Biochem. J. (1991) 280, 527–532 (Printed in Great Britain)

Aluminium transport in blood serum

Binding of aluminium by human transferrin in the presence of human albumin and citrate

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The binding of Al^{3+} by human serum transferrin has been investigated by u.v.–visible difference spectroscopy. In the presence of 25 mM-HCO_3^- at pH 7.4, the apparent association constants were found to be 1.69×10^{12} M^{-1} and 5.36×10^{11} M^{-1}. These association constants are pH-dependent, reducing with both increasing and decreasing pH. The apparent pK_a values were found to be 6.7 and 8.2. Competitive assays of binding of Al^{3+} to transferrin in the presence of citrate and human serum albumin at molar ratios corresponding to those found in normal plasma showed that a considerable amount of Al^{3+} was not bound to transferrin. Taking a concentration of 5 μM as a typical value observed for the plasma of patients on haemodialysis [Harris & Sheldon (1990) Inorg. Chem. **29**, 119–124] the competitive binding assays indicate that ~ 60% of it is bound to transferrin, ~ 34% to albumin and the remainder to citrate. These results therefore suggest that, although transferrin at pH 7.4 is the major Al^{3+}-binding component of plasma, an appreciable amount of Al^{3+} present in patients on haemodialysis may be bound to albumin.

INTRODUCTION

Recent reports suggest that Al^{3+} may be involved in the biochemistry of various animal diseases [see Kerr & Ward (1988), Perl (1988) and references therein]. Underpinning these reports are the undoubted facts that Al^{3+} can be taken up by animals and localized at various points within their bodies where biochemical lesions occur. In the present paper we address the question: how is Al^{3+} transported in blood serum?

Al^{3+} is not very soluble in water over the pH range 5–9 unless it is bound to a water-soluble ligand (Ganrot, 1986). Blood plasma contains many small molecules that are potential ligands (May *et al.*, 1977; Bell *et al.*, 1988; Harris & Sheldon, 1990), as well as the metal-binding proteins transferrin and albumin (Aisen & Listowsky, 1980; Brock, 1985; Peters, 1985). Thus a relatively large amount of Al^{3+} can be solubilized in blood plasma.

The uptake of Al^{3+} from the blood by tissues depends upon the properties of the Al^{3+}–ligand complexes. Thus the distribution of Al^{3+} between different ligands is valuable information. A variety of techniques have been applied to investigate the speciation of Al^{3+} in serum, including calculations based on known stability constants (Martin, 1980; Harris & Sheldon, 1990) and fractionation of serum by column chromatography (King *et al.*, 1979, 1982; Bertholf *et al.*, 1984; Farrar *et al.*, 1990). These studies have given conflicting results. For example, it has been suggested that the main carrier of Al^{3+} in normal human blood is human serum transferrin (HSTF) (Trapp, 1983; Rahman *et al.*, 1986), a combination of transferrin and human serum albumin (HSA) (Bertholf *et al.*, 1984), or a combination of HSTF and citrate, with HSTF being the carrier of ~ 80% of the Al^{3+} (Martin, 1980, 1988; Martin *et al.*, 1987; Wills & Savory, 1988). Because of the current uncertainty concerning the Al^{3+} carriers, we have re-examined this topic by a spectrophotometric study of Al^{3+} binding to HSTF in the presence and absence of citrate and HSA, and as a function of pH. Our present results are consistent with the view that Al^{3+} binding to HSTF is important and that Al^{3+} binding to HSA may also be significant.

MATERIALS AND METHODS

Iron-free citric acid, Tris, EDTA and dialysis tubing (M_r cut-off ~ 12000) were obtained from Sigma (Poole, Dorset, U.K.). $NaHCO_3$, $AlK(SO_4)_2$ and an atomic-absorption (a.a.) standard of $FeCl_3$ were supplied by BDH (Poole, Dorset, U.K.).

Before use of HSTF and HSA in the optical experiments, contaminating ions were removed by the following procedure: 0.1–0.2 g of the protein was dissolved in 5 ml of distilled water and dialysed against a 1% solution of EDTA at pH 7.4 and at 4 °C for 48 h; this was followed by dialysis against distilled water at pH 7.4 and at 4 °C for a further 48 h; the resulting solution was freeze-dried with an Edwards Modulyo freeze-drier; the dried proteins were then stored at −18 °C until required. Protein concentrations were determined by the Lowry method with Sigma kit P5656; BSA was used as standard.

