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Binding and transport of aluminum by serum proteins

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Contents

Abstract

There is a broad consensus that most of the aluminum in serum is bound to protein. A series of ultrafiltration studies indicate that about 85%–90% of the aluminum in normal serum is protein bound. This percentage drops to about 80% in uremic serum. It appears that most, if not all, of this aluminum is bound to the iron transport protein transferrin, which has an aluminum binding constant of about 10^{13}. The Al–transferrin complex binds to the transferrin receptor, and receptor-mediated cellular uptake appears to be an important factor in the uptake of aluminum by other tissues, including brain. There have been reports that aluminum is also bound in serum to albumin. While there is evidence for weak binding of aluminum to purified albumin, there is still no convincing evidence that albumin binds a significant amount of aluminum in serum. Administration of the iron chelator desferrioxamine (DFO) results in a sharp increase in ultrafilterable aluminum in serum. This is usually attributed to the formation of the very stable Al–DFO complex. However, some research groups have proposed that the administration of desferrioxamine results in the complexation of aluminum by either an 18 kDa protein named albindin, or by an even smaller 8 kDa protein.

Keywords: Metal ion binding; Metal ion transport; Alzheimer's disease; Complexation

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1. Introduction

Aluminum has long been regarded as a rather non-toxic element. This impression follows from the fact that Al is very abundant in the Earth's crust, it is extensively used in modern technology, and we are all exposed to relatively high levels of Al every day. However, the low oral toxicity of Al^{3+} stems largely from the very poor adsorption of Al from the gut [1–5]. If this absorption barrier is bypassed, then the toxicity of Al^{3+} becomes evident. A dramatic example of this is the high rate of neurological problems observed among long-term dialysis patients [1,5–7]. These problems arise in part from the Al content of the water used to prepare the dialysis solutions [5,8–10]. Al neurotoxicity has also been observed in patients on total parenteral nutrition [11], where once again the normal protection associated with low intestinal absorption has been circumvented. The routine use of oral aluminum drugs as phosphate binders also contributes to Al toxicity in dialysis patients [12,13]. A few cases of Al-related encephalopathy have even been observed in non-dialysis patients, especially children, who are taking high doses of oral Al drugs [14–16].

There has also been considerable interest in Al generated by proposals that the accumulation of this metal in the brain might be a factor in the development of Alzheimer's disease [17–19]. There is even some evidence that treatment of Alzheimer's patients with Al chelating drugs slows the progression of the disease [20]. However, the importance of Al as a causative factor in Alzheimer's disease has been seriously challenged [1,2,21–23].

The chemical speciation of Al in serum is obviously a critical point in trying to assess which specific compounds cross the blood–brain barrier and contribute to Al neurotoxicity. Other papers in this issue will address aluminum speciation with a greater emphasis on the low molecular weight fraction. Therefore in this paper we focus on the interactions of Al with serum proteins. The primary focus will be on the serum iron transport protein transferrin, since there is convincing evidence that this protein is the major Al transport agent in serum, and the metal complexation equilibria for Al–transferrin have been studied in some detail.

2. Serum transferrin

Based on the chemical similarities between Al^{3+} and Fe^{3+}, most bioinorganic chemists would immediately suspect that transferrin is likely to be a major factor in binding Al in serum. Thus it is not surprising that numerous studies have reported the detection of Al–transferrin in serum [24–31]. Serum transferrin (Tf) is a member of a small family of iron-binding proteins. The primary function of transferrin is to carry iron, as Fe^{3+}, through the blood between sites of utilization and storage [32–36]. The transferrins are characterized by the unique requirement that metals are only bound as a ternary complex between the metal, the protein and a carbonate anion, which is referred to as the synergistic anion. In the absence of carbonate, metal binding to form a simple binary metal–transferrin complex is usually so weak that it cannot compete with hydrolysis of the free metal ion. This is especially true

for hydrolytically unstable trivalent ions such as Fe^{3+} and Al^{3+}. Thus the addition of unchelated ferric ion to transferrin under carbonate-free conditions leads to the formation of insoluble $Fe(OH)_3$, rather than a binary Fe–transferrin complex.

Several crystal structures of transferrins have appeared recently [37–40]. Although transferrin consists of a single polypeptide chain, the protein folds into two distinct lobes, each of which contains one high-affinity metal binding site. In the case of the ferric complex, the metal ion is six-coordinate but is significantly distorted from a regular octahedral coordination geometry. The first coordination sphere contains two phenolate groups from tyrosine side-chains, one imidazole ligand from a histidine side-chain and one carboxylate ligand from an aspartic acid side-chain. The fifth and sixth coordination sites are occupied by two oxygens from the synergistic anion, which coordinates as a bidentate ligand to the iron and is also non-covalently linked to charged groups on the protein.

The concentration of transferrin in normal serum is about 37 μM [41], but since each transferrin molecule has two metal binding sites, the total binding capacity is about 74 μM. Iron binds to transferrin much more tightly than does Al^{3+} [42–44], and it would be essentially impossible for Al^{3+} to compete effectively with Fe^{3+} for the Tf binding sites. However, under normal conditions, transferrin is only about one-third saturated with iron [41]. This leaves a serum Al binding capacity of about 50 μM, well in excess of the normal Al concentration in serum which is only about 0.4 μM [2,25,29]. Even in dialysis patients, the Al concentration tends to fall in the range of 2–5 μM [25,29,45,46]. Thus there is sufficient binding capacity with transferrin to bind essentially all serum Al except at extremely high concentrations.

