serum transferrin, which contains an equivalent interdomain disulfide SS-7 (Cys137-Cys331) in N-lobe. Recent crystallographic analyses of mare lactoferrin show that both the N- and C-lobes are in the closed conformation either in the apo or holo form. For iron-transporter transferrins, the Fe$^{3+}$-loaded holo-form
shows a much higher affinity for the transferrin receptor than does the apo-form, strongly suggesting that iron-dependent conformational change is important for the cellular uptake of iron.\(^4\)\(^-\)\(^4\)\(^8\) Lactoferrin might lack, because of its non-iron-transporting nature, an open-close mechanism that may be important for the selective recognition of the holo-form by the receptor. It has been postulated that an equilibrium exists between the open and closed forms of apo lactoferrin in solution, and that the observed structure is selected by crystal packing.\(^3\)\(^1\)\(^7\)

### 3. Iron Release Mechanism

The rate for iron release from transferrin is accelerated by several factors, including endosomal low pH,\(^46\)\(^-\)\(^48\) association with the specific receptor at acidic pH,\(^45\) and binding of simple anions.\(^46\)\(^-\)\(^48\)

\textit{In vivo}, Fe\(^3+\) is released from transferrin in the endosome at acidic pH (pH 5.6). The apo and holo structure of the iron-transporter transferrins reveals that upon iron release the conformational change from the closed to open domain forms should be involved. As an iron release mechanism at low pH from the N-lobes of ovotransferrin and serum transferrin, Dewan \textit{et al}.\(^23\) have proposed the protonation mechanism of 'dilysine trigger' on the basis of structural finding: the two lysine residues Lys209-NZ and Lys301-NZ, which are located on the opposite domains of hen ovotransferrin N-lobe, are separated by 2.3 Å only in the holo structure. The interaction of the two lysine residues is abolished in the iron-released, domain-opened apo structure: Lys209-NZ to Lys301-NZ distance is 8.5 Å apart in this form.\(^49\) The dilysine trigger mechanism has been supported by the findings of the close proximity of Lys206-NZ...Lys296-NZ in serum transferrin N-lobe\(^50\) and of Lys209-NZ...Lys301-NZ in duck ovotransferrin N-lobe.\(^23\) The functional evidence that the rate for the iron release from human serum transferrin N-lobe is much slower for the dilysine mutants than for the wild protein counterpart\(^49\)\(^-\)\(^50\) further supports the mechanism.

The iron-binding stability at low pH of lactoferrin is much greater than serum- and ovotransferrins, and these iron-transporter transferrins generally show greater iron-binding stability at low pH in the C-lobe than in the N-lobe. The clear dilysine trigger structure is not found in the N- and C-lobes of lactoferrin and in the C-lobe of ovotransferrin. One of the two lysine residues is replaced by a different residue in the C-lobe of hen ovotransferrin\(^23\) (Gln541-OE1...Lys638-NZ), in the N-lobe (Arg210-NH2...Lys301-NZ) and C-lobe (Lys546-NZ...Asn644-ND2) of human lactoferrin,\(^22\) and in the C-lobes of bovine\(^46\) (Lys544-NZ...Asn642-OD1) and mare\(^25\) lactoferrin (Lys544-NZ...Asn642-ND2). Although the combination of two lysine residues can be found in the C-lobe of duck ovotransferrin\(^23\) (Lys541-NZ...Lys638-NZ) and in the N-lobes of bovine\(^26\) (Lys210-NZ...Lys301-NZ) and mare\(^25\) lactoferrin (Lys210-NZ...Lys301-NZ), no close interaction occurs in these lobes, indicating the absence of the dilysine trigger mechanism. The structural features for the dilysine trigger site are, therefore, consistent with the lower iron-binding stability in the N-lobe of iron-transporter transferrins.

