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Receptor-Modulated Iron Release from Transferrin: Differential Effects on N- and C-Terminal Sites†

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ABSTRACT: Iron release to PP_i from N- and C-terminal monoferric transferrins and their complexes with transferrin receptor has been studied at pH 7.4 and 5.6 in 0.05 M HEPES or MES/0.1 M NaCl/0.01 M CHAPS at 25 °C. The two sites exhibit kinetic heterogeneity in releasing iron. The N-terminal form is slightly less labile than its C-terminal counterpart at pH 7.4, but much more facile in releasing iron at pH 5.6. At pH 7.4, iron removal by 0.05 M pyrophosphate from each form of monoferric transferrin complexed to the receptor is considerably slower than from the corresponding free monoferric transferrin. However, at pH 5.6, complexation of transferrin to its receptor affects the two forms differently. The rate of iron release to 0.005 M pyrophosphate by the N-terminal species is substantially the same whether transferrin is free or bound to the receptor. In contrast, the C-terminal form releases iron much faster when complexed to the receptor than when free. Urea/PAGE analysis of iron removal from free and receptor-complexed diferric transferrin at pH 5.6 reveals that its C-terminal site is also more labile in the complex, but its N-terminal site is more labile in free diferric transferrin. Thus, the newly discovered role of transferrin receptor in modulating iron release from transferrin predominantly involves the C-terminal site. This observation helps explain the prevalence of circulating N-terminal monoferric transferrin in the human circulation.

The predominant pathway for uptake of iron by most vertebrate cells, receptor-mediated endocytosis of transferrin, involves two glycoproteins: transferrin and the transferrin receptor (Klausner et al., 1983; Dautry-Varsat et al., 1983). Transferrin, which functions to transport iron in the circulation, consists of a single 80-kDa polypeptide chain arranged in a bilobal structure. Each lobe is comprised further of two domains surrounding a cleft bearing a specific high-affinity iron-binding site (Anderson et al., 1987; Bailey et al., 1988). Although similar, the two sites differ in accessibility to iron chelates, binding strength, and spectroscopic properties (Aisen et al., 1978); kinetic lability (Baldwin & de Sousa, 1981; Bali

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& Harris, 1989); and response to changes in pH and salt concentration (Baldwin & de Sousa, 1981; Thompson et al., 1986a). The transferrin receptor, which functions to capture iron-bearing transferrin from the circulation for internalization by the cell, is a dimer of two identical 95-kDa subunits (Hu & Aisen, 1978; Seligman et al., 1979; Enns & Sussman, 1981; McClelland et al., 1984), linked by a pair of disulfide bonds (Jing & Trowbridge, 1987). The receptor bears complex and high-mannose oligosaccharides in N- and O-linkage (Omary & Trowbridge, 1981a; Do et al., 1990), which may serve to keep the transferrin-binding regions exposed to transferrin. Stable binding of transferrin by receptor appears to depend on its carbohydrate moieties (Reckhow & Enns, 1988). The transferrin-binding site of each subunit is located in a proteolytically cleavable 70-kDa fragment (Omary & Trowbridge, 1981b; Turkewitz et al., 1988a).

At extracellular pH, 7.4, iron-loaded transferrin binds strongly to its receptor on the plasma membrane. Iron-free transferrin has a much lower affinity for receptor at this pH so that it fails to compete effectively with diferric transferrin for binding to receptor (Young et al., 1984; Tsunoo & Sussman, 1983). The complex of transferrin and its receptor is internalized via a clathrin-coated pit into an endocytotic vesicle (Hanover et al., 1985) which matures into a proton pumping endosome (Klausner et al., 1983). The proton pump lowers the pH within the transferrin-bearing endosomes, thereby facilitating the dissociation of iron from transferrin but leaving iron-depleted transferrin still bound to receptor at the low pH of the endosome. Finally, the endosome returns to the cell surface where iron-depleted transferrin, upon encountering pH 7.4, is freed from the receptor. Both proteins then become available for a new cycle of iron transport.