3. Aluminum–transferrin complexation equilibria

Because of the unique involvement of the synergistic bicarbonate anion in the formation of metal–transferrin complexes, the overall equilibrium for binding of a trivalent metal ion to transferrin is expressed as

$$M^{3+} + \text{apoTf} + HCO_3^- \rightleftharpoons M^{3+}-CO_3^{2-}-\text{Tf} + 3H^+ \quad (1)$$

The three protons released by metal binding presumably arise from deprotonation of the bicarbonate anion and the two tyrosine ligands at each metal binding site. Metal and anion binding to transferrin has traditionally been regarded as an all-or-nothing event, with neither the metal ion nor the anion binding in the absence of the other. While it is true that most metal ions will not bind to apoTf in the absence of bicarbonate, it is now clear that bicarbonate binds to apoTf in the absence of a metal [47,48]. Bicarbonate–apoTf binding constants of $10^{2.5}$ for both the N- and C-terminal sites have been reported [47]. Furthermore, kinetic studies of the formation of ferric transferrin by the binding and subsequent oxidation of ferrous ion have shown that the binary carbonate–Tf species forms first, followed by complexation of the ferrous ion [49].

Most experimental studies of Tf binding report conditional or effective binding

constants which are valid only for a specific pH value and bicarbonate concentration. Since the metal ion is actually in equilibrium with a mixture of true apoTf and the binary HCO_3^-—Tf species, one can define [apoTf]′ as the sum of [apoTf] and [HCO_3^-—Tf]. The effective binding constant for the first equivalent of metal ion then becomes

$$K_1^* = \frac{[M{-}CO_3{-}Tf]}{[M][apoTf]'} \tag{2}$$

Since K_1^* is also conditional with respect to the solution pH, hydrogen ions are not explicitly included in Eq. (2). Similarly, for the binding of the second metal ion, one can define [$M{-}CO_3{-}Tf$]′ as the sum of [$M{-}CO_3{-}Tf$] and [$M{-}CO_3{-}Tf{-}HCO_3$], so that the effective binding constant for the second equivalent of metal ion becomes

$$K_2^* = \frac{[M{-}CO_3{-}Tf{-}CO_3{-}M]}{[M{-}CO_3{-}Tf]'[M]} \tag{3}$$

Since the bicarbonate–apoTf equilibria have been characterized, we can now treat the overall complexation reaction shown in Eq. (1) as two sequential equilibria.

$$HCO_3^- + apoTf \overset{K_C}{\rightleftharpoons} HCO_3^- - Tf \tag{4}$$

$$HCO_3^- - Tf + Al^{3+} \overset{K_M}{\rightleftharpoons} Al^{3+} - CO_3^{2-} - Tf \tag{5}$$

It is known that reactions (4) and (5) release a total of three protons [50]. However, it is not clear how the overall proton release is divided between the two sequential reactions. Both K_C and K_M are effective constants for pH 7.4, and hydrogen ions are not explicitly included in Eqs. (4) and (5).

The advantage of describing metal complexation by the sequential equilibria shown in Eqs. (4) and (5) is that the value of K_M is now independent of the solution bicarbonate concentration and provides a common frame of reference for comparing results from experiments conducted under different conditions. The addition of bicarbonate will increase the effective binding constant by shifting the equilibrium shown in Eq. (4) to the right, increasing the degree of saturation of the metal-free sites with bicarbonate. However, the effective binding affinity is not linearly related to the bicarbonate concentration. Instead, the constants K_M and K_1^* are related [51] by the equation

$$\log K_1^* = \log K_M + \log \alpha \tag{6}$$

where α is defined as

$$\alpha = \frac{K_C[HCO_3]}{1 + K_C[HCO_3]} \tag{7}$$

A similar set of equations can be used for K_2^*. Given that the value of $\log K_C$ is 2.5

for both Tf binding sites [47], one can easily convert experimental values of K^* into K_M values.

The Al–transferrin complex lacks the strong charge transfer transition that gives ferric transferrin its characteristic red color. Thus metal binding is usually followed via small changes in the UV spectrum that results from perturbation of the aromatic bands associated with the coordinated tyrosine residues. After a baseline of apoTf vs. apoTf has been recorded, the addition of Al^{3+} to the sample cuvette produces a characteristic difference spectrum, with a major peak near 240 nm and a broader, smaller peak around 290 nm. This type of spectrum is characteristic of the binding of essentially any metal ion, except in cases where there are overlapping metal–ligand charge transfer bands [52,53].

The sequential addition of Al^{3+} titrant produces a family of spectra such as that shown in Fig. 1 [44]. Titration curves are prepared by plotting absorptivity $\Delta\varepsilon$ vs. the equivalents of metal added. It is also possible to prepare both C-terminal and N-terminal monoferric transferrin, and to titrate the vacant binding site of each of these proteins with Al^{3+} [44]. Titration curves for apoTf and both forms of monoferric Tf are shown in Fig. 2.