The atomic-absorption standard solution of $FeCl_3$ was used as supplied. Stock solutions of $AlK(SO_4)_2$ in water were adjusted to the required concentration. Where necessary, Al^{3+} and Fe^{3+} concentrations were determined by a.a spectroscopy (a.a.s.) with a Pye–Unicam a.a. spectrophotometer equipped with a graphite furnace.

The optical titrations were carried out with a Hitachi 557 double-beam spectrophotometer. For the addition of Al^{3+} and Fe^{3+} to apotransferrin, samples were prepared by dissolving 6 mg of purified HSTF in 6 ml of 0.1 M-buffer. An equal volume of apotransferrin solution was added to both the sample and reference cuvettes and a baseline of protein against protein was recorded. Al^{3+} or Fe^{3+} solution was added in 5–10 μl aliquots to the sample cuvettes, and an equal volume of distilled water was added to the reference cuvette. It took 20–30 min for the solution to reach equilibrium after each addition, judging by the A_{240}; spectra were only recorded when the A_{240} was constant. Spectra were recorded from 340 to 200 nm for Al^{3+}, and from 500 to 200 nm for Fe^{3+}, after each addition. Titration proceeded up to a 3-fold metal/HSTF molar ratio, beyond which no change was observed in A_{240}.

Abbreviations used: HSTF, human serum transferrin; Al–HSTF, aluminium–HSTF complex(es); Fe–HSTF, iron–HSTF complex(es); HSA, human serum albumin; a.a.(s.), atomic-absorption (spectroscopy).

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Titrations of Al–HSTF complex with HSA or citrate were carried out in two ways. The first series of experiments were performed by adding either citrate or albumin to the preformed complex. This was prepared, in the cuvette, by adding 2 equiv. of Al–HSTF solution to the buffer. The solution was left for 1–2 h for complete equilibration of the complex. Aliquots of citrate or albumin (5–10 μl) in pH 7.4 Tris buffer were then added to the sample cuvette, and an equal volume of distilled water was added to the reference cuvette. A spectrum was recorded after each addition. Titration was continued up to a physiological HSTF/citrate or HSTF/albumin ratio.

The second series of experiments required 2.5 ml aliquots of HSTF (7.6 μM) in 0.1 M-Tris (containing 40 mM-NaCl and 25 mM-$NaHCO_3$ at pH 7.4). These were placed in a number of sample and reference cuvettes. To each of these an appropriate amount of sodium citrate, or HSA, or both (in 0.1 M-Tris containing 40 mM-NaCl at pH 7.4) was added to give a concentration of 22 μM-citrate or 13 μM-albumin. Aliquots (5–10 μl) of 1 mM-$AlK(SO_4)_2,12H_2O$ were then added to the sample cuvettes, and an equal volume of distilled water was added to the reference cuvette, and the difference procedure above was carried out.

The effect of pH on the Al–HSTF association was investigated in two ways. Firstly, the relationship between A_{240} and pH was investigated for a solution of transferrin fully saturated with Al^{3+}. Apotransferrin solution, at the same concentration but with no Al^{3+} added, was introduced into a reference cell as a blank. The pH of the solution in the sample cuvette was adjusted by adding HCl or NaOH, and after 1 h, to allow complete equilibration of the Al–HSTF complex to occur, spectra were taken from 340 to 200 nm.

Secondly, a series of samples was prepared by dissolving 6 mg of purified HSTF in 6 ml of 0.1 M-buffer at three different pH values: 6.5, 7.4, and 8.5. The procedure for monitoring the binding of Al^{3+} to HSTF described above was then carried out for each solution.

In studying the competitive binding of Fe^{3+} and Al^{3+} to HSTF, the following procedures were employed.

Firstly, a solution of HSTF (14 μM), in 0.1 M-Tris (containing 40 mM-NaCl and 25 mM-$NaHCO_3$ at pH 7.4), was equilibrated with 2 equiv. of Al^{3+} for 1–2 h. Equal volumes of this solution were added to both the sample and a reference cell, and a baseline of Al–HSTF against Al–HSTF was recorded. Fe^{3+} solution was then added, in 5–10 μl aliquots, to the sample cuvette, and equal volumes of distilled water were added to the reference cell. Spectra were taken 1 h after each addition. Titration was continued up to a Fe^{3+}/HSTF ratio of 2:1.