One problem with this simple overview of receptor-mediated endocytosis is that the entire cycle may be complete in as little as 1–3 min (Aisen, 1983), although spontaneous release of iron from diferric transferrin requires hours at pH 5.5 (Foley & Bates, 1988). Thus, some mechanism in addition to lowering of pH must be available to the cell to facilitate release of iron from transferrin. Evidence for a role of the transferrin receptor in modulating release of iron from transferrin has already been presented in the isolated receptor–transferrin complex (Bali et al., 1991a) and in transferrin bound to cell surface receptors (Sipe & Murphy, 1991). The receptor retards the rate of iron release from diferric transferrin at pH 7.4 and accelerates release at pH 5.6 (Bali et al., 1991a), thereby impeding iron release at the cell surface and promoting iron release within acidified endocytotic vesicles. In this paper we report the differential effects of receptor on the two sites of transferrin.

Materials and Methods

CHAPS[1] was purchased from Sigma Chemical Co., St. Louis, MO. Iron-free human serum apotransferrin (stated purity >98%, CALBIOCHEM Corp., La Jolla, CA) was purified as previously reported (Aisen et al., 1978). The Amersham Corp., Arlington Heights, IL, supplied ^{59}Fe as $^{59}FeCl_3$.

Radiolabeled Transferrins. C-Terminal monoferric (Tf-Fe_C) and diferric (Fe_N-Tf-Fe_C) transferrins were prepared from purified apotransferrin in 0.05 M HEPES/0.1 M NaCl buffer, pH 7.4, by adding the appropriate amount of ^{59}Fe as bis(nitrilotriacetato)ferrate(III) (Bali & Harris, 1990). Released NTA was removed by ultrafiltration as described. N-Terminal monoferric transferrin (Fe_N-Tf) was prepared from diferric transferrin by a published method (Baldwin & de Sousa, 1981), except that 0.05 M HEPES/NaCl buffer was used instead of Tris-HCl. Purity of transferrin preparations was checked by urea/PAGE. Specific activities were 1687, 1736, and 3480 cpm/μg of protein, respectively, for Fe_N-Tf, Tf-Fe_C, and Fe_N-Tf-Fe_C.

Transferrin Receptor. Human transferrin receptor was isolated from freshly frozen placentas by reported procedures entailing detergent solubilization, iron chelation, and affinity chromatography (Turkewitz et al., 1988a; Bali et al., 1991a). Use of human placentas for the isolation of the transferrin receptor was approved by the Committee on Clinical Investigation of the Albert Einstein College of Medicine. The isolation procedure often yielded receptor contaminated with a 70-kDa species, presumably its trypsin-cleavable fragment bearing the transferrin-binding site (Turkewitz et al., 1988a). This was removed by gel filtration chromatography on a Bio-Rad HPLC system using a preparative Bio-Sil TSK-250 column. Purified preparations showed a single band at 95 kDa by SDS–PAGE electrophoresis (Pharmacia Phastgel System), with only a single peak evident on gel filtration chromatography. Receptor concentration was determined from absorbance at 280 nm using a molar absorption coefficient of 360 000 M^{-1} cm^{-1}. This value was estimated with the Pierce Micro-BCA assay; a previously reported value of 250 000 M^{-1} cm^{-1} (Bali et al., 1991a) was found to be in error.

Receptor–Transferrin Complexes. The transferrin-binding activity of receptor preparations was assessed by gel filtration chromatography as described previously (Bali et al., 1991a). For studies of iron release, complexes of receptor with the three species of iron transferrins were isolated from the respective incubation mixtures of transferrin and receptor by gel filtration chromatography (Bali et al., 1991a). SDS–PAGE of these complexes revealed two bands at 95 and 80 kDa, corresponding to the receptor subunit and transferrin, respectively. Gel filtration chromatography of the purified complexes showed superimposable peaks of radioactivity and absorbance at 280 nm, each with a mobility of the transferrin receptor. The Bio-Sil TSK-250 column does not resolve transferrin receptor from the complex of transferrin and receptor; both appear in the excluded volume.