In plots of $\Delta\varepsilon$ vs. equivalents of Al, the initial slope of the titration curve for apoTf should be equal to the molar absorptivity $\Delta\varepsilon_M$ of the Al—Tf complex. In the study from which the data in Figs. 1 and 2 have been taken [44], the average molar absorptivity for Al—Tf was 14 800 M^{-1} cm^{-1} per binding site. The titration curve

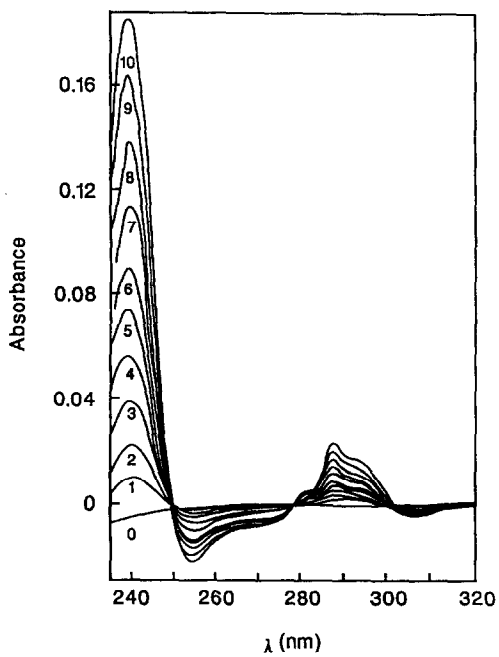

Fig. 1. Difference UV spectra generated by the addition of successive aliquots of Al^{3+} to a solution of apoTf in 0.1 M Hepes buffer at pH 7.4 and 25°C. Taken from Ref. [44].

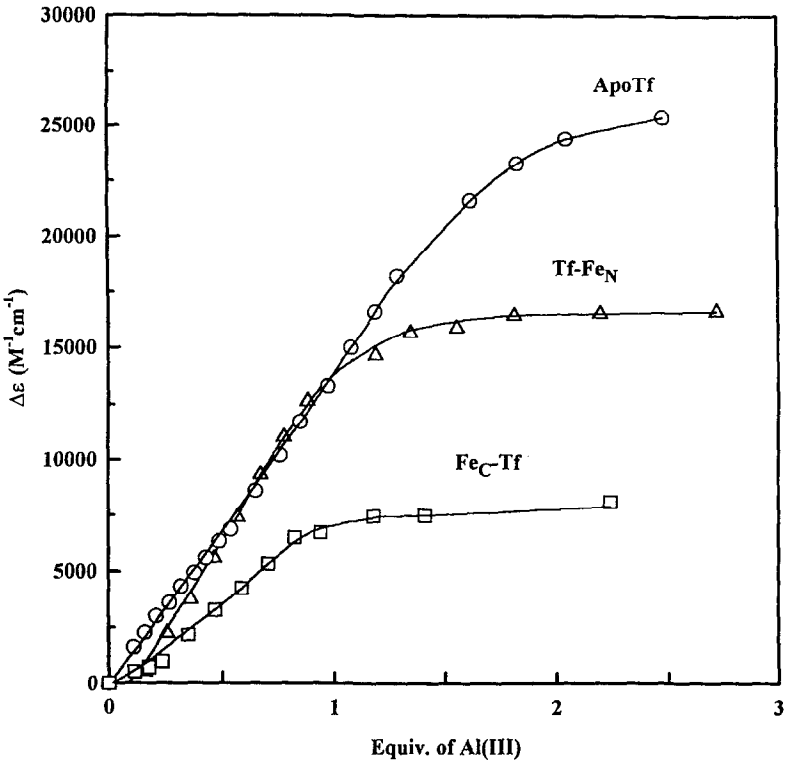

Fig. 2. Titration of apoTf (○), C-terminal monoferric Tf (□), and N-terminal monoferric Tf (△) with Al^{3+}. Δε is calculated as the absorbance at 242 nm divided by the total transferrin concentration. Data taken from Ref. [44].

for apoTf is linear out to one equivalent, which indicates that there is relatively strong binding of the first equivalent of Al. Based on the molar absorptivity of approximately 15 000 M^{-1} cm^{-1}, saturation of both binding sites at high Al concentrations would give a final absorptivity of about 30 000 M^{-1} cm^{-1}. However, the absorptivity levels off at about 25 000 M^{-1} cm^{-1}. Thus the second Al ion is bound more weakly than the first, and it is not possible to saturate both Tf binding sites with Al^{3+}.

Titrations of the monoferric transferrins confirm that one Tf binding site is stronger than the other. The titration of the vacant C-terminal site of Tf—Fe_N shows the same initial linearity as the titration of apoTf and levels off at about 16 000 M^{-1} cm^{-1}. This is consistent with the strong binding of one equivalent of Al at the C-terminal site. Conversely, the titration curve for the vacant N-terminal site of Fe_C–Tf levels off at only about 8000 M^{-1} cm^{-1}. This is consistent with weaker binding, such that competition from hydroxide becomes a significant factor.