Secondly, a similar titration was carried out for Fe–HSTF (2:1 molar ratio) using Al^{3+} solution as a titrant.

Thirdly, 3 ml of apotransferrin solution was added to both a sample and a reference cell, and Fe^{3+} solution was added to the sample cell up to 2 equiv. of Fe^{3+}/HSTF solution. The resulting solution was titrated with 5–10 μl aliquots of Al^{3+} solution up to an Al/Fe ratio of 50:1.

RESULTS AND DISCUSSION

Binding of Al^{3+} to HSTF

Addition of Al^{3+} to HSTF (in 0.1 M-Tris/40 mM-NaCl at pH 7.4 and at 25 °C) in the presence of HCO_3^- was accompanied by an increased absorbance in the difference spectrum at 240 and 290 nm. This is broadly in agreement with the observations of Cochran *et al.* (1984, 1987), Trapp (1983) and Tomimatsu & Donovan (1977). A typical series of spectra from 350 to 190 nm is shown in Fig. 1. A titration curve of the ratio of the measured absorbance maximum at 240 nm divided by the total HSTF concentration, to give a value for $\Delta\epsilon$, against the ratio of the total Al^{3+} concentration to the total HSTF concentration, is given in Fig. 2. The shape of the curve indicates strongly that Al^{3+} occupies both of the major metal-binding sites.

The apparent association constants were obtained from a Hill-plot analysis, taking the minimum and maximum absorbance values for the first and second Al^{3+} ions bound to be the absorbances at zero $[Al^{3+}]$ and at a Al^{3+}:HSTF ratio of 1:1, and the absorbances at a Al^{3+}:HSTF ratio of 1:1 and at 2:1. The values obtained for the two binding sites were $K_1' = 2.09 \times 10^5$ M^{-1} and $K_2' = 6.7 \times 10^4$ M^{-1}. These values assume that the Al^{3+} is distributed between HSTF-bound Al^{3+} and $[Al(H_2O)_6]^{3+}$ species only. However, this is not the case: various hydrolysed aluminium species are available. According to the procedure employed by Aisen *et al.* (1978) for Fe^{3+} binding to HSTF, the observed apparent association constants can be corrected for this by

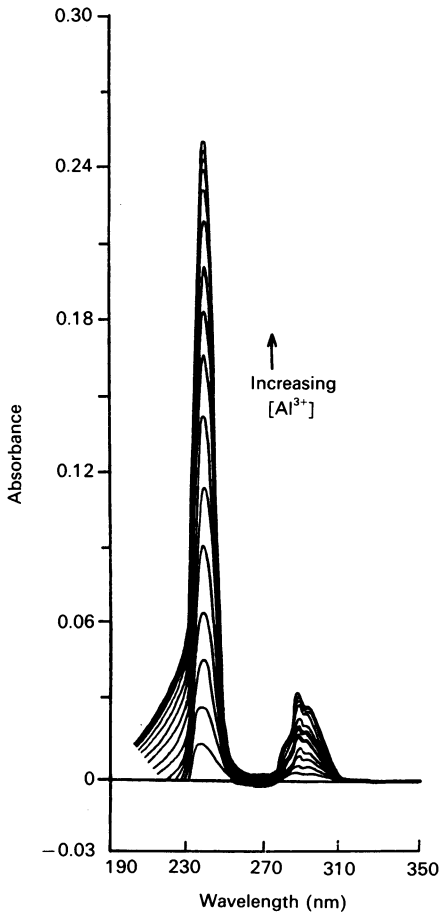

Fig. 1. A series of difference spectra for Al^{3+} binding to HSTF

Conditions were as follows: pH, 7.4; *T*, 25 °C; $[HCO_3^-]$, 25 mM; [HSTF], 10 μM; sample volume, 3 ml. The following Table shows the volumes of 1 mM-Al^{3+} added and the resulting concentration of Al^{3+} in the sample solution. Curve 16 is the topmost one.