Iron Removal from Monoferric Transferrins. Iron removal from free monoferric transferrins Fe_N-Tf and Tf-Fe_C, and their complexes with receptor [TfR–$(Fe_NTf)_2$] and [TfR–$(TfFe_C)_2$], was studied at pH 7.4 in 0.05 M HEPES/0.1 M NaCl/0.01 M CHAPS and at pH 5.6 in 0.05 M MES/0.1 M NaCl/0.01 CHAPS at 25 ± 1 °C by the method of PEG precipitation (Bali et al., 1991a). Reasonable rates of removal were achieved with 0.05 M PP_i at pH 7.4 and 0.005 M PP_i at pH 5.6. In all experiments, the concentration of free transferrin or transferrin in the receptor–transferrin complex was 0.2 μM.

Iron Removal from Diferric Transferrin. Iron removal from each site of Fe_N-Tf-Fe_C and [TfR–$(Fe_NTfFe_C)_2$] by 0.002 M PP_i at pH 5.6 was analyzed by urea/PAGE electrophoresis. Aliquots of 100 μL were withdrawn at 0, 0.5, 1.0, 1.5, and 3.0 min of incubation time and added to 60 μL of 5× loading

[1] Abbreviations: HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; NTA, nitrilotriacetic acid; PEG, poly(ethylene glycol); BCA, bicinchoninic acid; PAGE, polyacrylamide gel electrophoresis; PP_i, pyrophosphate; SDS, sodium dodecyl sulfate; Tf, human serum iron-free apotransferrin; Fe_N-Tf-Fe_C, diferric transferrin; Fe_N-Tf, N-terminal monoferric transferrin; Tf-Fe_C, C-terminal monoferric transferrin; TfR, transferrin receptor; [TfR–$(Fe_NTfFe_C)_2$], complex of receptor and diferric transferrin; [TfR–$(Fe_NTf)_2$], complex of receptor and N-terminal monoferric transferrin; [TfR–$(TfFe_C)_2$], complex of receptor and C-terminal monoferric transferrin.

Table I: Parameters Characterizing the Removal of Iron from C- and N-Terminal Monoferric Transferrins and Their Complexes with Transferrin Receptor

transferrin species	[PP_i] (mM)	pH	k (min^{-1})	B (%)
Fe_N-Tf	50	7.4	0.065	39
[TfR-(Fe_NTf)$_2$]	50	7.4	0.048	44
Tf-Fe_C	50	7.4	0.058	17
[TfR-(TfFe$_C$)$_2$]	50	7.4	0.055	52
Fe_N-Tf	5	5.6	0.91	40
[TfR-(Fe_NTf)$_2$]	5	5.6	1.01	40
Tf-Fe_C	5	5.6	0.30	50
[TfR-(TfFe$_C$)$_2$]	5	5.6	0.51	30

buffer (0.1 M Tris/0.01 M borate/0.0016 M EDTA/10% sucrose, pH 8.4) precooled to 4 °C in separate tubes. At 4 °C and pH 8.4, the reaction of iron release is effectively quenched (Thompson et al., 1986b). Electrophoresis was then carried out within 30 min by procedures published previously (Bali & Harris, 1990). Since staining with Coomassie blue R 250 produced bands that were too faint for photographic reproduction, silver staining with the Bio-Rad kit was employed to visualize bands.

Data Analysis. Radioactivity of iron bound to PEG-precipitable transferrin as a function of time was converted to a reaction coordinate R_t and normalized:

$$R_t(\text{ppt}) = 100 \times [(A_0 - A_t)/(A_0 - A_\infty)] \quad (1)$$

where A_0 = total radioactivity in transferrin at the start of each experiment, A_t = radioactivity at time t, and A_∞ = background radioactivity. When reactions were evaluated from PEG supernatants, representing iron released to the PP_i, the expression used for obtaining reaction coordinates was the complement to eq 1:

$$R_t(\text{spn}) = 100 - [100 \times [(A_0 - A_t)/(A_0 - A_\infty)]] \quad (2)$$