The inability to saturate both binding sites owing to hydrolysis of the metal has also been observed with the chemically similar Ga^{3+} ion [54]. Similar competition from hydroxide and/or carbonate has been observed in the transferrin binding of

Zn^{2+}, Cd^{2+}, Nd^{3+}, Sm^{3+} and Gd^{3+} [55–58]. This competition poses no major difficulty with respect to measuring the Al—Tf binding constants. However, it must be remembered when evaluating transferrin data that the very tight binding of two equivalents of metal ion, as is observed for ferric ion, is the exception rather than the rule in transferrin chemistry, and that the addition of excess metal ion to apoTf in no way guarantees the formation of the saturated M_2—Tf complex.

An alternative explanation for the difference between the monoferric titration curves in Fig. 2 would be that both sites bind Al^{3+} relatively tightly, but that the molar extinction coefficient for the N-terminal site is only about 8000 $M^{-1}\,cm^{-1}$. While we cannot conclusively eliminate this possibility, there is considerable evidence that this is not the case. For titrations of apoTf with Al^{3+}, as well as with Ga^{3+}, In^{3+}, Zn^{2+}, Lu^{3+}, Er^{3+} and Ho^{3+}, the initial linearity in the titration curve extends beyond one equivalent of metal ion [54,55,58,59]. This strongly suggests that the molar absorptivities of the two sites are very similar. In a study on Ga^{3+}, it was actually possible to calculate K_2^* from the titration of apoTf with unchelated Ga^{3+} by assuming equal molar absorptivies and explicitly including hydroxide in the calculations as the only competing ligand [54]. This type of calculation is possible for Ga^{3+} because the free Ga actually exists as the well-characterized and well-behaved $Ga(OH)_4^-$ anion. For most other metal ions a quantitative fit of the titration curves in the absence of a competing ligand such as NTA is precluded by the poorly defined hydrolysis and polymerization reactions of the free metal.

Although these different UV data indicate stronger binding at the C-terminal site, high-resolution ^1H NMR studies on solutions at pH 8.8 have detected preferential binding to the N-terminal site [60,61]. It was suggested that the NMR data might reflect a kinetic preference for binding at the N-terminal site [61]. This proposal was based in part on an analogy with iron chemistry, where it is known that the ferric ion in normal serum appears to be kinetically trapped in the thermodynamically less stable N-terminal binding site [62,63]. Other studies of the ferric ion have shown that the relative binding affinity of the two Tf binding sites reverses between pH 7 and pH 8 [64]. The C-terminal site is favored at lower pH, while the N-terminal site is favored at higher pH. Thus an alternative explanation for the apparent discrepancy between the difference UV and NMR data is that the site selectivity for Al also changes with pH, and that both studies are detecting the thermodynamically more stable site for their respective experimental conditions.

Over the past 15 years, several sets of Al—Tf binding constants have been reported [42,44,65–68]. Two of these studies [66,67] have been repeated by the original research groups, and only the more recent results [42,68] will be discussed here. Table 1 shows the set of reported Al—Tf binding constants. To facilitate the comparison, the experimental constants have also been converted to carbonate-independent K_M values. Cochran et al. [68] explicitly included bicarbonate as a reactant as in Eq. (1). Their constants have been reformulated to conform to the definition of K_M.

The Tf binding constants fall in the range from about 10^{12} to 10^{14}. Even with this spread in the values, it is clear that the Al binding constants are well below the value of about 10^{21} for the ferric ion [43]. There is no simple explanation for the discrepancies in the literature values for Al. Variations in bicarbonate concentration appear

Table 1.
Aluminum–transferrin binding constants

Ref.	Buffer	Temperature (°C)	log K_1^*	log K_2^*	log K_{M1}	log K_{M2}
[42]	100 mM Tris 5 mM HCO_3^-	25	12.2	11.6	12.4	11.8
[99]	100 mM Tris 100 mM NaCl 25 mM HCO_3^-	25	12.23	11.73	12.28	11.78
[44]	100 mM Hepes 5 mM HCO_3^-	25	13.5	12.5	13.7	12.7
[68]	25 mM Tris 100 mM NaCl 18 mM HCO_3^-	37	13.73[a]	13.72[a]	13.80	13.79

[a] The binding constants originally reported in this paper explicitly included bicarbonate as a reagent. These constants have been reformulated to correspond to the equilibrium expression in Eqs. (2) and (3).

to play a relatively minor role. Because all the studies used at least 5 mM bicarbonate, the bicarbonate corrections associated with Eq. (6) amount to less than 0.2 log units. Variations in temperature and ionic strength could be factors. It should be noted that the experimental procedure used in Ref. [65] varies significantly from those used in the other studies. The binding constants were determined from difference UV titration titrations but, unlike the other studies [42,44,68], no competing ligand was used.

The study by Cochran et al. [68], the only one conducted at 37 °C, is unusual in that essentially identical values are reported for both K_1 and K_2. For two identical metal binding sites, statistical factors would still lead a separation of 0.6 log units between log K_1 and log K_2 [43]. Thus equal values of K_1 and K_2 actually suggest some cooperativity, whereby the binding of the first Al^{3+} ion increases the binding affinity for the second Al^{3+} ion. The other Al binding studies show separations of 0.5–1.0 log units between the successive transferrin binding constants. Separations of this size have been observed for the binding of several other metal ions, all from studies conducted at 25 °C [43,54–56,69,70]. Further studies are needed to confirm the extent to which increasing the temperature reduces the separation in log K values.