Curve	Vol. (μl)	$[Al^{3+}]$ (μM)	Curve	Vol. (μl)	$[Al^{3+}]$ (μM)
1	5	1.66	2	10	3.32
3	15	4.97	4	20	6.62
5	25	8.2	6	30	9.90
7	35	11.53	8	40	13.15
9	45	14.77	10	50	16.39
11	55	18.00	12	60	19.60
13	65	21.20	14	70	22.80
15	75	24.39	16	80	27.55

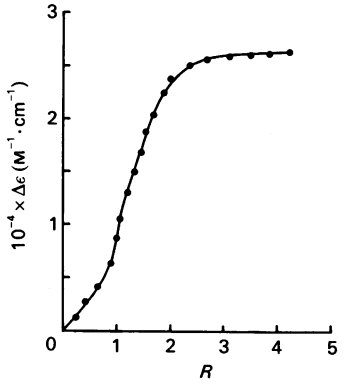

Fig. 2. Titration curve for the addition of Al^{3+} to apotransferrin

$\Delta\varepsilon$ is the absorbance at 240 nm divided by [HSTF]; R is $[Al^{3+}_t]/[HSTF]$, where $[Al^{3+}_t]$ is total Al^{3+} added.

taking account of the stepwise formation constants for the hydrolysis of $[Al(H_2O)_6]^{3+}$ as indicated by eqns. (1)–(3).

$$\alpha_{Al} = 1 + \frac{\beta_1^{OH}}{[H^+]} + \frac{\beta_2^{OH}}{[H^+]^2} + \frac{\beta_3^{OH}}{[H^+]^3} + \frac{\beta_4^{OH}}{[H^+]^4} \quad (1)$$

where

$$\beta_n^{OH} = \frac{[Al(OH)]_n[H^+]^n}{[Al(H_2O)_6]^{3+}} \quad (2)$$

$[Al(OH)_n]$ in eqn. (2) is written to indicate the number of ionized water molecules attached to the Al^{3+}. It does not indicate the total number of water molecules co-ordinated to the Al^{3+}: this is six at low pH and four at high pH.

$$K_n^* = \alpha_{Al} K'_n \quad (3)$$

where K'_n is the apparent association constant obtained from the data in Fig. 2 and K_n^* is the apparent association constant corrected for hydrolysis of the hydrated aluminium species.

Since our experiments involve relatively low concentrations of aluminium, we have disregarded polymeric hydrated species and thus considered only the species $[Al(H_2O)_6]^{3+}$, $[Al(H_2O)_5(OH)]^{2+}$, $[Al(H_2O)_4(OH)_2]^+$, $[Al(H_2O)_3(OH)_3]$ and $[Al(OH)_4]^-$. For these, using the data of Baes & Mesmer (1976), $\alpha_{Al} = 8 \times 10^6$ and therefore K_1^* and K_2^* are 1.69×10^{12} M^{-1} and 5.36×10^{11} M^{-1} respectively, and $\log K_1^* = 12.23$ and $\log K_2^* = 11.76$.

These values of $\log K_1^*$ and $\log K_2^*$ compare well with those reported by Martin *et al.* (1987) for solutions 5 mM in HCO_3^- (12.2 and 11.6 respectively), but differ from the values obtained by Harris & Sheldon (1990), also for solutions 5 mM in HCO_3^- (13.5 and 12.5 respectively) and from the values of Cochran *et al.* (1987) obtained with solutions 10 mM in HCO_3^- (both 13.5). The differences may be related to the ionic strength and composition of the solution. Apart from the HCO_3^- concentration, the NaCl concentration is different in the studies cited above, and in some of them nitrilotriacetic acid was used to complex the Al^{3+} not bound to HSTF. The concentrations of NaCl and HCO_3^- used in our study were chosen to resemble closely those present in normal serum and therefore we believe the values reported by us are the appropriate ones to use for speciation studies.

