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Binding Constants for Neodymium(III) and Samarium(III) with Human Serum Transferrin†

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Conditional equilibrium constants for the complexation of Nd^{3+} and Sm^{3+} by human serum transferrin in 10 mM hepes, pH 7.4, at 25 °C have been measured. Since added bicarbonate interfered with transferrin binding, apparently due to the formation of lanthanide–carbonato complexes, the solutions contained only the 0.2 mM bicarbonate that results from equilibration with atmospheric CO_2. The results are log K_1 = 6.09 ± 0.15 and log K_2 = 5.04 ± 0.46 for complexation of Nd^{3+} and log K_1 = 7.13 ± 0.24 and log K_2 = 5.39 ± 0.32 for complexation of Sm^{3+}. Linear free energy relationships for the complexation of trivalent lanthanides and actinides have been established. The relationships have been used to estimate transferrin binding constants of log $K_1 \simeq 6.5$ for both americium(III) and curium(III).

Serum transferrin is the mammalian protein whose normal function is the transport of ferric ion through the blood among sites of absorption, storage, and utilization.[1,2] Each transferrin molecule has two similar, but not identical, iron binding sites. The concomitant binding of one molecule of bicarbonate or other suitable anion at each binding site is required for the formation of a stable iron(III)–transferrin complex.

Since serum transferrin is not normally saturated with iron, it has an appreciable binding capacity for other hard metal ions that enter the blood. We are particularly interested in the role of transferrin in the metabolism of the actinides. It is known that serum transferrin carries tetravalent plutonium[3-6] as well as trivalent americium[7-9] in the blood. The high specific activity of these actinides precludes routine spectroscopic studies of their binding in vitro to transferrin. However, the strength of complexation of the actinides and lanthanides is expected to follow fairly simple trends based on the charge-to-radius ratio of the cation. Thus neodymium(III) (r = 0.983 Å) and samarium(III) (r = 0.958 Å) are expected to be good models for estimating the binding constants of americium(III) (r = 0.975 Å) and curium(III) (r = 0.970 Å).

Most studies of lanthanide–transferrin complexes have been concerned with the fluorescence properties of the lanthanide ions.[10-12] Using this type of probe, Meares and co-workers have measured the distance between the two metal-binding sites of the protein,[11] as well as the average distance of the binding sites from the outer surface of the protein.[12] Difference ultraviolet spectroscopy has also been used to detect lanthanide binding to transferrin.[10,13,14] When the metal ion displaces the proton from the binding-site tyrosine phenolic groups, there is a perturbation of the π to π^* absorption bands of the aromatic ring. This perturbation results in a strong absorbance band in the difference spectra of the metal–transferrin complex vs. apotransferrin, which can be used to calculate thermodynamic binding constants for metal ion–transferrin complexes.[15-17] This paper reports the binding constants for the transferrin complexes with neodymium(III) and samarium(III) and describes the use of a linear free energy relationship (LFER) to estimate the binding constants for americium(III) and curium(III).

Experimental Section

Human serum transferrin was purchased from Calbiochem and purified as previously described.[16] The concentration of each stock solution and the molar absorptivity of the apotransferrin at 278 nm were determined by titration with a standardized ferric ion solution containing a 2:1 ratio of nitrilotriacetic acid (NTA) to iron. C-Terminal monoferric transferrin was prepared by adding 1 equiv of $Fe^{III}(NTA)_2$ to an apotransferrin solution containing excess bicarbonate. The resulting solution was passed down a 2 × 20 cm Sephadex G-15 column to remove free NTA. N-Terminal monoferric transferrin was prepared by adding 1 equiv of ferrous ammonium sulfate to apotransferrin and was used without further purification. The ligands iminodiacetic acid (IDA), ((2-hydroxyethyl)imino)diacetic acid (HIDA), and ethylenediamine-*N*,*N*'-diacetic acid (EDDA) were commercially available with at least 98% purity and were used as received. Lanthanide binding constants for these ligands were taken from Martell and Smith.[18]

Stock solutions of neodymium and samarium chloride were prepared by dissolving weighed samples of the corresponding oxides in warm hydrochloric acid. Solutions were diluted to volume with distilled water and standardized by complexometric titration with EDTA using bromopyrogallol red as an indicator.[19]

Sample solutions were prepared by diluting the stock apotransferrin to volume with 0.010 M hepes buffer, pH 7.4. The concentration of apotransferrin in each sample was determined from the absorbance at 278 nm. Difference ultraviolet spectra were recorded on a Cary Model 219. Metal ion titrations were performed as previously described,[16] except that solutions were left at ambient bicarbonate concentration, which was calculated to be 0.2 mM on the basis of acid dissociation and Henry's law constants reported by Martell and Smith.[18]