R_t was then fit to a modified (truncated) single exponential expression by nonlinear regression using the Marquardt–Levenberg algorithm (SigmaPlot V. 4.0, Jandel Scientific):

$$R_t = B + (100 - B)\exp(-kt) \quad (3)$$

Under pseudo-first-order reaction conditions, monoferric transferrins should release iron by simple first-order processes. Because under our conditions reactions become immeasurably slow before completion, the simple first-order kinetic expression was modified to yield eq 3 in order to obtain reasonable fits to the experimental data. The phenomenological parameter B of eq 3 reflects the extent of reaction (a lower value of B indicates a reaction more nearly complete), while k is the apparent pseudo-first-order rate constant. The quality of the data and the limited number of data points do not warrant a mathematically sophisticated kinetic treatment of a reversible first-order reaction or analysis by the method of initial rates. We attribute the apparent failure of iron release reactions to proceed to near completion to the concentration of PP_i chosen to attain reasonable initial rates of release. Higher concentrations of the competing complexing agent lead to more nearly complete release but at the expense of initial rates of release too fast for our methods of study. Despite these limitations, the experimental conditions and data analysis offer reproducible comparisons of relative release rates from N- and C-terminal sites.

Results

Iron Removal from Monoferric Transferrins. Experimental data, as well as calculated fits to eq 3 for iron removal from

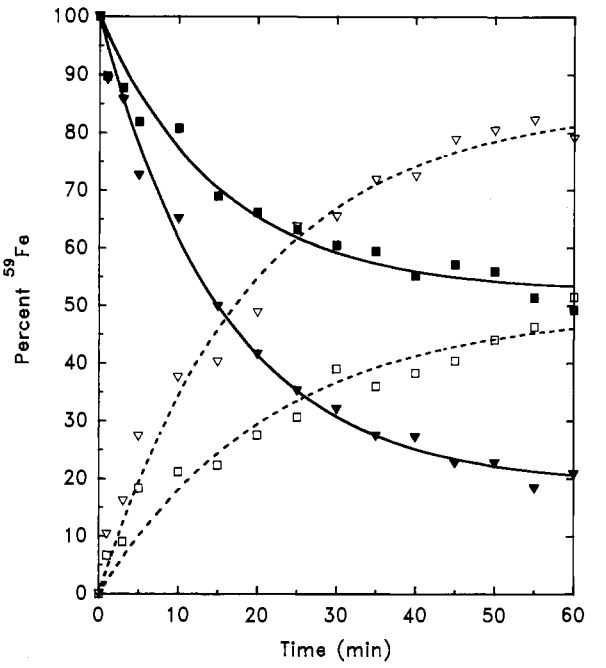

FIGURE 1: Plots of normalized reaction coordinate (percent ^{59}Fe) as a function of time for the removal of iron from Tf-Fe_C (▽, ▼) and [TfR-(TfFe$_C$)$_2$] (□, ■) by 0.05 M PP_i at pH 7.4 in 0.05 M HEPES/0.1 M NaCl/0.01 M CHAPS. Activity in PEG precipitates (▼, ■, —); activity in PEG supernatants (▽, □, ---). Symbols are experimental data points, while lines are calculated curve fits from eq 3 using parameters listed in Table I.