The differences between K_1^* and K_2^* for the binding of a range of metal ions by transferrin (Williams *et al.*, 1978; Brock, 1985), and the differences between K_1^* values and between K_2^* values for the same metal ions binding to transferrin and lactoferrin (Brock, 1985), may have their origin in outer-sphere effects. The X-ray structures of transferrin and lactoferrin reveal that the two metal-binding sites on each of two proteins provide the same

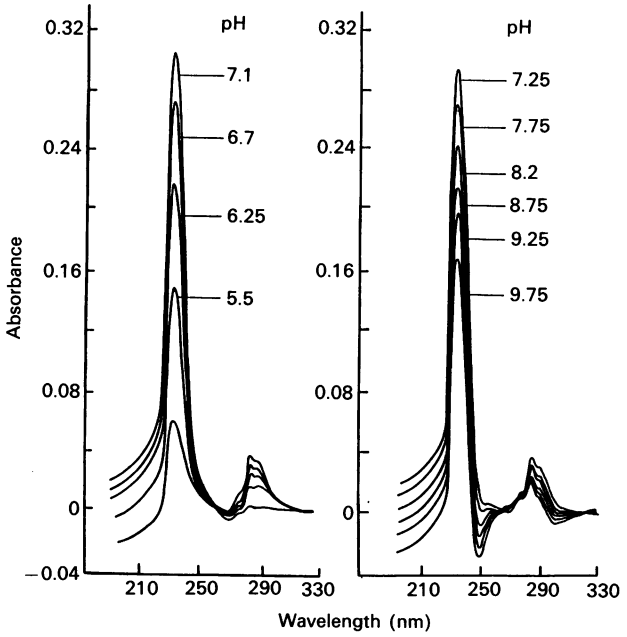

Fig. 3. Effect of pH variation on the Al^{3+}–HSTF (ratio 2:1) binding sites

Conditions were as follows: [HSTF], 11.6 μM; $[Al^{3+}]$, 23.2 μM; $[HCO_3^-]$, 25 mM.

type of groups for co-ordinating the metals (Anderson *et al.*, 1987; Bailey *et al.*, 1988). This situation resembles the variation in redox potential for haemoproteins with common haem-binding sites; for example, class I cytochromes *c*. In such cases the redox-potential variation is largely due to electrostatic effects modulating the stabilities of the Fe^{3+} ions [see Moore & Pettigrew (1990) and references cited therein]. Similarly, outer-sphere environmental effects may affect the intrinsic metal-binding constants of the transferrins.

Competitive equilibrium between Al^{3+} and Fe^{3+} for the binding sites of transferrin

The Fe–HSTF absorption band in the visible region at 470 nm was used to monitor the competitive binding of Fe^{3+} and Al^{3+} to HSTF. The u.v. region was not used because the binding of both Fe^{3+} and Al^{3+} to HSTF involves the phenolic groups of tyrosine side chains, and therefore similar absorbance changes occur in this region for both ions.

No decrease in the binding of Fe^{3+} by HSTF (concentration ratio 2:1) was observed, even though the total Al^{3+}/Fe^{3+} ratio was increased up to 50-fold. In another experiment, the presence of the Al^{3+} ion in the HSTF solution produced no effect on the titration of HSTF by Fe^{3+}. Therefore both sets of results indicate that Al^{3+} cannot displace Fe^{3+} and that Fe^{3+} can readily replace Al^{3+}.

Effect of pH on Al^{3+}–HSTF binding

The influence of pH on the Al^{3+}-binding properties of HSTF was investigated in the absence of chelating agents.

HSTF was fully saturated with Al^{3+} (in 0.1 M-Tris/40 mM-NaCl) at pH 7.4 and 25 mM-$NaHCO_3$, and the pH was then varied. A typical series of spectra, at different pH values, is shown in Fig. 3. The A_{240} of the solution decreased from the value obtained at pH 7.4 as the pH changed. The pH profile of absorbance is bell-shaped, with a maximum at about pH 7.4 (Fig. 4). This indicates that HSTF releases Al^{3+} when the pH varies from 7.4.

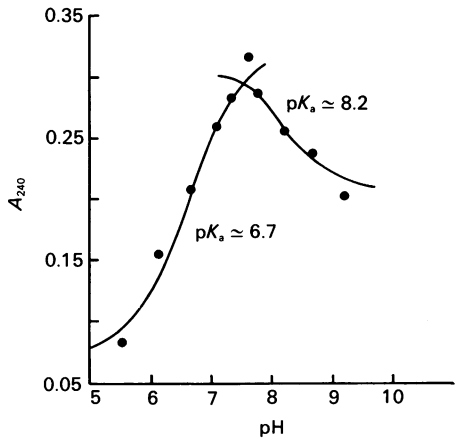

Fig. 4. pH profile of absorbance of Al^{3+} binding to HSTF

The points show the experimental values taken from the titration curve shown in Fig. 3. The continuous lines represent the theoretical curves calculated for the two pK_a values shown.

