Biochem. J. (1992) 287, 515–520 (Printed in Great Britain)

Interaction of aluminium citrate with horse spleen ferritin

Daniel J. DEDMAN, Amyra TREFFRY and Pauline M. HARRISON
Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2UH, U.K.

Horse spleen ferritin was found to bind aluminium poorly after equilibrium dialysis with buffered aluminium citrate solutions. Not more than 10 aluminium atoms/ferritin molecule were bound from a 25 μM-aluminium solution, pH 7.4, and the degree of binding was dependent on the method used to prepare the aluminium citrate solution. Up to 120 aluminium atoms/molecule were bound when ferritin iron cores were reconstituted by the addition of 3000 Fe atoms to apoferritin in the presence of aluminium citrate. Comparison of previously published binding constants of ferritin and citrate for aluminium suggests that, in the cell, the prevalence of small ligands effectively prevents the association of large amounts of aluminium with ferritin.

INTRODUCTION

Aluminium is known to be toxic both to plants and animals. In humans it has been implicated in Alzheimer's disease (although not necessarily as a cause) by its association with the two major neuropathological features, the neurofibrillary tangle-bearing neurons [1] and senile plaque cores, where it is present as aluminosilicate [2]. Aluminium neurotoxicity, a progressive and fatal encephalopathy, was seen in patients undergoing long-term renal dialysis who had received aluminium-containing dialysate or large oral doses of aluminium hydroxide given to limit phosphate absorption. (Aluminium-containing phosphate-binding compounds are still given to patients to decrease phosphate absorption [3].) In these patients a range of disorders are found: osteodystrophy, microcytic anaemia and parathyroid suppression.

Quantitative studies [4] have shown that transferrin, the Fe(III)-transport protein, is also the strongest binder of and therefore the ultimate carrier of Al^{3+} in the blood. Citrate, present at a concentration of 100 mM in plasma, is the most likely small Al^{3+} binder [5]. Al^{3+}–transferrin is bound efficiently by the transferrin receptor and is taken up by the rat K562 cell line almost as efficiently as Fe^{3+}–transferrin [6]. It has been shown [7] that, in renal-dialysis patients, high cortical aluminium concentrations are associated with regions rich in transferrin receptors, e.g. cerebral cortex, supporting the notion that Al^{3+}–transferrin is the means by which aluminium gains entry to cells. Al^{3+} may thus be internalized by the same mechanism as Fe^{3+}, namely receptor-mediated endocytosis of the transferrin–metal complex followed by release of the metal from the complex in an acidic non-lysosomal compartment [8,9].

Once in the cell, some of the iron may be utilized for production of haem and non-haem iron-containing enzymes, but in most animal cells the bulk of the iron is found in the storage pool as ferritin or haemosiderin [10]. Studies *in vitro* suggest that the formation of the iron core in ferritin involves the binding and oxidation of Fe^{2+} [11]. A similar mechanism could not apply to aluminium since the +2 oxidation state is not found naturally. However, a significant amount of aluminium could be incorporated into ferritin cores in place of Fe^{3+} atoms. A low-molecular-mass iron pool exists in the cytoplasm, which allows transport of iron between intracellular compartments and enables regulation of intracellular iron concentrations [10]. The exact nature of this pool is unknown, but small ligands such as citrate may be involved. Like Fe^{3+}, Al^{3+} in biological systems is expected to associate with small oxygen-donor ligands such as phosphates, nucleotides and carboxylates [5] and so there are a large number of potential Al^{3+} binders within the cell, in addition to ferritin.