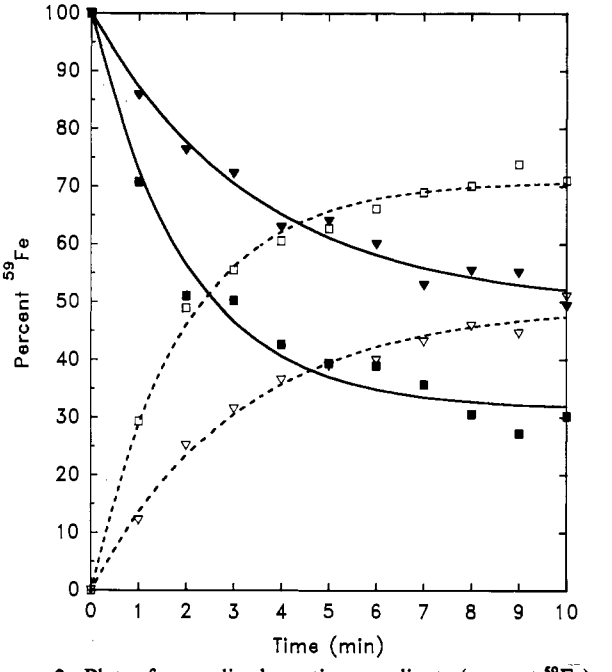

FIGURE 2: Plots of normalized reaction coordinate (percent ^{59}Fe) as a function of time for the removal of iron from Tf-Fe_C and [TfR-(TfFe$_C$)$_2$] by 0.005 M PP_i at pH 5.6 in 0.05 M MES/0.1 M NaCl/0.01 M CHAPS. Symbols and lines as in Figure 1.

Tf-Fe_C and [TfR-(TfFe$_C$)$_2$] by 0.05 M PP_i at pH 7.4, and by 0.005 M PP_i at pH 5.6, are shown in Figures 1 and 2, respectively. Corresponding plots for iron removal from Fe_N-Tf and [TfR-(Fe_NTf)$_2$] are given in Figures 3 and 4, respectively. Calculated values of the parameters k and B for various reactions are listed in Table I.

Urea/PAGE Analysis of the Iron Removal from Diferric Transferrin. Iron removal from free diferric transferrin and the complex of receptor and diferric transferrin by 0.002 M PP_i at pH 5.6 was analyzed by urea/PAGE. Polyacrylamide

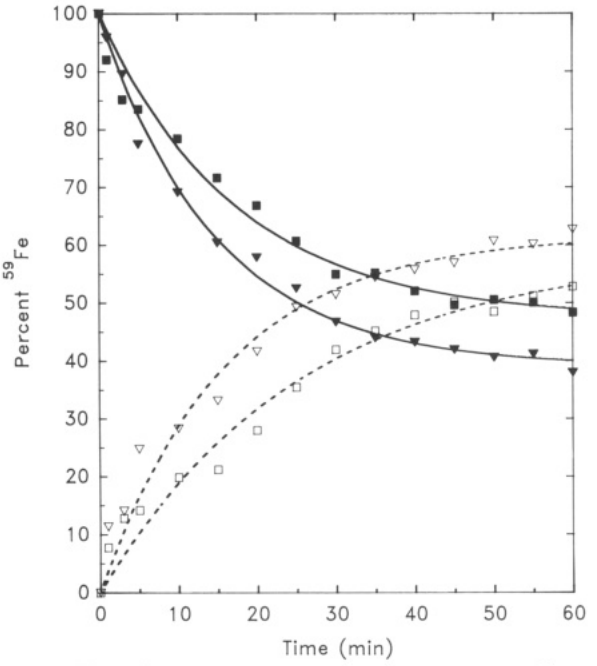

FIGURE 3: Plots of normalized reaction coordinate (percent ^{59}Fe) as a function of time for the removal of iron from Fe_N-Tf and [TfR–(Fe_NTf)$_2$] by 0.05 M PP_i at pH 7.4 in 0.05 M HEPES/0.1 M NaCl/0.01 M CHAPS. Symbols and lines as in Figure 1.