As an alternative factor, simple anions have been demonstrated to facilitate iron release from ferric transferrins \textit{in vitro}.\(^46\)\(^-\)\(^48\) The requirement of anion for the Fe\(^3+\) release \textit{in vitro} has also been demonstrated for the Fe\(^3+\)-transferrin-receptor complex,\(^22\) suggesting the participation of some anion in the cellular Fe\(^3+\) release mechanism. Structural evidence for the anion binding sites has been first demonstrated for ovotransferrin N-lobe by crystallographic analysis using apo crystal, grown in ammonium sulfate solution.\(^53\) By the electron density map, the existence of
three bound SO$_4^{2-}$ anions has been clearly detected with reasonably low B-factors in the opened inter-domain cleft. Iron release from the ovotransferrin N-lobe is accelerated by a variety of simple anions including sulfate anion.\textsuperscript{50} Two of the binding sites (site-1 and site-2) may be directly implicated for the anion-dependent Fe$^{3+}$-release mechanism. The surface accessibilities of the protein groups of these sites are greatly increased by domain opening. It is therefore very likely that the anion bindings to the two sites stabilize the open conformation. Furthermore, site-1 and site-2 include the protein groups that make functionally important interactions in transferrins.

Site-1 includes Ser 91-OG and His 250-NE2. The latter protein group is a consensus Fe$^{3+}$-coordinating ligand.\textsuperscript{20-29} Furthermore, both Ser 91 and His 250 are localized in the hinge strands\textsuperscript{25} which can undergo a conformational change upon domain opening and closure. The occupation of site-2 by anion may facilitate both the domain opening and carbonate anion release. Site-2 comprises Ser 122-N, Arg 121-NE, and Arg 121-NH2. Arg 121-NE and Arg 121-NH2 are consensus CO$_3^{2-}$-anchor groups for holo transferrins.\textsuperscript{20-29} Anion binding to site-2 may therefore facilitate the release of the synergistic carbonate anion from transferrin. A protein group, Ser 122-N, forms a hydrogen bond in the holo structure\textsuperscript{27} with an oxygen atom (OD1) of Asp 60; the Asp residue is the only iron-coordinating ligand from domain 1 (see Fig. 1) and plays a central role in the domain opening and closure.\textsuperscript{55} The anion binding to site-2 should, therefore, disrupt the Ser 122-N and Asp 60 interaction, facilitating domain opening. Taken together, it can be reasonably hypothesized that the cooperative anion binding to site-1 and site-2 induces a domain opening and carbonate anion release, and hence iron release.

4. Alternative Iron-loaded Structures as Functional Intermediates

On the basis of X-ray crystallographic (see Table 1) and solution scattering\textsuperscript{29-36} analyses, it has been a consensus structural feature of iron-transporter transferrins that upon Fe$^{3+}$ uptake and release, the proteins undergo a large-scale conformational transition between the domain-opened apo and closed holo structures. This implies the presence of iron-loaded, domain-opened form as an intermediate for Fe$^{3+}$ uptake and release. Differential domain and hinge locations of the four protein ligands (see Fig. 1 for ovotransferrin N-lobe: Asp 60 in the domain 1, Tyr 191 in the domain 2, and Tyr 92 and His 250 in different hinges) inevitably require an alternative Fe$^{3+}$ coordination structure for the Fe$^{3+}$-loaded, domain-opened intermediate.

Attempts have been made to isolate a proteolytically derived fragment that may mimic the intermediate structure and to analyze its functional implications by a biochemical approach.\textsuperscript{56,57} As the first clue by X-ray crystallography, Lindley \textit{et al.} prepared an 18-kDa polypeptide fragment by limited proteolysis of duck ovotransferrin and solved its structure at 2.3 Å resolution.\textsuperscript{53} The fragment retains Tyr 92 and Tyr 191 ligands but lacks Asp 60 and His 250 ligands, and hence it corresponds to a protein segment comprising domain 2 and one of the two hinge strands. The crystal structure revealed that Fe$^{3+}$ is coordinated by Tyr 92 and Tyr 191 residues and CO$_3^{2-}$ in the same way except for the absence of the coordination by Asp 60 and His 250 ligands as in the canonical holo structure as shown in Table 2. This led them to hypothesize the participation of the two Tyr residues for the iron binding ligands of the intermediate.

