The Anion Requirement for Iron Release from Transferrin Is Preserved in the Receptor–Transferrin Complex†

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ABSTRACT: Rates of iron release from both sites of free transferrin at pH 7.4 are critically dependent upon ionic strength, because release appears to require binding of a simple nonchelating anion such as chloride to a kinetically active site of the protein. This site is distinct from the synergistic anion-binding site, occupancy of which is required for binding of iron to occur at all. Complexing of transferrin to its receptor also modulates release of iron, but in a more complex fashion. At extracellular pH, 7.4, receptor retards release, but at the pH of the endosome in which release occurs within the cell, 5.6, receptor accelerates release. The present study was undertaken to determine whether the kinetically active anion requirement is maintained at pH 5.6 and whether the effects of anion binding and receptor binding are independent of each other. A spectrofluorometric method was developed to monitor release of iron from C-terminal monoferric human transferrin and its complex with the transferrin receptor. At pH 5.6, as at pH 7.4, profiles of iron release to pyrophosphate from free and from receptor-complexed monoferric transferrin show curvilinear dependence on pyrophosphate concentration, consistent with a previously described kinetic scheme and suggestive of a similar release mechanism in all cases. Furthermore, at pH 5.6 release rates depend upon anion (chloride) concentration in free and in receptor-complexed transferrin as in free transferrin at pH 7.4, extrapolating nearly to zero as chloride concentration approaches zero. The enhancing effect of receptor on release is displayed at all concentrations of chloride tested, indicating that the release-promoting effects of receptor and chloride are independent of each other. Since release is thought to occur from a lobe of transferrin when the two domains enclosing the binding site of the lobe rotate about their hinging strands to an "open" conformation, one possibility is that in the C-terminal lobe the anion- or receptor-binding sites, or both, are located in the hinging strands.

The human transferrin molecule consists of a single polypeptide chain of molecular weight near 80 000, with over 40% sequence identity between N- and C-terminal halves (MacGillivray et al., 1983). Each half is structured in a lobe consisting of two hinged domains surrounding a cleft bearing a single specific iron-binding site (Anderson et al., 1987; Bailey et al., 1988). Despite their sequence homologies and identical iron-binding ligands, the two sites differ in their spectroscopic, kinetic, and thermodynamic properties and in the relative occupancies by iron in the circulation; for recent reviews, see Brock (1985), Aisen (1989), and Harris and Aisen (1989). During its lifetime, the transferrin molecule may experience over 100 cycles of iron uptake and release (Katz, 1961). Because of the indispensable role of transferrin in iron transport and the regulation of iron metabolism, considerable attention has been directed to mechanisms entailed in uptake and release of iron by the protein.

Two factors modulating the kinetics of iron release from transferrin have recently been identified, one involving simple nonchelating anions and the other the transferrin receptor. At pH 7.4, release is dependent upon ionic strength: release from either site to a synthetic tricatecholate sequestering agent extrapolate to zero as the ionic strength of the supporting buffer nears zero (Kretchmar & Raymond, 1988). Further investigation of this remarkable observation showed that the effects of ionic strength could be rationalized by postulating a kinetically active anion-binding site on the transferrin molecule (Egan et al., 1992). For release of iron to occur, the kinetic site must be occupied by a simple nonchelating, nonsynergistic anion, such as chloride or perchlorate. Such occupancy is increased as ionic strength (or anion concentration) is increased, accounting for the dependence of observed release rates on ionic strength. The kinetically active anion-binding site is distinct from the more familiar synergistic anion-binding site which must be occupied for binding of iron to occur at all (Schlabach & Bates, 1975). In all cases, release requires a suitable iron-sequestering agent, such as pyrophosphate (PP_i)[1] to trap released iron and prevent its rebinding to transferrin.

The transferrin receptor also affects release rates from transferrin, but in a more complex fashion. At the pH of the cell surface, where the transferrin receptor first encounters iron-laden transferrin, the receptor slows release to PP_i from both sites of transferrin (Bali et al., 1991). In contrast, at the pH of the endosome, where iron release occurs within the cell, the receptor accelerates release, particularly from the C-terminal site (Bali & Aisen, 1991), thereby helping to account for the rapidity with which transferrin relinquishes iron to the iron-dependent cell.