The appropriate mass balance equations were used to calculate the molar concentrations of free metal ion, apotransferrin, and ligand on the basis of a set of initial guesses for the metal–transferrin binding constants. An absorptivity was calculated for each data point by using the equation

$$\Delta\epsilon_{calcd} = \frac{\Delta\epsilon_M K_1[M][apoTr] + 2\Delta\epsilon_M K_1 K_2[M]^2[apoTr]}{[Tr]_{tot}} \quad (1)$$

where [M] and [apoTr] refer to the molarities of free metal ion and free apotransferrin, $[Tr]_{tot}$ refers to the analytical concentration of transferrin, K_1 and K_2 are the stepwise metal-binding constants defined in eq 4 and 5, and $\Delta\epsilon_M$ is the molar absorptivity per binding site of the metal–transferrin complex in the difference UV spectrum. The two equilibrium constants were varied to minimize the sum of the squares of the residuals between the observed and calculated absorptivities.

Results

The addition of neodymium chloride to a solution of apo-

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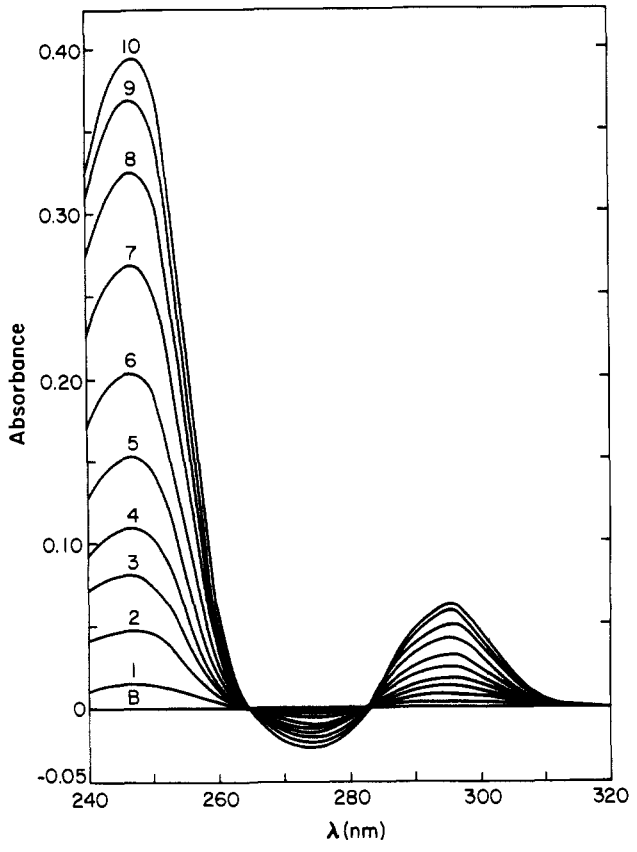

Figure 1. Difference ultraviolet spectra produced by the addition of $NdCl_3$ to 2.0 mL of apotransferrin in 10 mM hepes buffer, pH 7.4, at 25 °C with ambient bicarbonate. [Tr] = 1.637×10^{-5} M; [$NdCl_3$] = 3.30×10^{-4} M. Curve B is the base line of apotransferrin vs. apotransferrin. Other curves: (1) 5 μL Nd; (2) 15 μL; (3) 25 μL; (4) 35 μL; (5) 50 μL; (6) 70 μL; (7) 100 μL; (8) 140 μL; (9) 200 μL; (10) 290 μL.