The participation of the two Tyr residues as the iron-binding ligand in the intermediate has been proved by the crystallographic analysis of an intact lobe molecule.\textsuperscript{38} When an apo crystal of ovotransferrin N-lobe is soaked with Fe$^{3+}$-nitrilotriacetate complex (Fe$^{3+}$-NTA), the soaked form shows almost exactly the same overall open structure as the iron-free apo form but the presence of an iron atom with the coordination by the two protein ligands of Tyr 92-OH and Tyr 191-OH (Table 2). Other Fe$^{3+}$ coordination sites are occupied by nitrilotriacetate (NTA) anion, which is stabilized through the hydrogen bonds with the peptide NH groups of Ser 122, Ala 123, and Gly 124 and a side chain group of Thr.

| Table 2. Fe$^{3+}$-Coordinating and Anion-binding Residues in the Iron-loaded Forms of Transferrin N-Lobe |
|----------------|-----------------|----------------------|-----------------|
| Transferrin forms | Domain conformation | Iron-coordinating ligand residue* | Synergistic anion binding residue** | PDB code (Reference) |
| ovotransferrin | | | | |
| Holo form | Closed | Asp60/Tyr92/Tyr191/His250/CO$_3^{2-}$ | Thr117/Arg121/Ala123/Gly124 | INNT(27) |
| Iron-soaked form | Open | Tyr92/Tyr191/NTA | Thr117/Ser122/Ala123/Gly124 | INF(58) |
| 18-kDa domain 2 fragment | — | Tyr92/Tyr191/CO$_3^{2-}$ | Thr117/Arg121/Ala123/Gly124 | I0VB(32) |
| human serum transferrin | | | | |
| Conformer-A | Closed | Asp63/Tyr95/Tyr188/His249/CO$_3^{2-}$ | Thr120/Arg124/Ala126/Gly127 | IAS/1AF |
| Conformer-B | Closed | Asp63/Tyr95/Tyr188/His249/CO$_3^{2-}$ | Thr120 or Thr120/Arg124 | (29) |

* Iron-coordinating groups are Asp-OD2, Tyr-OH, His-NE. For the coordination by anion, two oxygen atoms of CO$_3^{2-}$ or three oxygen and one nitrogen atoms of NTA participated.

** The protein groups interacting with anion are side chain groups of Thr-OG1, Arg-NE, and Arg-NH2, and main chain groups of Ala-N, Gly-N, and Ser-N.
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117. There is, however, no clear interaction between the NTA anion and the synergistic anion binding site, Arg 121.\(^{18}\)

For loading \(\text{Fe}^{3+}\) onto apo transferrin (apo-Trf), a ferric chelate, most widely \(\text{Fe}^{3+}\)-NTA complex, is used.\(^{3}\) The binding reaction yields a ternary complex consisting of transferrin, \(\text{Fe}^{3+}\) and NTA molecules (Trf-\(\text{Fe}^{3+}\)-NTA) in the absence of another synergistic anion such as carbonate anion:\(^{13}\)

\[
\text{apo-Trf} + \text{Fe}^{3+}\)-NTA ⇔ Trf-\(\text{Fe}^{3+}\)-NTA
\]

The Trf-\(\text{Fe}^{3+}\)-NTA complex is a stable form. When a high concentration of bicarbonate is added, however, NTA is replaced by \(\text{CO}_3^{2-}\); this reaction yields the physiological holo form consisting of transferrin, \(\text{Fe}^{3+}\), and \(\text{CO}_3^{2-}\) (Trf-\(\text{Fe}^{3+}\)-\(\text{CO}_3^{2-}\)):\(^{14}\)

\[
\text{Trf-Fe}^{3+}\)-NTA + \text{CO}_3^{2-} ⇔ \text{Trf-Fe}^{3+}\)-\(\text{CO}_3^{2-}\) + NTA
\]