The question then arises whether the kinetic effects of simple anions and the transferrin receptor are independent of each

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[1] Abbreviations: PP_i, pyrophosphate; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid]; MES, 2-(*N*-morpholino)-ethanesulfonic acid; Tf-Fe_C, C-terminal monoferric transferrin; [TfR–(Tf-Fe_C)$_2$], complex of transferrin receptor and C-terminal monoferric transferrin.

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other, especially at pH 5.6, or whether they share a common mechanism. Accordingly, we have undertaken a study of the effects of chloride and receptor, singly and in combination, at pH 7.4 and at pH 5.6. Our observations suggest that the kinetic effects of anion binding and complex formation with receptor are each preserved in the presence of the other and are therefore independent.

MATERIALS AND METHODS

Proteins. Transferrin receptor was isolated from human placentas using minor modifications of the affinity chromatographic procedures reported by Turkewitz et al. (1988a). Each series of experiments used receptors obtained from two different placentas. Tf-Fe_C was prepared by established methods (Baldwin & de Sousa, 1981) from commercially available transferrin (Boehringer). No impurities were detected in the transferrin by SDS–polyacrylamide gel electrophoresis. The complex of receptor and Tf-Fe_C, [TfR–(Tf-Fe_C)$_2$], was isolated by gel-filtration chromatography as previously described (Bali et al., 1991) and shown by SDS–polyacrylamide gel electrophoresis on a Pharmacia Phast-System to be free of contaminating proteins. Repeated gel-filtration chromatography demonstrated that the isolated complex in detergent solution is stable to dissociation. Receptor concentration was determined using a Bio-Rad DC protein assay kit. The extinction coefficient of the receptor at 280 nm ascertained with this method (200 000 M^{-1} cm^{-1}) is at variance with the value of 300 000 M^{-1} cm^{-1} obtained using the Pierce micro BCA protein assay kit (Bali et al., 1991). We have used the former value throughout this study because the background signal given by the Bio-Rad test is much lower than that obtained with the Pierce method, and the Bio-Rad extinction coefficient is in good agreement with the value calculated (Wetlaufer, 1962) from the numbers of tyrosines, tryptophans, and phenylalanines in the receptor sequence (McClelland et al., 1984). The extinction coefficient of [TfR–(Tf-Fe_C)$_2$] was assumed to be the sum of those for Tf-Fe_C and the receptor. On the basis of these extinction coefficients, the concentration of receptor complex or free transferrin used in our studies was close to 40 nM. It should be noted, however, that since the concentrations of PP_i and Cl^- were always in vast excess over that of the protein, our findings or inferences do not depend on a precise knowledge of the concentrations of transferrin and transferrin receptor used.

Spectrofluorometry and Data Analysis. In this work the kinetics of iron release from [TfR–(Tf-Fe_C)$_2$] and Tf-Fe_C to PP_i were followed using intrinsic protein fluorescence (due to tryptophan and tyrosine residues). This technique is able to detect iron binding and release because the binding of iron to transferrin quenches the intrinsic fluorescence of protein due to nonradiative (Förster) energy transfer from the fluorophores to the visible charge-transfer band of bound Fe(III) (Lehrer, 1969). The method is highly sensitive, yielding measurable results with concentrations of transferrin in the range 10–100 nM, and therefore particularly valuable given the small amounts of receptor (about 2–3 mg) obtained per placenta. Previous studies from this laboratory on iron release from [TfR–(Tf-Fe_C)$_2$] to PP_i were carried out using poly(ethylene glycol) precipitation of ^{59}Fe-labeled transferrin to distinguish released iron from bound iron. The current spectrofluorometric method has the dual advantage of economy in effect while providing continuous progress curves of the reaction kinetics, hence allowing a more accurate determination of rate constants.

C-Terminal monoferric transferrin was chosen for the present study because this species has been the subject of previous mechanistic investigations (Egan et al., 1992) and is more influenced by binding to receptor than its N-terminal counterpart (Bali & Aisen, 1991). Interpretation of data from diferric transferrin is complicated by site–site cooperativity and strong positive correlations of rate constants obtained with nonlinear curve-fitting algorithms (Bali & Harris, 1989).