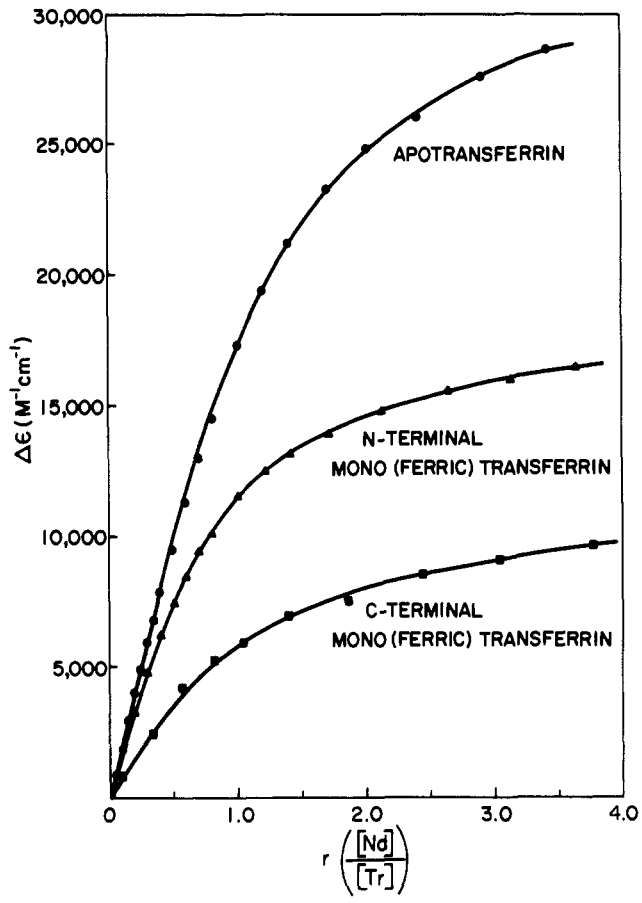

Figure 2. Titration of apotransferrin and both forms of monoferric transferrin with $NdCl_3$ in 10 mM hepes buffer, pH 7.4, at 25 °C and ambient bicarbonate. Δε is the absorbance divided by the analytical transferrin concentration at each point, and r is the ratio of total neodymium to total transferrin.

transferrin generates the series of spectra shown in Figure 1. The bands at 247 and 295 nm are characteristic of metal ion binding to the tyrosine residues at the two transferrin binding sites.[10,14,20] The absorbance at each point in the titration was divided by the analytical transferrin concentration to give a value of Δε. This procedure normalizes the data to account for variations in the concentration of transferrin from run to run. Titration curves were prepared by plotting Δε vs. r, which was defined as the ratio of total metal to the analytical transferrin concentration. Titrations of apotransferrin and both forms of monoferric transferrin are shown in Figure 2. Since neodymium would not be expected to displace ferric ion from its very stable transferrin binding sites, titrations of the monoferric transferrins were assumed to involve the binding of neodymium only at the remaining iron-free site.

If these metal ions were strongly complexed by transferrin, one would expect the titration curve to consist of two straight-line segments. The first would extend from $r = 0$ to $r = 2.0$ with a slope equal to the molar absorptivity of the transferrin complex per binding site. The second segment would continue from $r = 2.0$ with a slope near zero, since the metal-binding sites would be saturated and the free metal ions do not absorb strongly in this wavelength range.

The initial slope of the Nd–apotransferrin titration curve is linear out to an r value of 0.6 with an initial slope of 18 700 M^{-1} cm^{-1}. This slope is slightly greater than the molar absorptivity of 15 400 M^{-1} cm^{-1} previously reported for the neodymium complex of the diphenolic ligand *N,N'*-ethylenebis[(*o*-hydroxyphenyl)glycine],[14] which is frequently used as a model compound for metal–transferrin complexes. Beyond an r value of 0.6, the apotransferrin titration begins to curve downward, reaching a final value of about 28 000 M^{-1} cm^{-1} after the addition of almost 4 equiv of neodymium. Such a result is indicative of rather weak binding of neodymium(III) to serum transferrin. Even a twofold excess of neodymium fails to produce the Δε of 37 400 M^{-1} cm^{-1} that would be expected for fully formed dineodymium–transferrin. The ultimate flattening of the titration curve appears to be related to the limited solubility of neodymium carbonate, which has a K_{sp} of 10^{-33}. Thus even at the ambient bicarbonate concentration of about 0.2 mM, the free-neodymium concentration is limited to about 10^{-7} M.

The titrations of the two monoferric transferrins produce very different titration curves. When neodymium is added to the vacant C-terminal site of N-terminal monoferric transferrin, the initial slope is close to that observed for apotransferrin, but the curve levels off at about 16 000 M^{-1} cm^{-1}. This can be accounted for by relatively strong binding of approximately 1 equiv of neodymium at a site with a molar absorptivity of 18 700 M^{-1} cm^{-1}. In contrast, titration of the vacant N-terminal site begins with a much lower slope and reaches a maximum of only 10 000 M^{-1} cm^{-1}. Thus the N-terminal site appears to have a lower binding constant and/or a lower absorptivity.

Similar results were obtained from titrations with samarium chloride, as shown in Figure 3. The initial slope of the apotransferrin titration curve is linear from 0 to ~0.8 with a slope of 21 000 M^{-1} cm^{-1}. The curve for samarium binding to the vacant C-terminal site has an initial slope of ~20 000 M^{-1} cm^{-1} and reaches a plateau at about 17 000 M^{-1} cm^{-1}. Thus there appears to be fairly strong binding of samarium at the C-terminal site. Titration of the N-terminal site produces an initial slope of only 6500 M^{-1} cm^{-1}, which quickly curves downward and reaches only about 8600 M^{-1} cm^{-1} after the addition of over 4 equiv of samarium. Thus there is a clear heterogeneity in the binding of both neodymium and samarium at the two metal-binding sites of serum transferrin.