The Al^{3+} ion, with an ionic radius of only 0.535 Å [71], is significantly smaller than the ferric ion ($r = 0.645$ Å). It might be suspected that the much weaker binding of Al to transferrin reflects a steric effect, wherein the smaller Al^{3+} ion does not fit properly into the protein binding site. However, smaller Al binding constants are also characteristic of low molecular weight ligands. This is most easily illustrated by the linear free-energy relationship (LFERs) between Fe^{3+} and Al^{3+} [44,72,73]. A typical LFER based on a series of low molecular weight ligands which bind through a combination of oxygen and nitrogen donor atoms is shown in Fig. 3. This plot is described by

$$\log K_{Al} = 0.622 \log K_{Fe} + 1.53 \quad (8)$$

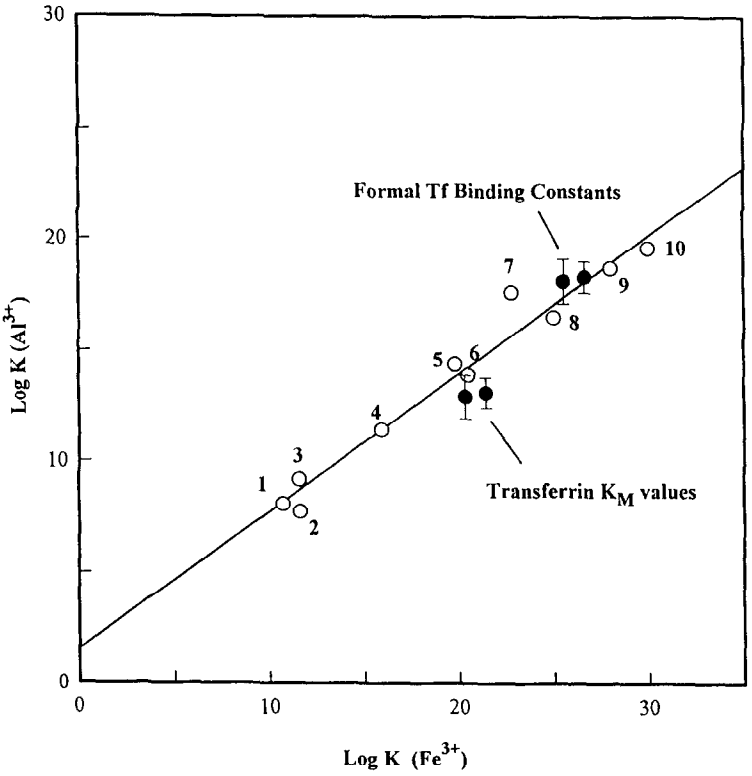

Fig. 3. LFER for the complexation of Fe^{3+} and Al^{3+}. Each data point represent a low molecular weight ligand which binds metals through a combination of oxygen and nitrogen donors. The vertical brackets show the range of the reported Al–Tf binding constants. The lower set represent values for log K_{M1} and log K_{M2} (see Eq. (5)). The higher set correspond to K_M values which have been converted from effective binding constants to formal stability constants as described in the text. The ligands included in the LFER are as follows: (1) iminodiacetic acid; (2) *N*-(2-hydroxyethyl)iminodiacetic acid; (3) sulfoxine, 1:1 complex; (4) nitrilotriacetic acid (nta); (5) *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N*′,*N*′-triacetic acid (hedta); (6) (1,10-diaza-4,7-dioxadecane-1,1,10,10-tetraacetic acid (egta); (7) sulfoxine, 2:1 complex; (8) ethylenediaminetetraacetic acid (edta); (9) diethylenetriaminepentaacetic acid (dtpa); (10) *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (cdta).

Similar values for the slope and intercept have been reported in other studies [44,73]. Based on this equation, log K for Al^{3+} for a typical ligand is only about 70%–80% of log K for Fe^{3+}. Based on a value of $10^{21.4}$ for the K_M value for Fe^{3+} [53], we would predict an Al—Tf binding constant of about $10^{14.8}$. Thus the experimental log K_M values fall slightly below the line in Fig. 3.

These LFERs are based on formal stability constants, which are always expressed in terms of the fully deprotonated form of the ligand. Martin [72] has pointed out that the effective binding constants for transferrin should be adjusted to account for the degree of protonation of the protein ligating groups. In Tf the Al is coordinated to two tyrosine residues, which would have an estimated pK_a of 10. Thus to con-

vert the effective binding constants at pH 7.4 to more formal stability constants, one would add twice the net difference between the pK_a and the solution pH ($(10 - 7.4) \times 2 = 5.2$ log units). These formal Tf binding constants bracket the line in Fig. 3. Thus essentially all of the difference of 8 log units between the Tf binding constants of Al and Fe appears to be due to the usual difference in coordination chemistry of these two metal ions. There appears to be little or no specific steric effect associated with the protein binding site. A similar LFER analysis of Cd^{2+} vs. Zn^{2+} indicated that there were no steric effects associated with the binding of the larger Cd^{2+} ion [56]. However, one should be cautious concerning this type of LFER analysis. The slope and intercept of Eq. (9) are different for an $Fe^{3+}-Al^{3+}$ LFER based on ligands that bind exclusively through oxygen donor atoms [44,73]. It is not always clear which type of low molecular weight ligand is most appropriate for predicting Tf binding constants [59].