Progress curves were monitored using an SLM 8000 spectrofluorometer with the excitation wavelength set at 280 nm and the emission wavelength at 330 nm. For studies at pH 5.6 (where reactions are relatively fast) both excitation monochromator resolution settings were 16 nm, while the emission entry and exit settings were 16 and 8 nm, respectively. These settings maximize the intensity of light reaching the detector, with consequent optimization of signal-to-noise. Photomultiplier voltage was set at 800 V and the gain at 10. Signal integration time was 0.5 s and the digital sampling interval was 1 s (except for the fastest reactions, where a setting of 0.6 s was used). Shorter integration times and sampling intervals did not significantly alter the values of the rate constants obtained but did lead to an increase in noise. For the slower reactions at pH 7.4, narrower entry and exit resolutions were used (excitation, 4 and 16 nm, respectively; emission, 4 and 8 nm) with a photomultiplier voltage of 850 V and gain of 10. The narrower resolutions were found to decrease drift in the signal over the long time course, although noise levels increased. This decrease in drift probably results from a decrease in signal contribution from the Rayleigh scatter line (centered at the excitation wavelength) as a result of the improved wavelength resolution. The intensity of the scatter line is particularly sensitive to the presence of particulate matter or turbidity in the solution under study. These can generate large noise spikes, mandating abandonment of a kinetic run and wastage of material. Most problems were alleviated by submicrometer filtering (Uniflow, 0.45 μm) of buffer, pyrophosphate, and chloride solutions and thorough washing of cuvettes with detergent and water.

Temperature in the sample compartment was maintained at 25 °C with a circulating water bath. Reactions were carried out with continuous stirring in buffers specified previously (Bali et al., 1991) and were initiated by addition of protein to the reaction mixture. Pyrophosphate solutions were prepared by adjusting the pH of pyrophosphoric acid with NaOH. Appropriate chloride concentrations were obtained by addition of NaCl solution to the reaction mixture.

Observed pseudo-first-order rate constants (k_{obs}) were obtained from the exponential spectrofluorometric progress curves by nonlinear least-squares fitting:

$$F(t) = F_f - (F_f - F_0) \exp(-k_{obs}t) \quad (1)$$

in which $F(t)$ is the fluorescence intensity at time t, F_f is the equilibrium fluorescence intensity at the end of each reaction, and F_0 is the initial fluorescence intensity. The curve-fitting facility of the SigmaPlot graphics software, based on the Marquardt–Levenberg algorithm, was used. Coefficients of variation in the fitting of eq 1 to experimental data were typically 1–3% for data obtained at pH 5.6. At pH 7.4 in the case of the receptor complex these rose to 3–12% because of increased noise associated with narrower slits. In addition, for the slowest reactions, data were collected for only about two half-lives (approximately 2 h), as drift inevitably interfered with the data if longer measurements were carried out.

Equation 1 is only valid for reactions involving essentially complete removal of Fe(III) from transferrin, so that the back

reaction of iron rebinding to transferrin is negligible. At pH 5.6 the magnitude of the total fluorescence change, $\Delta F = (F_f - F_0)$, is largely independent of $[PP_i]$, at least for concentrations above 0.005 M. For [TfR–$(Tf\text{-}Fe_C)_2$] in 0.0025 M Cl^-, ΔF is 4439 arbitrary fluorescence units at 0.005 M PP_i, 4242 ± 612 (mean ± SEM, n = 3) at 0.03 M PP_i, and 5266 ± 352 at 0.05 M PP_i. At 0.100 M Cl^-, ΔF ranges from 4436 at 0.005 M PP_i to 4034 at 0.05 M PP_i. Thus, for all of these reactions the variation in ΔF is comparable to the variation observed for experiments at fixed $[PP_i]$. Within the constraints of experimental error, therefore, iron release in all of these reactions appears to go to completion. Only in the case of 0.0025 M Cl^- at 0.005 and 0.001 M PP_i at pH 5.6 is there evidence that the reaction may not reach completion in either free transferrin or the receptor–transferrin complex. For these conditions k_{obs} may be overestimated, since using eq 1 to fit the progress curve of a reaction that fails to reach completion results in a value of k_{obs} which is too large. At pH 7.4 reactions proceed to completion when $[PP_i] \geq 0.005$ M (Bali & Harris, 1989; Egan et al., 1992), and therefore all of our experiments at that pH have been carried out at or above 0.005 M PP_i.