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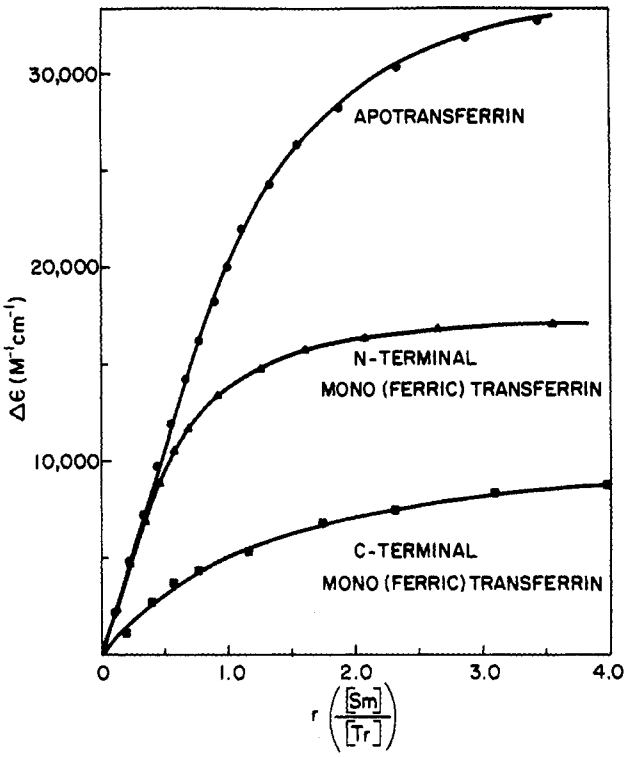

Figure 3. Titration of apotransferrin and both forms of monoferric transferrin with $SmCl_3$ in 10 mM hepes buffer, pH 7.4, at 25 °C and ambient bicarbonate. $\Delta\epsilon$ is the absorbance divided by the analytical transferrin concentration at each point, and r is the ratio of total samarium to total transferrin.

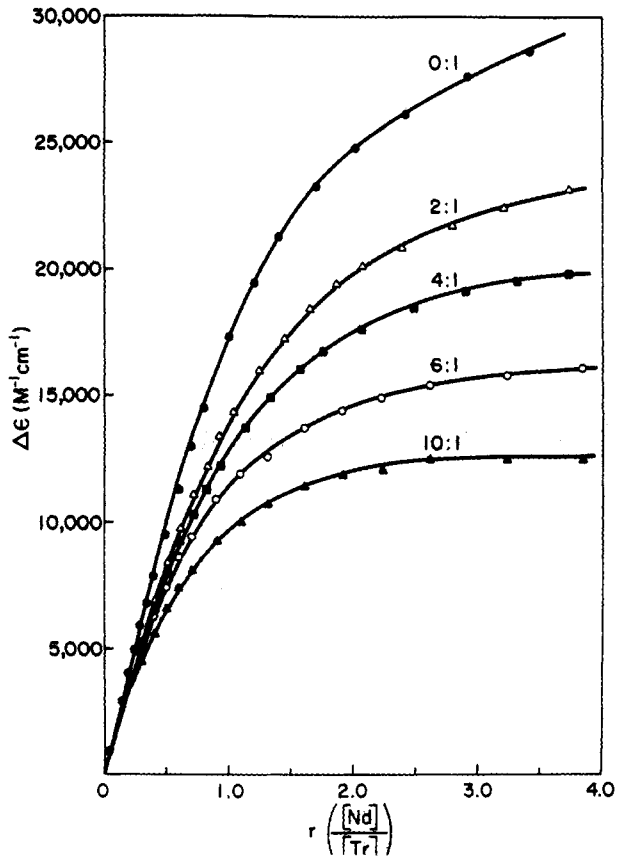

Figure 4. Titration of apotransferrin with solutions of neodymium chloride containing various concentrations of iminodiacetic acid. [Tr] $\simeq 1.5 \times 10^{-5}$ M; [Nd] = 3.30×10^{-4} M; the ratios represent [IDA]:[Nd] in the titrant. Solution conditions are the same as those for Figure 1.