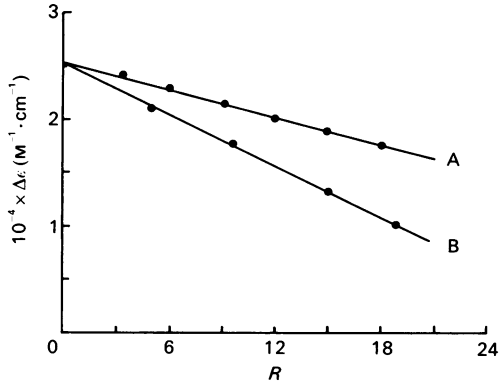

Fig. 5. Titration curves for the addition of citrate (A) and HSA (B) to Al–HSTF at ratios of 2:1

Conditions were as follows: pH, 7.4; *T*, 37 °C; [HCO_3^-], 25 mM; [HSTF], 7.6 μM. Δε is the absorbance at 240 nm divided by [HSTF]; *R* is [citrate]/[HSTF] for line A and [HSA]/[HSTF] for line B.

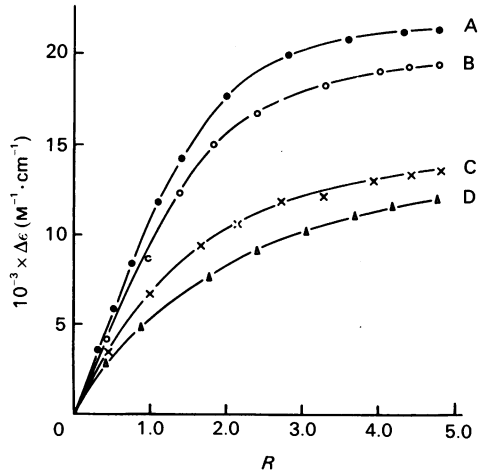

Fig. 6. Titration curves for the addition of Al^{3+} to HSTF (A), HSTF + citrate (B), HSTF + HSA (C) and HSTF + citrate + HSA (D)

Conditions were as follows: pH, 7.4; *T*, 37 °C; [HCO_3^-], 25 mM; [HSTF], 7.6 μM; [citrate], 21.7 μM; [HSA], 132 μM. Δε is the absorbance at 240 nm divided by [Al^{3+}].

Assuming that only the association constant for Al^{3+} binding to HSTF is pH-dependent, and not the 240 nm absorption coefficient of HSTF or Al–HSTF, the bell-shaped curve can be analysed to yield two apparent pK_a values: 6.7 and 8.2.

The general appearance of the acid part of the titration resembles that observed for the 470 nm absorbance of iron-saturated transferrin (Lestas, 1976). In both cases, at pH 5.5, the absorbance is reduced to ~ 25% of its maximum value, which occurs at about pH 7.5. A major difference between the two, however, is that whereas the absorbance decreases sharply as the pH is reduced below 7.5 for Al–HSTF, for Fe–HSTF the absorbance remained constant until about pH 6.4. This may be related to the fact that the Fe–HSTF study was carried out with solutions lacking HCO_3^- (Lestas, 1976). If so, this suggests the pK_a of 6.7 detected in the present Al–HSTF study may be the protonation of the bound carbonate.

Competitive binding assay of HSTF, albumin and citrate for Al^{3+}

This series of experiments was carried out in order to determine which of HSTF, HSA and citrate might be the dominant Al^{3+} binder in blood.

HSTF and citrate. A spectrophotometric titration of 14 μM-HSTF, at pH 7.4 (0.1 M-Tris/40 mM-NaCl) and 37 °C in the presence of 25 mM-$NaHCO_3$, on its own and with the addition of 100 μM-citrate, was carried out. Because of the interest in the metal-binding capabilities of HSTF in blood plasma, a similar titration was performed at the physiological concentrations of HSTF (35 μM) and citrate (100 μM).