Because of the extremely low concentrations of protein used, it was necessary to exercise great care in acquisition, analysis, and interpretation of the data. Formation of turbidity in the solution can lead to a significant increase in the signal (due to increased light scattering), which can in turn lead to erroneous data. An initial set of data obtained with [TfR–$(Tf\text{-}Fe_C)_2$] at pH 5.6 and 0.600 M Cl^- gave misleading results indicating that k_{obs} at this chloride concentration was almost identical to that at 0.100 M Cl^-. This error appears to have resulted from the formation of turbidity upon dilution of the 0.01 M CHAPS detergent by addition of 2 M NaCl which did not contain detergent (presumably resulting in aggregation of some of the protein), combined with a sampling frequency which caused most of the data points to be lost during the fast reactions with half-lives of 5–10 s.

Representative reaction profiles of fluorescence as a function of time, and results of nonlinear curve fitting, are shown in Figure 1; linear semilogarithmic plots of the data, corroborating the first-order nature of the reactions, are displayed to the inset to Figure 1. In these and all other cases, data were taken for analysis 6 s after initiation of reaction to assure completeness of mixing.

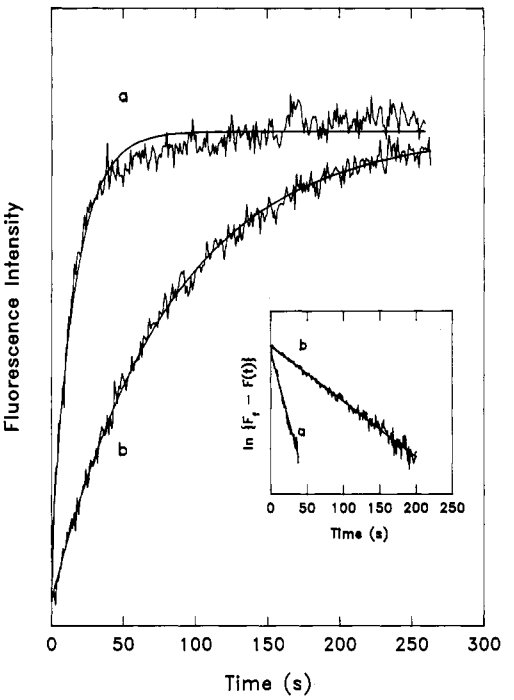

FIGURE 1: Typical fluorescence profiles for iron release from [TfR–$(Tf\text{-}Fe_C)_2$], the complex of $Tf\text{-}Fe_C$ with transferrin receptor: (a) $[PP_i]$ = 0.01 M, $[Cl^-]$ = 0.600 M, pH 5.6; (b) $[PP_i]$ = 0.01 M, $[Cl^-]$ = 0.100 M, pH 5.6. Fluorescence intensity scales in arbitrary units are the same for both curves. (Inset) Semilogarithmic plots of the first 90% of each of the reactions presented in the main figure. Coefficients of variation for these fitted curves were less than 1%.

RESULTS AND DISCUSSION

Dependence of k_{obs} on Pyrophosphate Concentration at Fixed Chloride Concentration. At pH 5.6, k_{obs} for release of iron from free C-terminal monoferric transferrin under pseudo-first-order conditions increases with increasing concentration of PP_i (Figure 2, top). The dependence of observed rate constant on $[PP_i]$ is preserved in the complex of transferrin with receptor (Figure 2, bottom). In both preparations, the effect of PP_i on iron release is amplified by increasing chloride concentration. Binding to receptor further accelerates iron release from C-terminal monoferric transferrin by a factor of 7 or 8, in qualitative accord with results from earlier studies which were not carried out for sufficiently long times to obtain accurate estimates of release constants (Bali & Aisen, 1991). Overall shapes of the k_{obs} versus $[PP_i]$ curves for free and receptor-complexed transferrin are similar at each concentration of chloride used, suggesting that the release mechanisms are also similar in free and receptor-complexed transferrin at pH 5.6.

The curvatures in the data plots at pH 5.6, most evident at 0.6 M chloride, and the amplifying effects of chloride on iron release rates are qualitatively consistent with the "saturation-linear" dependence of k_{obs} on $[PP_i]$ observed in studies of iron release from free C-terminal monoferric transferrin at pH 7.4 (Bali & Aisen, 1991). Such dependence is predicted by a kinetic scheme in which release to a suitable iron-sequestering agent occurs only when a kinetically active anion-binding site on transferrin is occupied by an anion which may be a simple species, like chloride or perchlorate, or the iron-sequestering anion itself (Egan et al., 1992). Definitive proof of saturation-linear dependence could not be obtained in our studies, however, because of scatter in the data resulting from the low concentrations of protein used to conserve receptor. Nevertheless, the increasing rate of iron release with increasing chloride concentration is evident at pH 5.6 in receptor-complexed and free $Tf\text{-}Fe_C$ alike.