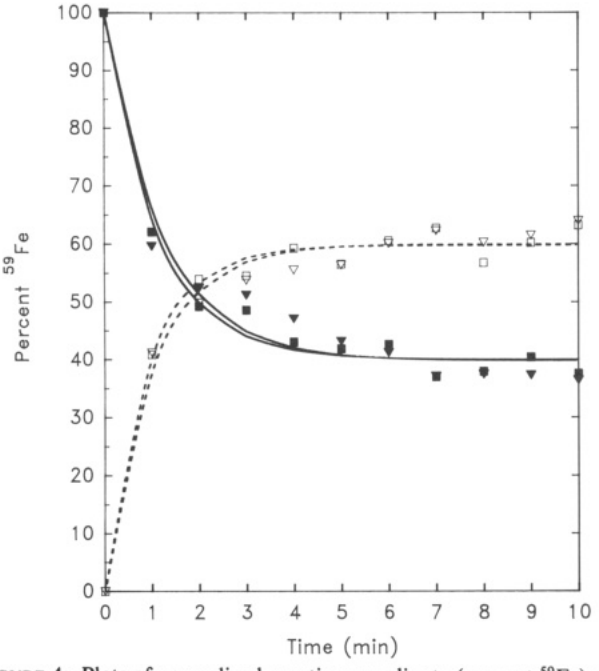

FIGURE 4: Plots of normalized reaction coordinate (percent ^{59}Fe) as a function of time for the removal of iron from Fe_N-Tf and [TfR–(Fe_NTf)$_2$] by 0.005 M PP_i at pH 5.6 in 0.05 M MES/0.1 M NaCl/0.01 M CHAPS. Symbols and lines as in Figure 1.

gel electrophoresis in 6 M urea resolves the four possible species of transferrin: apo-Tf, Tf-Fe_C, Fe_N-Tf, and Fe_N-Tf-Fe_C (Makey & Seal, 1976; Aisen et al., 1978; Evans & Williams, 1980). This makes it possible to distinguish relative rates of iron removal from each site of diferric transferrin. (It should be noted that a band corresponding to receptor subunits does not appear in urea gels, presumably because urea-denatured receptor fails to enter the gels.) A silver-stained gel, depicting the time course of iron release, is shown in Figure 5. In the case of free diferric transferrin, the band due to diferric transferrin at zero time (lane A1) almost disappears by 0.5 min, while a band of comparable intensity due to monoferric

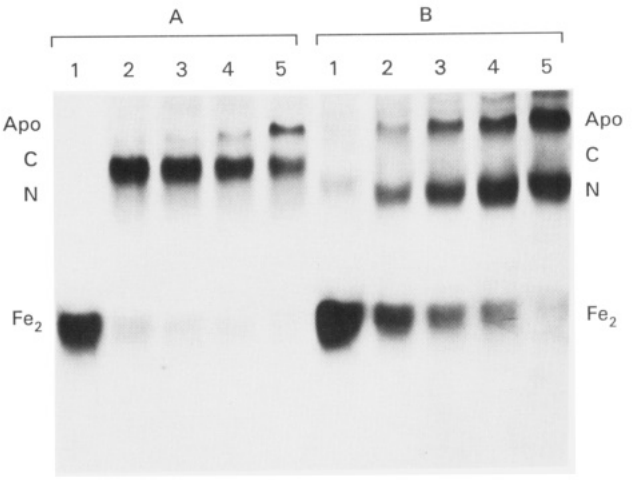

FIGURE 5: Silver-stained urea/PAGE gel showing time course of iron release to 0.002 M PP_i, at pH 5.6 in 0.05 M MES/0.1 M NaCl/0.01 M CHAPS from (A) free diferric transferrin and (B) the complex of diferric transferrin with its receptor. Lane 1, zero time; lane 2, 0.5 min; lane 3, 1.0 min; lane 4, 1.5 min; and lane 5, 3.0 min. Positions marked Apo, C, N, and Fe_2 are for apo-Tf, Tf-Fe_C, Fe_N-Tf, and Fe_N-Tf-Fe_C transferrins, respectively.

C-terminal transferrin appears (lane A2). In lanes A3–A5, bands representing Tf-Fe_C decay as bands due to apo-Tf appear. Virtually no band corresponding to monoferric N-terminal transferrin can be distinguished at any time, indicating that release is predominantly from the N-terminal site.

In contrast, during iron removal by 0.002 M PP_i at pH 5.6 from diferric transferrin complexed to the receptor (lanes B1–B5), bands due to diferric transferrin decay with time, while bands corresponding to Fe_N-Tf and apo-Tf build up. No band corresponding to the C-terminal monoferric transferrin is seen. Thus, release of iron occurs preferentially from the C-terminal site when diferric transferrin is bound to the receptor.