4. Serum fractionation studies

The separation and identification of the Al–protein species in serum has not been a simple task. There are several complicating factors. There is no convenient radioisotope for Al, and so serum fractionation studies must be conducted using cold Al. In addition, Al contamination is ubiquitous, which makes it especially difficult to measure low Al concentrations accurately [74]. This problem is nicely documented by Ganrot [2], who shows that the values reported for the concentration of Al in normal human serum have dropped from about 10 μM in studies performed between 1940 and 1970 down to an average of 0.4 μM for 24 studies from the early 1980s. This decline is attributed to better analytical methods and an increasing awareness of sample contamination.

4.1. Ultrafiltration

The most reliable data on the fraction of high molecular weight Al in normal serum come from ultrafiltration studies [25,29,45,75–77]. In three of these studies [45,75,76], normal serum was spiked with Al to a concentration of 2–7 μM. The other analyses were conducted on normal serum without adding any Al [25,29,77]. There is an overall average of 18% ultrafilterable or low molecular weight Al. This average is elevated by one high value of 46% from Ref. [29], with the remaining values averaging only 12% ultrafilterable Al. The high value from Ref. [29] has been challenged by Perez Parajon et al. [45] as possibly being due to Al contamination. In addition, other reports [78,79] of the percentage of ultrafilterable Al in normal serum (not spiked) have been disregarded here because the reported total serum Al concentrations are too high for normal serum, which again suggests that there are problems with sample contamination. Thus there is general agreement that about 85%–90% of the Al in normal serum is bound fairly tightly to proteins.

There have also been several ultrafiltration studies of Al in uremic serum from patients with chronic renal disease [25,29,45,46,80–85]. The concentration of Al in

these samples in typically about 4 μM. The average of 23% ultrafilterable Al is slightly larger than the value of 12% for normal serum. Two studies which include data on both normal and uremic serum report that the percentage of ultrafilterable Al^{3+} is five percentage points higher in uremic serum (15% vs. 20% [25] and 8% vs. 13% [45]).

The increase in low molecular weight Al species in uremic serum could be related to a higher total Al concentration. Both an aluminum citrate trimer $Al_3(H_{-1}cit)_3(OH)^{4-}$ [86,87] and a binuclear Al phosphate complex $Al_2(PO_4)(OH)_2^+$ [88] have been reported. Elevating the total aluminum concentration should shift the complexation equilibria in favor of these polynuclear species and thus increase the low percentage of low molecular weight Al species. However, serum fractionation studies usually show a modest negative correlation between the percentage of ultrafilterable Al and the total Al concentration [29,46,75,85]. An alternative explanation for the larger fraction of low molecular weight Al species in uremic serum is that it reflects the higher concentration of phosphate (and possibly other Al chelating agents) in uremic serum [89].

4.2. *Size exclusion chromatography*

There have been numerous attempts to obtain a profile of the Al species in serum by size exclusion chromatography (SEC). However, several problems have been associated with SEC studies on serum Al. Quite often there is an exchange of Al between the serum sample and the column packing [24,28–30,90]. In most cases the serum sample has eluted excess Al from the column, although in one study [28], in which the column had been washed with EDTA, the serum sample lost Al to the column. It is largely because of this exchange of Al between the column and the sample that greater weight is given to ultrafiltration data for determining the percentage of protein-bound Al in serum. In one SEC study by Kiersse et al. [90], in which the researchers were very careful to document 100% recovery from the column, there was approximately 25% low molecular weight Al species, in reasonably good agreement with the ultrafiltration studies described above.

The SEC analysis of the protein binding of Al in serum is further complicated by the critical role of bicarbonate in the binding of Al by TF. If the eluting buffer does not contain bicarbonate, one would expect the effective binding affinity of Tf for Al to decrease dramatically as the sample progresses down the column, leading to enhanced dissociation of the metal from Tf. Indeed, there is a consistent difference between the SEC profiles of Al in serum depending on whether or not bicarbonate has been included in the eluting buffer. In the absence of bicarbonate, more peaks and a greater proportion of low molecular weight Al species were observed [24,26,91–94]. In some cases the Al appears to be spread more or less evenly throughout the eluent fractions [24,91]. In contrast, those studies in which bicarbonate was added to the eluting buffer tend to show fewer peaks and always show the largest peak in the region where Tf and albumin elute [25,28,29,95,96]. In some cases this peak in the transferrin/albumin region is the only Al peak in the chromatogram [28,29].

5. Aluminum binding to albumin

There have been several proposals, based on SEC chromatograms that show an overlap between the elution of Al and albumin, that a portion of the Al in serum binds to albumin [26,27,92,93,97]. The two earliest reports of albumin binding were from Savory and co-workers at the University of Virginia [92,93]. However, in more recent papers, the Virginia group [1,42] and others [25,28,30,94] have pointed out that the SEC columns used in these studies were not capable of resolving albumin and Tf.