Titrations were conducted with solutions of neodymium and samarium that contained various concentrations of competitive

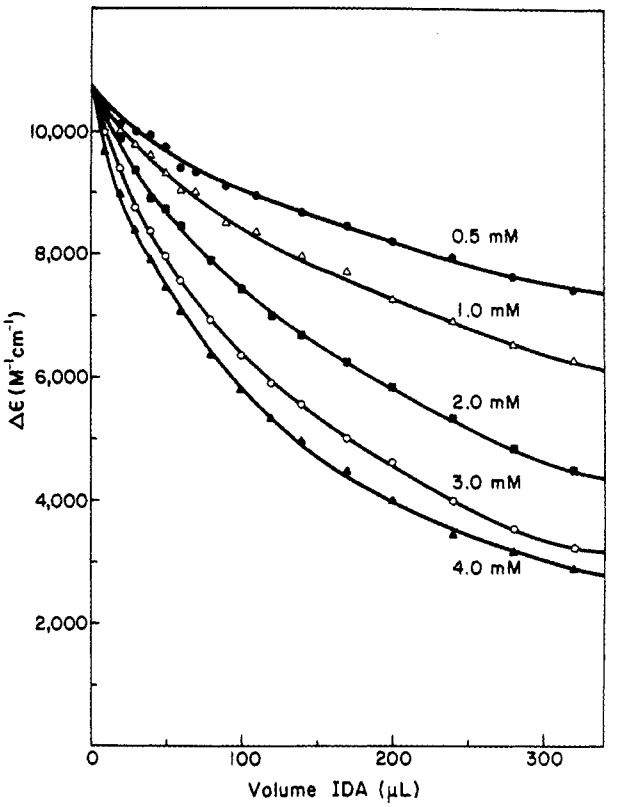

Figure 5. Titration of neodymium–transferrin solutions with a series of solutions of iminodiacetic acid. [Nd–Tr] $\simeq 1.5 \times 10^{-5}$ M; [IDA] is shown in the figure. Solution conditions are the same as those for Figure 1.

chelating agents. At any point in the titration there is a distribution of the metal ion between the transferrin and the low molecular weight chelating agent, resulting in lower values of $\Delta\epsilon$ as the ligand:metal ion ratio increases. A typical series of curves for the titration of transferrin with solutions of Nd–IDA are shown in Figure 4. Neodymium binding was also studied with HIDA and EDDA as competing ligands. Samarium binding was evaluated by using EDDA and IDA.

Titrations were also run by adding aliquots of the free ligand to a solution of the preformed metal–transferrin complex. Typical titration curves for neodymium and IDA are shown in Figure 5. Satisfactory results were obtained only when the initial neodymium:transferrin ratio was below 1. These data can be used to calculate the first stepwise metal–transferrin binding constant K_1 (eq 4). By measuring K_1 from both forward and reverse titrations, one can be assured that the results represent true equilibrium. For K_2 one can compare values obtained from forward titrations using three different ligands for neodymium and two ligands for samarium.

The binding of metal ions to transferrin almost invariably involves the displacement of protons from the protein and the concomitant binding of one molecule of (bi)carbonate per metal ion.[1,2] Kinetic studies indicate that formation of a binary transferrin–bicarbonate intermediate precedes metal binding,[21] so the overall process can be described by

$$HCO_3^- + \text{apoTr} \overset{K_C}{\rightleftharpoons} HCO_3^-\text{–Tr} \qquad (2)$$

$$M^{n+} + HCO_3^-\text{–Tr} \overset{K_{eq}}{\rightleftharpoons} M^{n+}\text{–}HCO_3^-\text{–Tr} + nH^+ \qquad (3)$$

Although the formation of a ternary metal–transferrin–bicarbonate complex has not been demonstrated specifically for neodymium and samarium, the necessity of a synergistic anion for metal binding is a well-established feature of transferrin chemistry, and it is assumed that the equilibria shown in eq 2 and 3 are valid

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Table I. Binding Constants of Neodymium(III) and Samarium(III) with Human Serum Transferrin[a]

	Nd^{3+}			Sm^{3+}		
	n[b]	log K_1	log K_2	n	log K_1	log K_2
M(IDA) + Tr	8	5.98 (5)	4.76 (7)	14	7.06 (24)	5.20 (14)
M(HIDA) + Tr	5	6.22 (10)	4.69 (17)			
M(EDDA) + Tr	9	6.10 (21)	5.48 (40)	9	7.28 (13)	6.59 (28)
M-Tr + IDA	6	5.99 (3)		8	6.91 (2)	
M-Tr + HIDA	6	6.10 (14)				
M-Tr + EDDA	9	6.18 (7)		9	7.29 (3)	
grand means	43	6.09 (15)	5.04 (46)[c]	40	7.13 (22)	5.39 (32)[d]

[a] Numbers in parentheses are the standard deviation in the least significant digit. [b] n = number of replicate titrations. [c] Based on n = 22. [d] Based on n = 23.