At pH 7.4, rates of iron release from receptor-complexed $Tf\text{-}Fe_C$ showed a curvilinear dependence on $[PP_i]$ (Figure 3, top) consistent with saturation-linear kinetics previously observed for free $Tf\text{-}Fe_C$ (Egan et al., 1992), although again scatter in the data precluded least-squares fitting to the appropriate kinetic equation. For purposes of comparison, studies were also undertaken with free $Tf\text{-}Fe_C$ (Figure 3, bottom) with results in accord with published data (Bali & Harris, 1989; Egan et al., 1992) obtained from spectrophotometric measurements requiring concentrations of transferrin nearly 3 orders of magnitude greater than that used in our investigations.

Both receptor-complexed and free transferrin showed an approximately 2–3-fold acceleration in iron release when chloride concentration is increased from 0.1 to 0.6 M, indicating that at pH 7.4 the anion-accelerating effect is displayed in the receptor complex as well as in free transferrin. We note also that receptor acts to retard release of iron from transferrin at 0.6 M chloride as it does at physiological chloride concentration (Bali & Aisen, 1991), with release rates

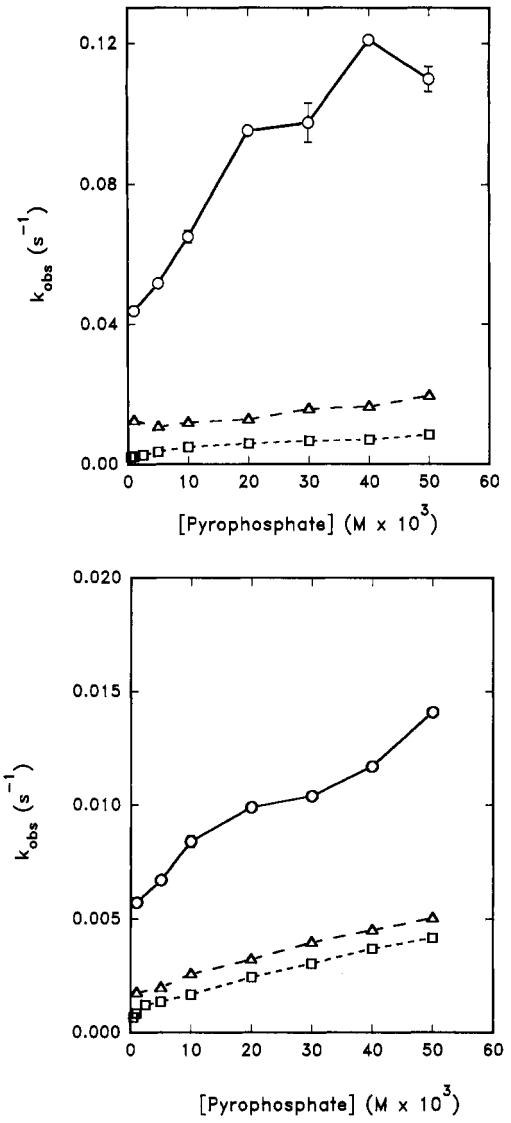

FIGURE 2: (Top) Dependence of k_{obs} on [PP_i] at three different Cl^- concentrations for [TfR–(Tf-Fe_C)$_2$]. (□) 0.0025 M Cl^-; (△) 0.1 M Cl^-; (○) 0.6 M Cl^-. Error bars (±1 SEM) were obtained for each data set at 0.01 (n = 4), 0.03 (n = 3), and 0.05 (n = 3) M PP_i. Error bars for the lower two curves at 10, 30, and 50 mM PP_i, and for the uppermost curve at 10 mM PP_i, are smaller than the plot symbols. All reactions were carried out in 0.05 M MES buffer and 0.01 M CHAPS, pH 5.6. For clarity in this and subsequent figures, experimental points or means of experimental points are joined by straight lines. (Bottom) Dependence of k_{obs} on [PP_i] at three different Cl^- concentrations for Tf-Fe_C. (□) 0.0025 M Cl^-; (△) 0.1 M Cl^-; (○) 0.6 M Cl^-. Error bars for all Cl^- concentrations (n = 3), at [PP_i] = 10 and 40 mM, are smaller than the plot symbols. Reactions were in 0.05 M MES buffer and 0.01 M CHAPS, pH 5.6.