Conversely, when a high concentration of NTA is added to the holo form (Trf-\(\text{Fe}^{3+}\)-\(\text{CO}_3^{2-}\)), ion is released,\(^{20}\) probably according to the reverse reactions. It is therefore very likely that the \(\text{Fe}^{3+}\)-soaked structure corresponds to an intermediate structure for the uptake and release of iron. Arg 121-NE and \(\text{NH}_2\), which are the anchoring sites for \(\text{CO}_3^{2-}\) in the holo form, are both vacant in the \(\text{Fe}^{3+}\)-soaked form (Table 2). Upon the addition of bicarbonate in the second reaction, therefore, an initial entry of \(\text{CO}_3^{2-}\) into the Arg 121 anchor site and then the total replacement of NTA by \(\text{CO}_3^{2-}\) would occur. This reaction should yield a short-lived Trf-\(\text{Fe}^{3+}\)-\(\text{CO}_3^{2-}\)-NTA complex with the open conformation, in which only four of the six \(\text{Fe}^{3+}\)-coordination sites are occupied by Tyr 92 and Tyr 191 and bidentate \(\text{CO}_3^{2-}\) as in the 18 kDa domain 2 fragment structure (Table 2).

Altered synergistic-anion binding structures have also been found in the two different crystals (orthorhombic and tetragonal) of the iron-loaded N-lobe of recombinant human serum transferrin.\(^{20}\) Both the crystals consist of two conformers, A and B, with different structural states of synergistically bound carbonate anion. The conformer A assumes the canonical \(\text{Fe}^{3+}\) coordinating and synergistic anion binding structure of the holo transferrins: the \(\text{Fe}^{3+}\) coordinating carbonate anion makes hydrogen bonds with the side chains of Thr 120 and Arg 124 and with the main chain nitrogen atoms of Ala 126 and Gly 127 (Table 2). In the conformer B, however, the carbonate anion is rotated 30° relative to \(\text{Fe}^{3+}\) and the interactions of the anion to the main chain nitrogens are abolished (Table 2). The authors of the paper\(^{20}\) have pointed out that this structure corresponds to the protonation of the carbonate and resulting partial removal of the anion as an intermediate prior to cleft opening and \(\text{Fe}^{3+}\) release.

5. Conclusion

The overall and \(\text{Fe}^{3+}\) coordinating structures are almost indistinguishable for the holo forms of serum transferrin, ovotransferrin, and lactoferrin. However, the extent of domain movement upon the uptake and release of \(\text{Fe}^{3+}\) and also the structural mechanism for \(\text{Fe}^{3+}\) release at acidic pH are not identical for different transferrins and lobes. Previous kinetic studies have revealed that both the \(\text{Fe}^{3+}\) uptake and release pathways include multiple reaction steps and hence the multiple structural states as reaction intermediates.\(^{54,59,60}\) For better understanding the structural mechanism of \(\text{Fe}^{3+}\) uptake and release, therefore, the analysis of the three-dimensional structures of many intermediates should be crucial for variety of transferrin species. Although the previous crystal structures of mutant transferrins for \(\text{Fe}^{3+}\) coordinating\(^{61,62}\) and synergistic anion binding\(^{63}\) residues show the canonical holo-like closed conformations, the site-directed mutagenesis should no doubt be one of the most promising approaches for producing many stable structural states that mimic the reaction intermediates. On the basis of the accumulated structural and functional evidence, mutations at variety of transferrin sites would be reasonably designed for preparing useful protein forms. For the cellular \(\text{Fe}^{3+}\) transport and release mechanism, the transferrin receptor plays a central role. The crystal structure of the ectodomain of the receptor that has been solved very recently\(^{64,65}\) should provide a crucial hint for the transferrin mechanism in vivo.

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