for both neodymium and samarium. Since the complexation equilibria described here were all measured at pH 7.4 and 0.2 mM HCO_3^-, the binding can be described by conditional constants that are valid only under these prescribed conditions. These conditional constants are expressed as

$$K_1 = \frac{[\text{M-Tr}]}{[\text{M}][\text{Tr}]} = \frac{\alpha K_{1eq}}{[\text{H}^+]^n} \quad (4)$$

$$K_2 = \frac{[\text{M-Tr-M}]}{[\text{M}][\text{M-Tr}]} = \frac{\alpha K_{2eq}}{[\text{H}^+]^n} \quad (5)$$

where [Tr] represents the sum of apotransferrin and the binary bicarbonate–transferrin species and α is the fraction of total free transferrin that exists as the binary bicarbonate species.

Values of K_1 and K_2 were calculated by least-squares techniques that varied these constants to minimize the sum of the squares of the residuals between $\Delta\epsilon_{obsd}$ and $\Delta\epsilon_{calcd}$ for both the forward and reverse titrations. This calculation requires a known value for the molar absorptivity per binding site of the metal–transferrin complexes. The initial slopes of the apotransferrin titration curves can be taken as the molar absorptivity for both sites. The resulting equilibrium constants are shown in Table I.

Discussion

Equilibrium in the transferrin titrations was approached from two different initial states, either by adding metal complex to apotransferrin or by adding free ligand to metal–transferrin. There were no significant differences in the transferrin stability constants determined by these procedures. For neodymium, the average value determined by titration of apotransferrin with metal chelates was log K_1 = 6.09 ± 0.18, compared to a value of log K_1 = 6.10 ± 0.12 determined by titration of the metal–transferrin complex with free ligand. For samarium, the values were log K_1 = 7.15 ± 0.23 and log K_1 = 7.11 ± 0.20, respectively. Thus it appears that the results do represent a true equilibrium between the metal ions and transferrin.

The attainment of this equilibrium between the lanthanides and transferrin appears to depend on maintaining the metal ions in a suitably complexed form. Titration of apotransferrin with free metal ion in the absence of any ligand gave unsatisfactory results. Problems also appeared in the reverse titrations where the initial metal:transferrin ratio exceeded about 1.0. This precluded the determination of log K_2 values by titration with the free ligand, since very little of the 2:1 metal–transferrin complexes formed when less than 1 equiv of metal ion was added.

The values of log K_2 determined by titrations with the metal chelate are much less precise than the corresponding log K_1 values. The standard deviations for titrations with each ligand are greater for K_2. In addition, there is a greater range in the values of log K_2 determined by titrations with the different competing ligands. The difficulties in determining precise values of log K_2 are ascribed to the weaker binding of the second equivalent of metal ion and to competition from the formation of lanthanide–carbonato complexes. The K_{sp} values of neodymium carbonate and samarium carbonate are 10^{-33} and $10^{-32.5}$, respectively.[18] At 0.2 mM bicarbonate and pH 7.4, one would predict formation of insoluble carbonate species instead of lanthanide–hydroxo complexes as the concentration of free metal ion increases. Initially, transferrin was titrated with neodymium in the presence of 5 mM bicarbonate, which was added to enhance the degree of formation of the ternary metal–bicarbonate–transferrin complex. However, the added bicarbonate actually reduced the degree of complexation to transferrin, as indicated by smaller $\Delta\epsilon$ values, compared to titrations with no added bicarbonate. This contrasts with the results obtained for titrations with zinc(II) and nickel(II), where increasing the bicarbonate concentration to 15 mM significantly enhanced metal binding to transferrin.[15,22]

The titration curves for neodymium and samarium both level off before reaching the theoretical maximum $\Delta\epsilon$ value equal to twice the molar absorptivity of the metal–transferrin complex. This is attributed to competition from bicarbonate, which should put an upper limit of about 10^{-7} M on the concentration of the free metal ions. A similar leveling effect observed in both the Zn– and Ga–transferrin systems[15,16] was attributed to the formation of carbonato and hydroxo complexes, respectively.

The titration curves for the monoferric transferrins indicate that the C-terminal site forms a thermodynamically more stable complex with both neodymium and samarium. The same site preference has been observed for iron(III)[24] and zinc(II).[15] Thus far, only nickel(II) has shown a thermodynamic preference for binding to the N-terminal site.[22] The procedures used to prepare the monoferric transferrins are expected to give 80–90% labeling of the desired site.[24,25] Any mixing of ferric ion between the two sites would be expected to reduce the observed differences in metal binding at the vacant sites of the monoferric transferrins. Thus the possibility of some scrambling of ferric ion between the two sites does not weaken the basic conclusion that the sites are different, with stronger binding at the C-terminal site.