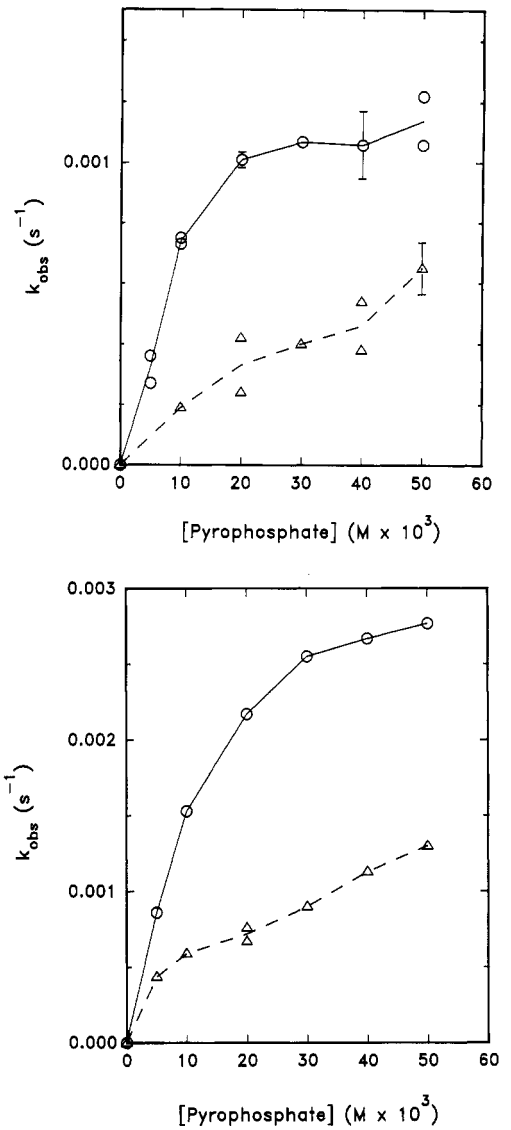

FIGURE 3: A. (Top) Dependence of k_{obs} on [PP_i] at two Cl^- concentrations for [TfR–(Tf-Fe_C)$_2$]. (△) 0.1 M Cl^-; (○) 0.6 M Cl^-. All reactions were in 0.05 M HEPES buffer and 0.01 M CHAPS, pH 7.4. (Bottom) Dependence of k_{obs} on [PP_i] at two Cl^- concentration for Tf-Fe_C. (△) 0.1 M Cl^-; (○) 0.6 M Cl^-. All reactions were in 0.05 M HEPES buffer and 0.01 M CHAPS, pH 7.4.

generally 2.5 times faster in the free protein for the near-linear portions of the data plots in the present studies at both concentrations of chloride. To a first approximation, then, the effects of chloride and receptor may be considered as independent, rather than competitive or synergistic.

Dependence of k_{obs} on Chloride Concentration at Fixed Pyrophosphate Concentration. The acceleration of iron release rates by chloride from free Fe_C-transferrin and from the complex of transferrin with receptor at pH 5.6 recall the observation of Kretchmar and Raymond on the dependence on ionic strength of iron release from transferrin at pH 7.4 (Kretchmar & Raymond, 1988). Accordingly, the dependence of k_{obs} on concentration of chloride (as NaCl) at a fixed concentration of PP_i, 0.01 M, was evaluated at pH 5.6 for free and for receptor-complexed Tf-Fe_C. In both cases, a linear relationship between release rates and chloride concentration was observed, with k_{obs} nearing zero as chloride concentration approaches zero (Figure 4). The accelerating effect of receptor on iron release is again revealed, with the slope of the regression line for the receptor complex about 20 times that of the slope for free transferrin. At zero chloride concentration the offset from zero in both curves is substantially accounted for by the contribution to ionic strength of the PP_i, I = 0.042, and MES buffer, I = 0.012. Thus, binding to receptor does not substitute for binding of an anion, the requirement for which is preserved in Fe_C-Tf complexed to receptor.