Discussion

Iron Release from Free Monoferric Transferrins. Monoferric transferrins are known to release iron at slightly different rates at pH 7.4 depending upon the identity of the iron acceptor, its concentration, and the site of bound iron (Bali et al., 1991b; Bali & Harris, 1989; Baldwin & de Sousa, 1981). In the present study as well, the two forms exhibit kinetic differences in releasing iron to PP_i. At pH 7.4, C-terminal monoferric transferrin releases iron faster than the N-terminal form; about 80% iron is removed from the C-site in 1 h, compared to about 60% from the N-site. However, this effect is largely attributable to difference in the extent of reaction (B values of 17% and 39% for C- and N-forms, respectively) rather than in apparent release rates (k values of 0.058 min^{-1} versus 0.065 min^{-1}). These results are consistent with a previous report based on spectrophotometric studies of iron removal from monoferric transferrins by PP_i (Bali & Harris, 1989).

In contrast, at pH 5.6, the accepted pH of the endosome, iron removal from N-terminal monoferric transferrin by 0.005 M PP_i is 3 times faster than from the C-terminal species. This difference is largely in the rates of reaction (k values of 0.91 min^{-1} and 0.30 min^{-1}) rather than the extents (B values of 50% and 40%), as expected from the known acid lability of the N-terminal site (Baldwin et al., 1982).

Iron Removal from Complexes of Receptor with Monoferric Transferrins. Rates of iron release to 0.05 M PP_i at pH 7.4 from [TfR–(Fe_NTf)$_2$] and [TfR–(TfFe$_C$)$_2$] are slower than those from corresponding free monoferric transferrins. In the

case of the C-terminal form the effect is largely in the extent rather than the apparent rate of reaction (compare B values of 17% to 52% and k values of 0.058 min^{-1} to 0.055 min^{-1} for free and receptor-complexed C-terminal monoferric transferrin, respectively). In contrast, the retardation in rate of reaction of N-terminal monoferric transferrin complexed to receptor, compared to free protein, is predominantly in the apparent first-order rate constant (k values of 0.065 min^{-1} and 0.048 min^{-1}, and B values of 39% and 44%, for free and receptor-bound N-terminal monoferric transferrin, respectively). Slowing of the rate of iron removal from diferric transferrin complexed to the receptor as compared to free transferrin has previously been observed for iron removal by 0.05 M PP_i at pH 7.4 (Bali et al., 1991a).

At pH 5.6, binding of monoferric transferrins to receptor affects the rate of iron release to 0.005 M PP_i from the two forms of the protein differently. The k and B parameters from iron removal from the N-terminal monoferric transferrin remain substantially the same whether the transferrin is receptor-bound or free. However, the rate and extent of iron release to 0.005 M PP_i by C-terminal monoferric transferrin increase by about 70% when the protein is complexed to its receptor compared to when it is free (k values of 0.51 min^{-1} and 0.30 min^{-1}, and B values of 30% and 50%, respectively, for receptor-bound and free C-terminal monoferric transferrin). Acceleration in rate of iron release to 0.001 M PP_i from diferric transferrin when complexed to the receptor at pH 5.6 has been reported earlier (Bali et al., 1991a). We conclude, therefore, that binding of transferrin to receptor predominantly affects the C-terminal domain.

Iron removal from Fe_N-Tf, Tf-Fe_C, [TfR–(Fe_NTf)$_2$], and [TfR–(TfFe_C)$_2$] by 0.005 M citrate has also been studied at pH 5.6 (data not shown). Results were similar to those obtained with PP_i, in that the rate of iron removal from the N-site was not altered by binding of transferrin to receptor, while release from the C-site was accelerated when transferrin was receptor-bound. The rate of iron removal by 0.005 M citrate was similar to that obtained with 0.002 M PP_i. Accordingly, the site-specific effect of receptor binding is not dependent on iron removal by PP_i alone.