When other types of chromatography have been used, the case for the binding of serum Al^{3+} by albumin becomes much weaker. Blanco Gonzalez et al. [30] used ion exchange chromatography to separate the Al binding components of serum. Unlike SEC, ion exchange cleanly separates Tf and albumin. Their results show very strong binding to Tf and little, if any binding to albumin. In addition, two studies using immunoaffinity chromatography have reported that essentially all the protein-bound Al in serum is bound to Tf [29,98].

There are indications that Al will bind to purified albumin. Fatemi et al. [99] measured the binding of Al to albumin by ^{27}Al NMR spectroscopy. In the presence of albumin, they observed a peak at -7 ppm, quite distinct from the $Al(OH)_4^-$ peak at 80 ppm, which they assigned to an Al–albumin complex. Titration of albumin with Al^{3+} led to the binding of three Al^{3+} ions upon addition of a 10-fold excess of Al, but no binding constants were reported.

The Al^{3+} binding site on serum albumin has not been well characterized. The high-affinity Cu^{2+}/Ni^{2+} binding site of serum albumin consists of the N-terminal tripeptide sequence asp-ala-his [100]. Martin [101] has pointed out that one would not expect a metal such as Al^{3+} to bind strongly at this type of site, and a recent ^1H NMR study has now confirmed that Al^{3+} does not bind at the N-terminal Cu binding site [102]. The ^{27}Al NMR results of Fatemi et al. [99] indicate that the Al^{3+} is bound to six oxygen ligands in an octahedral site, and they suggested that the Al might be binding at Ca^{2+} binding sites. There are reports of from four to twelve Ca^{2+} binding sites on albumin, with binding constants in the range from 10^2 to 10^3 [100]. The hypothesis that Al^{3+} binds at the Ca^{2+} binding sites is consistent with a report by Cochran et al. [28] that the weak binding of Al^{3+} to albumin is eliminated by the addition of 0.2 mM Ca^{2+}.

There are also two reports of stronger binding of Al^{3+} to albumin. One the earliest studies of Al–albumin used a Chelex resin to study the binding of Al to both Tf and albumin and reported similar binding constants for both proteins [66]. However, Martin [101] has pointed out that there appear to be experimental problems with the use of Chelex as the competitive ligand. Fatemi et al. [65], using difference UV spectroscopy to monitor the degree of saturation of the transferrin, reported direct competition for the Al between Tf and albumin. They concluded that albumin was in fact a stronger Al chelator than citrate, and estimated that it should carry about 34% of the Al in serum. However, their experimental design requires the measurement of small changes in the difference spectrum of Al—Tf at 240 nm in the presence of

up to a 20-fold excess of serum albumin. It is not clear how one can accurately correct for the large background absorbance of serum albumin at this wavelength.

The Ca^{2+} sites on albumin can easily account for the relatively weak binding of about three Al^{3+} ions detected by ^{27}Al NMR [99]. Although the trivalent Al^{3+} ion would certainly bind more strongly than Ca^{2+}, it is unlikely that these sites could compete with the binding constant of 10^{13} for Tf. Thus the ^{27}Al NMR data are not inconsistent with the arguments of Martin and co-workers [42,72,101] that the binding to albumin is too weak to be significant in the presence of the competing chelating agents which Al encounters in serum.

6. Interactions with transferrin receptors

The toxic effects of Al, particularly as related to chronic renal failure, have been very well documented [1,2,5,10]. There is evidence that the binding of Al to Tf and the subsequent recognition and transport of Al—Tf by the Tf receptor is an important factor in Al toxicity. Several studies have reported that there is relatively little difference between the binding of Fe—Tf and Al—Tf to the Tf receptor [103–106]. It has also been shown in a variety of cell lines that Al uptake is enhanced by the addition of apoTf, presumably due to receptor-mediated endocytosis of the Al—Tf complex [107–109].

Of particular importance because of the neurotoxicity of Al are observations that the passage of Al across the blood–brain barrier also appears to be mediated by Tf receptors [1,105,110,111]. There is relatively little difference in the binding of Fe—Tf or Al—Tf to the Tf receptors in normal or Alzheimer's brain cells [107] or in neuroblastoma cells [106]. In addition, the regional distribution of Al within the brain matches the distribution of Tf receptors [112]. There is also a study, based on the uptake of ^{67}Ga as a model for Al, which suggests that this receptor-mediated movement across the blood–brain barrier is unidirectional, which could lead to the gradual accumulation of Al in the brain [18]. The rate of uptake appears to be high enough to account for the Al found in the brains of Alzheimer's victims [18].

One of the central themes for the computational studies of the speciation of Al in serum has been the identification of neutral low molecular weight Al species which might be responsible for transporting Al across the blood–brain barrier [88,89,113–115]. Several papers have emphasized the role of citrate as the primary low molecular weight carrier of Al [24,114–116]. However, citrate is unable to transport Al across a simple lipid bilayer [117] and does not facilitate cellular uptake of Al [108,109,118–120]. Similarly, the addition of phosphate, another possible Al carrier in serum, does not markedly enhance the cytotoxicity of Al [121]. These observations are most easily explained as resulting from hydrolysis of the neutral Al chelates of both citrate and phosphate to give anionic species at neutral pH [89]. Thus citrate and/or phosphate binding of Al in serum may actually be beneficial in terms of removing the metal from Tf, which appears to be more effective at transporting Al into the brain.