Dependence of Release Rates on pH. Because release of iron from transferrin is retarded by complexing of the protein to its receptor at pH 7.4 but accelerated at pH 5.6, we examined release rates from free and receptor-bound Fe_C-Tf at fixed concentrations of chloride (0.1 M) and PP_i (0.01 M) as a function of pH (Figure 5). A crossover is found near pH 6.1, consistent with earlier studies showing the accelerating influence of receptor on release from diferric transferrin to persist at pH 6.5 (Bali et al., 1991). No abrupt transition is

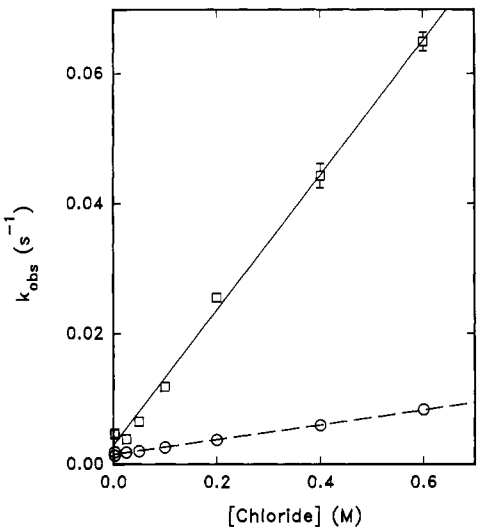

FIGURE 4: Dependence of k_{obs} on [Cl^-] in 0.01 M PP_i for [TfR–(Tf-Fe_C)$_2$] (□) and Tf-Fe_C (○). Error bars were obtained at 0.1 M Cl^- (n = 4 for [TfR–Tf-Fe_C)$_2$], n = 3 for Tf-Fe_C); 0.4 M Cl^- (n = 3), and 0.6 M Cl^- (n = 4 for [TfR–(Tf-Fe_C)$_2$]; n = 3 for Tf-Fe_C). In some cases they are smaller than the plot symbols. Reactions were in 0.05 M MES buffer and 0.01 M CHAPS, pH 5.6.

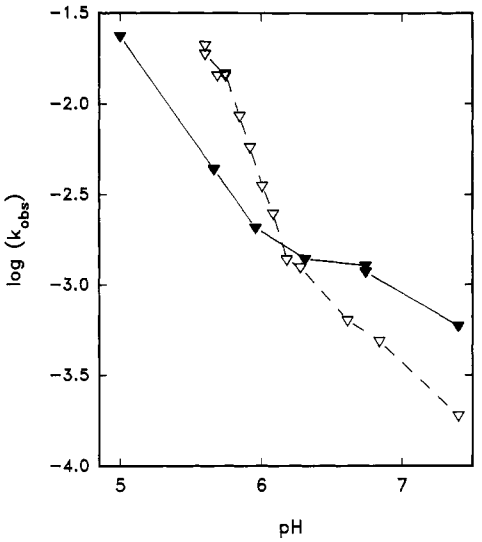

FIGURE 5: Dependence of log k_{obs} on pH at 0.01 M PP_i concentration and 0.1 M Cl^- (▽) [TfR–(Tf-Fe_C)$_2$]; (▼) Tf-Fe_C.

apparent in either free or receptor-bound transferrin, but the latter shows a steeper dependence on pH throughout the range of values studied. Since transferrin is known to undergo changes in conformation and iron-binding properties as pH is lowered, and a reversible conformational change in receptor when pH falls below 6 has been described (Turkewitz et al., 1988b), we cannot say whether the crossover point predominantly reflects changes in transferrin or changes in receptor. Possibly, both are involved.

Possible Anion- and Receptor-Binding Loci on Transferrin. Each lobe of the transferrin molecule bears an iron-binding site situated in the cleft formed by two surrounding domains (Anderson et al., 1987; Bailey et al., 1988). A pair of hinging β-strands connects the domains, allowing their rotation with respect to each other. This pivoting permits an "open-jaw" or "closed-jaw" conformation of the binding cleft. When the iron-binding site is vacant, the open conformation is favored, but binding of Fe(III) promotes the closed state (Anderson et al., 1990; Grossman et al., 1992). Release of iron from transferrin is thought to be facile from the open conformation but impeded by the closed conformation, in accord with early kinetic studies of iron release from transferrin predicting release only when an apotransferrin-like conformational state of the protein is achieved (Cowart et al., 1982). We suggest, therefore, that binding of receptor to transferrin at pH 5.6, or binding of anions to the kinetically active anion-binding sites of transferrin at pH 5.6 as well as at pH 7.4, promotes accessibility of transferrin to the open conformation.