The changes in absorbance at 240 nm (Δε) for a 2:1 [Al^{3+}_t]/[HSTF] solution, against an increasing molar ratio of citrate to HSTF were measured (Fig. 5, line A). Metal exchange reactions involving HSTF and citrate can be quite slow, so special care was taken to ensure that the results reflected the thermodynamic equilibrium, rather than kinetic factors, by carrying out experiments with citrate added to Al–HSTF, and with Al^{3+} added to a mixture of citrate and HSTF.

These results show, in good agreement with the findings of Cochran *et al.* (1987), that the citrate ion inhibits the binding of Al^{3+} to HSTF, by competing with HSTF in binding Al^{3+}. The present results show that the concentration of the Al–HSTF complex is dependent upon the molar ratio of citrate to HSTF. For example, the data indicate that the citrate ion binds to more than 40% of the total Al^{3+} present in solution at a citrate/HSTF molar ratio of 18:1, whereas it binds to only 10% at the physiological molar ratio of 3:1.

HSTF and albumin. A similar experiment was carried out to investigate the binding of Al^{3+} to HSTF in the presence of HSA. The results of this titration are shown in Fig. 5 (line B).

A comparison of the citrate and HSA results reveals that HSA binds more Al^{3+} than does citrate when both are at the same molar ratio with HSTF. These results are consistent with those of King *et al.* (1979), who found, using gel chromatography, that a large proportion of the Al^{3+} in normal serum is bound to HSA. Although HSA, at the plasma molar ratio of 15.9:1 ([HSA] = 550 μM; [HSTF] = 35 μM), has a significant effect on Al^{3+}/HSTF binding compared with citrate, HSTF still binds to about 50% of the total Al^{3+} present in the solution.

These results are in good agreement with those obtained in previous studies (King *et al.*, 1982; Trapp, 1983; Bertholf *et al.*, 1984; Farrar *et al.*, 1990) employing ultrafiltration, dialysis and gel chromatography, which indicate that Al^{3+} in serum is highly protein bound, to both HSA and HSTF, at the protein concentrations found in blood plasma. This study therefore

confirms that HSA and HSTF may serve as carriers in the biological transport of Al^{3+} in blood plasma.

HSTF, albumin and citrate. In order to define more clearly the binding of Al^{3+} to serum constituents, the affinity for Al^{3+} of a mixture of HSTF (7.6 μM) with either HSA (132 μM), or citrate (21.7 μM), or both, was investigated.

Addition of aliquots of the stock $[Al(H_2O)_6]^{3+}$ solution to the above mixtures produced a family of spectra. The absorbance maxima at 240 nm were used to calculate $\Delta\epsilon$, and titration curves were prepared by plotting $\Delta\epsilon$ against R, the ratio of $[Al_t^{3+}]/[HSTF]$. The results of the four spectrophotometric titrations are shown in Fig. 6. These suggest that HSTF, HSA and citrate ('cit' below) will all bind Al^{3+}. HSA competes more successfully than citrate. Both, however, compete with HSTF in binding Al^{3+}.

The values of K' were determined and are as follows:

$$\text{Curve A: } K'_{Al(HSTF)} = 3.2 \times 10^5\ M^{-1}$$

$$\text{Curve B: } K'_{Al(HSTF+cit)} = 2.8 \times 10^5\ M^{-1}$$

$$\text{Curve C: } K'_{Al(HSTF+HSA)} = 9.5 \times 10^4\ M^{-1}$$

$$\text{Curve D: } K'_{Al(HSTF+HSA+cit)} = 5.6 \times 10^4\ M^{-1}$$

These values need to be corrected for the non-HSTF association reactions of Al^{3+}, according to the following equations:

$$K^*_{Al(HSTF)} = K'_{Al(HSTF)} \cdot \alpha_{Al} \quad (4)$$

$$K^*_{Al(HSTF)} = K'_{Al(HSTF+cit)} \cdot \alpha_{Al} \cdot \alpha_{cit} \quad (5)$$

$$K^*_{Al(HSTF)} = K'_{Al(HSTF+HSA)} \cdot \alpha_{Al} \cdot \alpha_{HSA} \quad (6)$$

$$K^*_{Al(HSTF)} = K'_{Al(HSTF+HSA+cit)} \cdot \alpha_{Al} \cdot \alpha_{HSA+cit} \cdot \alpha_{cit} \quad (7)$$

where $\alpha_{cit.}$, α_{HSA} and $\alpha_{HSA+cit}$ account for the non-hydrolysis association reactions of Al^{3+} in expts. B–D of Fig. 6.