Al exposure can also result in anemia. There are conflicting reports on whether

Al—Tf inhibits cellular uptake of iron [103,109,122,123]. It appears that the primary effect of Al on iron metabolism is related more directly to inhibition of intracellular iron release from Tf and alterations in the intracellular distribution of iron [103,122,123]. It has been suggested that cells may have difficulty in removing Al from the receptor-bound Al—Tf because, unlike Fe—Tf, the Al^{3+} ion cannot be reduced to a weakly bound divalent oxidation state [103,107]. It has also been suggested that Al—Tf may down-regulate the expression of Tf receptors [122]. The presence of Tf has also been shown to enhance the inhibitory effect of Al on erythropoiesis [118,124] as well as the proliferation of bone cells [104] and lymphocytes [120].

7. Low-molecular-weight aluminum-binding proteins

In 1980, Ackrill et al. [125] reported that the clinical symptoms of Al toxicity in patients on long-term dialysis could be improved dramatically by adding the siderophore desferrioxamine (DFO) to the dialysate solution. The structure of DFO is shown in Fig. 4. The ligand coordinates trivalent metal ions through the six oxygens of the three hydroxamic acid functional groups. DFO is a powerful Al-chelating agent with a stability constant of 10^{24} [126]. Computer simulations show clearly that DFO should be able to remove essentially all the Al from serum transferrin under in vivo conditions with very little competition from serum Zn^{2+} or Ca^{2+} [75].

DFO has now become the drug of choice for treating Al toxicity. The use and efficacy of DFO therapy has recently been reviewed [5,12,127] and will not be described in detail here. In general, regular treatment with DFO has been very effective in reversing a broad range of clinical symptoms associated with Al toxicity. There is even a report that DFO therapy may slow the progression of Alzheimer's disease [20].

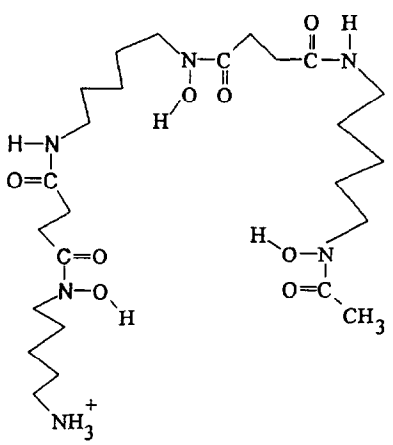

Fig. 4. Structural formula for the siderophore desferrioxamine B which is used in aluminum chelation therapy.

From the studies of DFO chelation therapy, it is very well established that the administration of DFO results in a large increase in the fraction of ultrafilterable Al in serum [25,45,82,83,96,98,127–131]. This increase is routinely attributed to the formation of the Al–DFO chelate by removal of Al^{3+} from Tf and/or mobilization of Al from other tissues. Leung and co-workers [25,96] have used SEC to study the Al species in the serum of dialysis patients before and after DFO therapy. They reported that the major new Al peak in the SEC produced by treatment with DFO did not coincide with any protein absorption at 280 nm but did coincide with the position of an Al–DFO standard measured at 214 nm.

However, the SEC peak identified as Al–DFO by Leung and co-workers [25,96] eluted well before the peak identified as Al citrate, which would be consistent with a much higher molecular weight than Al–DFO. McLachlan and co-workers [94,128] conducted similar SEC studies using a different type of column. They also find that treatment with DFO produces a large Al peak which is not associated with any strong protein absorption at 280 nm. However, in this case the new Al peak did not co-elute with an Al–DFO standard [128]. In addition, they report that this Al peak appears in the SEC of serum from workers with very high serum Al levels even without treatment with DFO [128], and that the peak can be generated by treating serum in vitro with aluminum lactate [94]. They have proposed that this peak is due to the formation of an Al complex with a small protein, which they have named albindin [94,128]. The molecular weight of albindin is estimated to be around 18 kDa [94].

There are also reports of a second small Al-binding protein [83,132]. Just as with albindin, this species is observed at high serum Al concentrations prior to DFO treatment and is greatly enhanced by DFO therapy. Ultrafiltration and SEC indicate an apparent molecular weight of about 8 kDa [83,132]. These researchers have suggested that Al may induce the de novo synthesis of the protein as a means of storing and detoxifying the metal, and that administration of DFO stimulates the release of the Al–protein complex from its storage sites, possibly as a ternary complex between the protein, Al and DFO [83].

As yet there is little characterization of these purported low molecular weight proteins beyond estimated molecular weights. Favarato et al. [94] have reported an amino acid analysis of albindin, but the results show reasonable amounts of aromatic phenylalanine and tyrosine residues, which seems inconsistent with the absence of a 280 nm absorbance. Given the absence of a UV chromophore at 280 nm in association with the Al peak and the appearance of these peaks at high Al concentrations only, one might suspect that some of the chromatography peaks assigned to a low molecular weight protein actually represent some sort of Al polymer with hydroxide and/or phosphate. Further work is needed to confirm the existence of these low molecular weight Al-binding proteins.

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