Chloride binding and receptor binding both modulate the kinetics of iron release from transferrin, and the effect of each is maintained in the presence of the other. Furthermore, anion binding remains a prerequisite for iron release even in the presence of receptor. The likelihood, then, is that the binding sites for chloride and for receptor are distinct from each other so that chloride and receptor can function in concert, neither impeding the action of the other. At present, no information is available to localize the binding site for either chloride or receptor. One possibility is that the hinging strands about which rotation of the two domains occurs are involved in binding chloride (or other simple anions), or receptor, or both. The interdomain hinging strands and sequences leading into them, representing residues 403–433 and 588–590 in the C-terminal lobe of human transferrin, are rich in charged residues, any of which is a candidate anion-binding ligand: Lys 433, Lys 434, Lys 557, Arg 568, and Lys 569, for example. Conceivably, also, binding of receptor to either or both of the connecting strands disrupts stabilizing interactions between them at pH 5.6, thereby facilitating access to the open conformation of the lobe from which release is facile. We anticipate that site mutagenesis studies will help in revealing the mechanisms of anion and receptor effects on iron release from human transferrin.

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REFERENCES

Aisen, P. (1989) in *Iron Carriers and Iron Proteins* (Loehr, T. M., Gray, H. B., & Lever, H. B. P., Eds.) pp 353–371, VCH Publishers, Weinheim, Germany.

Anderson, B. F., Baker, H. M., Dodson, E. J., Norris, G. E., Rumball, S. V., Waters, J. M., & Baker, E. N. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 1769.

Anderson, B. F., Baker, H. M., Norris, G. E., Rumball, S. V., & Baker, E. N. (1990) *Nature 344*, 784.

Bailey, S., Evans, R. W., Garratt, R. C., Gorinsky, B., Hasnain, S., Horsburgh, C., Jhoti, H., Lindley, P. F., Mydin, A., Sarra, R., & Watson, J. L. (1988) *Biochemistry 27*, 5804.

Baldwin, D. A., & de Sousa, D. M. R. (1981) *Biochem. Biophys. Res. Commun. 99*, 1101.

Bali, P. K., & Harris, W. R. (1989) *J. Am. Chem. Soc. 111*, 4457.

Bali, P. K., & Aisen, P. (1991) *Biochemistry 30*, 9947.

Bali, P. K., Zak, O., & Aisen, P. (1991) *Biochemistry 30*, 324.

Brock, J. (1985) in *Metalloproteins, Part 2* (Harrison, P. M., Ed.) pp 195–262, Verlag Chemie, Weinheim, Germany.

Cowart, R. E., Kojima, N., & Bates, G. W. (1982) *J. Biol. Chem. 257*, 7560.

Egan, T. J., Ross, D. C., Purves, L. R., & Adams, P. A. (1992) *Inorg. Chem. 31*, 1994.

Grossmann, J. G., Neu, M., Pantos, E., Schwab, F. J., Evans, R. W., Townes-Andrews, E., Lindley, P. F., Appel, H., Thies, W.-G., & Hasnain, S. S. (1992) *J. Mol. Biol. 225*, 811.

Harris, D. C., & Aisen, P. (1989) in *Iron Carriers and Iron Proteins* (Loehr, T. M., Gray, H. B., & Lever, A. B. P., Eds.) pp 239–351, VCH Publishers, Weinheim, Germany.
Katz, J. H. (1961) *J. Clin. Invest. 40*, 2143.
Kretchmar, S. A., & Raymond, K. N. (1988) *Inorg. Chem. 27*, 1436.
Lehrer, S. S. (1969) *J. Biol. Chem. 244*, 3613.
MacGillivray, R. T. A., Mendez, E., Shewale, J. G., Sinha, S. K., Lineback-Zins, J., & Brew, K. (1983) *J. Biol. Chem. 258*, 3543.
McClelland, A., Kühn, L. C., & Ruddle, F. H. (1984) *Cell 39*, 267.
Schlabach, M. R., & Bates, G. W. (1975) *J. Biol. Chem. 250*, 2182.
Turkewitz, A. P., Amatruda, J. F., Borhani, D., Harrison, S. C., & Schwartz, A. L. (1988a) *J. Biol. Chem. 263*, 8318.
Turkewitz, A. P., Schwartz, A. L., & Harrison, S. C. (1988b) *J. Biol. Chem. 263*, 16309.
Wetlaufer, D. B. (1962) *Adv. Protein Chem. 17*, 303.