Values of α were calculated to be:

$$\alpha_{cit} = 1.14$$

$$\alpha_{HSA} = 3.37$$

$$\alpha_{(HSA+cit)} = 5.71$$

The data analysis shows that:

$$K^*_{Al(HSTF)} \approx K'_{Al(HSTF+HSA+cit)} \cdot \alpha_{Al} \cdot \alpha_{HSA} \cdot \alpha_{cit}$$

Therefore the effect of citrate and HSA on Al^{3+} binding to HSTF is additive, not synergistic.

General discussion

The competitive binding studies described in the present paper indicate that, although Al^{3+} binds relatively tightly to HSTF under conditions of temperature, pH and HCO_3^- concentration similar to those found for normal blood plasma, there is appreciable binding to HSA at an HSA/HSTF molar ratio similar to that in normal serum. Taking 5 μM-Al^{3+} as the total serum concentration, Fig. 6 indicates that $\sim$ 60% of it is bound to HSTF, $\sim$ 34% to HSA and the remainder to citrate. At a concentration of 7.6 μM-Al^{3+}, only $\sim$ 50% of it is bound to HSTF.

The above values for Al^{3+} binding can be compared with the speciation calculations described by Harris & Sheldon (1990). These workers took a concentration of 5 μM-Al^{3+} and calculated that 94.2% of it would be bound to HSTF in normal serum at pH 7.4. The large difference between their value and our experimental value of $\sim$ 60% is partly because we have not taken account of other serum constituents in our experiments, such as Fe^{3+}, Ca^{2+}, Mg^{2+} SO_4^{2-}, lactate and glycine, and partly because Harris & Sheldon (1990) have neglected to take account of Al^{3+} binding to HSA. This latter factor is probably the most significant. A ^{27}Al-n.m.r.-spectroscopy study has shown that HSA can bind about three Al^{3+} ions per molecule (S. J. A. Fatemi, D. J. Williamson & G. R. Moore, unpublished work). Thus even though the binding appears to be weaker than that to HSTF, since there is a large excess of HSA over HSTF, there is a large pool of potential non-HSTF Al^{3+}-binding sites in serum. Some of these HSA sites may be occupied by Ca^{2+} or Mg^{2+} in serum, as probably happens with the citrate-binding sites, but to counterbalance this $\sim$ 30% of the HSTF is saturated with Fe^{3+} (Chasteen, 1977).

The observation that, even at the relatively high concentrations of citrate used for our experiments with HSTF and HSA, comparatively little Al^{3+} is bound by it, implies that citrate is not a significant Al^{3+}-binding component in plasma. Much of the plasma citrate will be bound to Mg^{2+}, and thus not free to bind to Al^{3+}, and so our data overstate considerably the amount of Al^{3+} that will be bound to citrate in plasma.

The effect of pH on the Al^{3+} binding to HSTF is striking (Figs. 3 and 4). It appears as though small variations in pH away from 7.4 significantly reduce the binding affinity of HSTF for Al^{3+}. Unless the affinities of other potential Al^{3+}-binding components are similarly affected, then varying the serum pH will lead to a shift of Al^{3+} away from HSTF. This may be important if Al^{3+} uptake into cells is largely a result of receptor-mediated endocytosis of $(Al^{3+})_2$–HSTF.

In conclusion, it seems probable that an appreciable amount of Al^{3+} present in serum at levels observed for patients on haemodialysis [see Harris & Sheldon (1990) and references cited therein] is bound to HSA. However, at pH 7.4, HSTF probably represents the major Al^{3+}-binding component.

We thank the Wellcome Trust for post-doctoral support for one of us (F.H.A.K.) and the Science and Engineering Research Council for support of the University of East Anglia Centre for Metalloprotein Spectroscopy and Biology through its Molecular Recognition Initiative.

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Received 1 May 1991/18 June 1991; accepted 25 June 1991