Iron Release from Diferric Transferrin at pH 5.6. The apparent fast decay at pH 5.6 of the band due to free diferric transferrin, and concomitant increase in the band for monoferric C-terminal transferrin (Figure 5A), indicates that iron is lost predominantly if not exclusively from the N-terminal site of the diferric protein. This accords well with the known acid lability of the N-terminal site (Aisen et al., 1978). However, the pattern of iron removal changes when diferric transferrin is complexed to receptor. The relatively rapid buildup of bands due to Fe_N-Tf (Figure 5, lanes B1–B5) indicates predominant removal of iron from the C-terminal site of the diferric protein. Total iron removal in 3 min from the complex of diferric transferrin and receptor is also greater than from free diferric transferrin, as previously observed (Bali et al., 1991a). Furthermore, experiments with transferrin bound to receptors at the surface of K562 cells demonstrated enhanced release of iron at pH 6 or below (Sipe & Murphy, 1991), reinforcing inferences about the physiological consequences of transferrin binding to receptor.

The receptor-induced switch in site lability is an intriguing but not yet explainable phenomenon. The kinetic data for monoferric transferrins, summarized in Table I, do not conform to the patterns of iron release from diferric transferrin at pH 5.6 as shown in Figure 5. Iron release is more rapid from monoferric N-terminal transferrin than from the C-terminal form, whether the proteins are free or complexed to receptor, but receptor-bound diferric transferrin relinquishes iron much more readily from the C-terminal site. This surprising finding might reflect receptor-induced conformational changes in transferrin that are not observed in monoferric forms, as well as cooperative interactions between the sites. A site–site cooperativity has previously been noted for iron removal from transferrin at pH 7.4. Iron is released more rapidly from the C-terminal site when the N-terminal site is occupied than when it is vacant (Bali & Harris, 1989; Bali et al., 1991b). Such a site–site interaction may be even more pronounced at pH 5.6 in differric transferrin complexed to receptor.

Proposed Mechanism for the Effect of Receptor on Iron Release. We have suggested that receptor binding to diferric transferrin may force the latter into a labile or "open" conformation, thereby facilitating the release of iron (Bali et al, 1991a). The results of the present study suggest that the effect may largely pertain to the C-terminal domain, which appears especially sensitive to its environment. Release of iron from the C-terminal site has previously been found to be facilitated by high salt concentration (Baldwin & de Sousa, 1981; Harris & Bali, 1988), while separation of the C-terminal lobe from the parent protein weakens its iron-binding properties, particularly at low pH (Zak & Aisen, 1985). The C-terminal site of lactoferrin, which has a crystal structure very similar to that of rabbit serum transferrin (Bailey et al., 1988), persists in the "closed" conformation even in the absence of iron (Anderson et al., 1990). The transferrin receptor, and in particular the 70-kDa fragment of its extracellular domain which carries the transferrin-binding region, has been reported to undergo a conformational transition below pH 6 (Turkewitz et al., 1988b). Such a conformational transition in the receptor may induce a secondary conformational change in bound transferrin, particularly in the C-terminal domain. This could then lead to opening of the otherwise narrow mouth of the cleft in the C-terminal domain, thereby enhancing the accessibility of iron in this site to iron chelators. Possibly, too, the C-terminal lobe may be the principle contributor to the receptor-binding region of transferrin.

Also to be considered is the possibility that monoferric Fe_N-Tf binds poorly to receptor at pH 5.6, thereby accounting for failure of receptor to modulate iron release from the protein. No experimental data bearing on this point are now available, but the question is currently under study in our laboratory.

Physiological Implications. Several studies indicate that iron is not distributed randomly between the two sites of transferrin in the human circulation (Zak & Aisen, 1986; Williams & Moreton, 1980; van Eijk & van Noort, 1986). The N-terminal site is predominantly occupied in the majority of healthy subjects, although the C-terminal site binds iron 6 times more strongly under physiological conditions (Aisen et al., 1978). The results of the present study offer an explanation of this previously enigmatic observation. Facilitated release of iron from the C-terminal site of transferrin bound to its receptor in acidified endosomes would lead to a preponderance of iron at the N-terminal site in exocytosed transferrin, and hence in the circulation.

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