Lanthanide–transferrin titration curves have been previously reported, although no binding constants were calculated.[10] The maximum absorptivities (in M^{-1} cm^{-1}) were reported to be 13 400 for Pr^{3+}, 17 000 for Nd^{3+}, 31 600 for Eu^{3+}, and roughly 37 000 for Tb^{3+}, Ho^{3+}, and Er^{3+}. It was suggested that the lower absorptivity values for the larger Pr^{3+} and Nd^{3+} ions were due to a size restriction that limited metal binding to only one of the two binding sites on the transferrin. Difference UV spectra on the Eu^{3+}–transferrin system were interpreted in terms of asymmetric binding, with coordination to two tyrosines at one site and coordination to only one tyrosine at the other.[10]

The results of the present study indicate that these previous data on lanthanide binding have been misinterpreted. The earlier studies were conducted at pH 8.5 and 5 mM bicarbonate. We have now shown that decreasing the bicarbonate concentration leads to an increase in the maximum absorptivity for Nd–transferrin from 13 400 to 30 000 M^{-1} cm^{-1}. The present results also demonstrate quite clearly that Nd^{3+} binds at both metal-binding sites on transferrin. The increase in absorptivity observed by Luk for the smaller lanthanide parallels an increase in the solubility product of the lanthanide–carbonate species.[18]

It has also been proposed that Th^{4+} binds to both transferrin sites but that it coordinates to two tyrosines at one site and only one tyrosine at the second site.[23] Since the molar absorptivity of each binding site is due to perturbations of the tyrosine aromatic groups, such asymmetric binding would lead to inequivalent absorptivities for the two sites. Although the titrations on Nd^{3+} and Sm^{3+} clearly show binding to both sites, it is still possible that size restrictions could lead to the type of asymmetric binding proposed for Eu^{3+} and Th^{4+}. The results listed in Table I were obtained by using an "equivalent" model in which both transferrin

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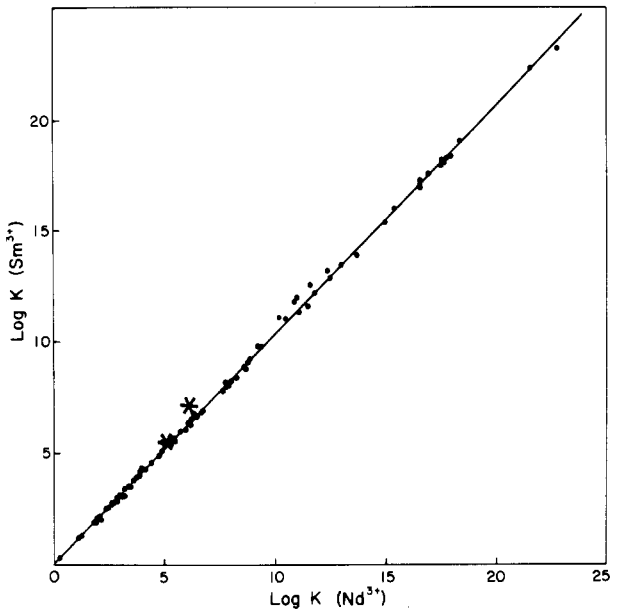

Figure 6. Linear free energy relationship for the complexation of Nd and Sm by 88 low molecular weight ligands. Asterisks represent log K_1 and log K_2 values for serum transferrin.

binding sites are assigned the same molar absorptivity. The binding data on neodymium and samarium were also refined by using a "nonequivalent" model, where the molar absorptivity of the C-terminal site was set equal to the initial slope of the apotransferrin titration curve, while that for the more weakly binding N-terminal site was set to half this value.

The least-squares refinements of the data using these two models are of comparable quality. The value for log K_1 changes very little between the two models. The principal effect of assuming the lower value for the molar absorptivity of the second site is a significant increase in the value of K_2 due to a strong negative correlation between this parameter and $\Delta\epsilon_M$. The results for the equivalent model are shown in Table I. The mean log K values calculated by using the nonequivalent model are log K_1 = 6.09 ± 0.15 and log K_2 = 5.5 ± 0.43 for Nd and log K_1 = 7.32 ± 0.22 and log K_2 = 6.01 ± 0.33 for Sm.

The nonequivalent model leads to a decrease in the separation between log K_1 and log K_2 from 1.05 to 0.59 for Nd and from 1.74 to 1.31 for Sm. Previous thermodynamic studies have shown separations between successive log K values for transferrin of 0.87 for nickel(II),[22] 0.85 for dioxovanadium(V),[17] 0.95 for gallium(III),[16] 1.3 for iron(III),[24] and 1.4 for zinc(II).[15] If the second binding site involves coordination of only one tyrosine to neodymium and samarium, one would expect much weaker complexation compared to the two-tyrosine site. This should result in a significantly larger separation in log K values. The separations between log K_1 and log K_2 with the nonequivalent model are 1.31 for Sm, which falls within the usual range, and 0.59 for Nd, which is actually smaller than those for the other metal ions. Therefore we favor the equivalent model in which the absorptivities of the two site are equal.

There may still be less drastic steric hindrance to binding of the larger cations at both transferrin binding sites. One way to assess this factor is by comparing the log K values of Nd and Sm. A linear free energy relationship between Nd and Sm constructed by using equilibrium data on 88 low molecular weight ligands is shown in Figure 6. As one would expect from the strong chemical similarity between these two cations, there is an excellent correlation between the data on these two metal ions. The relationship can be described by the linear equation

$$\log K_{Sm} = (1.033 \pm 0.003) \log K_{Nd} + 0.013 \pm 0.18 \quad (6)$$

The fit of the data to this equation is quite good, with an R factor of 0.018 and Pearson correlation coefficient of 0.9995.[25] Using eq 6 and the measured Nd–transferrin binding constants, one calculates values for the Sm–transferrin binding constants of log K_1 = 6.32 ± 0.28 and log K_2 = 5.23 ± 0.52. The difference between the log K_2 values obtained from the titrations and the LFER is not significant. However, the difference in log K_1 values (7.13 vs. 6.32) is statistically significant at $\alpha < 0.001$ due to the high precision of the LFER for these two metal ions.

Table II. Parameters for Linear Free Energy Relationships for Complexation of Trivalent Lanthanides and Actinides[a]

ref ion	unknown	n^b	slope	int	R^c	r^d
Nd	Am	18	1.084 (20)	−0.56 (55)	0.048	0.9974
Nd	Cm	13	1.107 (27)	−0.75 (67)	0.047	0.9966
Sm	Am	19	1.034 (16)	−0.17 (47)	0.043	0.9981
Sm	Cm	14	1.043 (12)	−0.21 (33)	0.025	0.9992

[a] Numbers in parentheses are the standard deviation in the least significant digit. [b] Number of data points. [c] Crystallographic R factor; $R^2 = \sum(\text{obsd} - \text{calcd})^2/\sum(\text{obsd})^2$.[26] [d] Pearson correlation coefficient.[26]

While the results from the Nd–Sm LFER suggest a size discrimination at the C-terminal site, this conclusion must remain tentative due to the small size difference of only 0.025 Å between the 8-coordinate trivalent cations. Comparable data on lanthanide cations with a larger variation in size are not yet available. An attempt was made to compare the data for Fe^{3+} and Nd^{3+}. A LFER between these two ions predicts values of $10^{13.5}$ and $10^{12.7}$ for the successive Nd-binding constants, which are about 7 log units greater than the observed values. However, the LFER relating these two cations is of much lower quality, with an R factor of 0.133 and a Pearson correlation coefficient of 0.967, and the predicted values have an uncertainty of about 2 log units. Again there is the suggestion that the binding of the larger lanthanides is hampered by size restrictions, but a more quantitative assessment is needed before any firm conclusions can be reached. Studies in this area are in progress.

LFER's have been prepared relating the binding constants of Nd^{3+} and Sm^{3+} to the limited data available on Am^{3+} and Cm^{3+}. The parameters describing these LFER's are listed in Table II. Values for the actinide–transferrin binding constants can be calculated by inserting the appropriate slope and intercept values from Table II into eq 6. The results are log K_1 values for Am–transferrin of 5.95 ± 0.67 and 7.11 ± 0.65 based on neodymium and samarium, respectively. The values for the Cm–transferrin binding constant based on the LFER's are 5.84 ± 0.79 and 7.10 ± 0.55. Simple averages of the two values for each actinide are log K_1 = 6.5 for both Am and Cm, with an estimated standard deviation of about 1 log unit.

If one accepts that the difference between the LFER estimates for each actinide is due in part to the differences in ionic radius of the two lanthanide ions, then it is reasonable to calculate the Am and Cm values by a linear interpolation between neodymium and samarium based on the ionic radii, rather than averaging. The values obtained by such a procedure are log K_1 = 6.3 ± 0.7 for Am and log K_1 = 6.5 ± 0.8 for Cm. Additional studies on other lanthanides are in progress to establish more firmly the validity of such an interpolation procedure.

(26) Hamilton, W. C. *Statistics in Physical Science*; Ronald: New York, 1964.