In 1989, Sczekan & Joshi [12] reported the binding of about 160 aluminium atoms to horse spleen ferritin (HSF) with a $K_d = 4.5 \times 10^{-5}$ M. Similar binding affinities were reported for the binding of other metal ions (Be^{2+}, Cd^{2+}, Zn^{2+}) to HSF, phytoferritin and to synthetic iron cores. The authors concluded that ferritin was capable of acting as a detoxicant for many metal ions including Al^{3+}. Another group [13] observed the binding of 98 aluminium atoms/molecule to HSF after incubation at 37 °C with aluminium citrate-containing buffer, at pH 7.4, and also suggested that ferritin could play a protective role by binding aluminium in intestinal mucosal cells. Absorption of aluminium into the body would then be prevented through the sloughing of these cells. However, as pointed out by Martin in his excellent reviews of the bioinorganic chemistry of aluminium [14,15], it appears that many investigators have performed experiments with Al^{3+} without fully appreciating its complex solution chemistry.

In the present investigation we have attempted to verify and extend the studies mentioned above. We have examined the binding of aluminium to HSF by equilibrium dialysis. Citrate was used to approximate to the conditions *in vivo* found where the small amounts of aluminium are likely to be complexed with ligands such as carboxylates and phosphates, with which ferritin must compete. We show that the behaviour of the aluminium citrate system depends on its method of preparation and that this in turn influences the amount of aluminium binding to ferritin, apoferritin and the dialysis membrane and also the amount of aluminium retainable by a 3500-Da molecular-mass cut-off membrane. We have also examined the binding of aluminium during core formation, by adding aluminium citrate together with ferrous iron to horse spleen apoferritin (AF). Discrepancies between the results obtained here and those of previous studies are discussed with reference to the solution chemistry of aluminium.

MATERIALS AND METHODS

All chemicals were AnalaR grade. Purified HSF was obtained from Boehringer. The iron core was reduced to provide apoferritin by dialysis against 0.1 M-thioglycollic acid/0.1 M-sodium acetate, pH 4.25, at 4 °C. The buffer was changed once, after the brown coloration of the samples was no longer visible (1–2 h). The apoferritin was dialysed extensively against 9 g of NaCl/l and centrifuged at 2000 *g* for 5 min at room temperature

Abbreviations used: HSF, horse spleen ferritin; AF, horse spleen apoferritin.

to remove any denatured protein. Further traces of thioglycollic acid were removed by precipitating the apoferritin with 400 g of $(NH_4)_2SO_4$/l.

Aluminium solutions

Three methods were used to prepare aluminium citrate solutions. All stocks were prepared using Milli-Q water (Millipore).

Method A. $AlNH_4(SO_4)_2,12H_2O$ (22.7 mg) was dissolved in 90 ml of 5 mM-HCl, and 2 ml of 25 mM-trisodium citrate was added, followed by 2.313 g of solid Mops (sodium salt). The pH was adjusted with HCl to 6.8–7.4, as required, and the volume made up to 100 ml with water. This gave a stock 0.5 mM-aluminium citrate solution in 100 mM-Mops buffer (referred to as aluminium solution A).

Method B. A stock solution of 10 mM-$AlNH_4(SO_4).12H_2O$/10 mM-citric acid/200 mM-Mops (free acid), pH 2.3, was prepared. The solution was diluted 1:1 with water and then 1:4 with 100 mM-Mops buffer at the required pH, to give a 1 mM-aluminium citrate solution (referred to as aluminium solution B).

Method C. This method is the same as that described in ref. [13]. Equal volumes of stock 10 mM-$AlK(SO_4)_2.12H_2O$ (pH 3.7) and 10 mM-trisodium citrate (pH 7.9) were mixed and this solution was diluted 1:4 in 100 mM-Mops buffer. NaOH (1 M) was added to give a 1 mM-aluminium citrate solution at the appropriate pH (referred to as aluminium solution C).

The aluminium solutions A, B and C were diluted with 100 mM-Mops buffer at the specified pH at the time of the experiments.

Aluminium determination by graphite furnace atomic absorption spectroscopy

Total aluminium determination in samples was carried out by G. A. Taylor at the MRC Neurochemical Pathology Unit using a Perkin–Elmer 3030 atomic absorption spectrophotometer with HGA graphite furnace and AS40 autosampler. Aluminium concentrations were determined from an aqueous standard curve prepared from an aluminium nitrate standard solution (Spectrosol, BDH). The concentration of aluminium in a solution of 3.7 μM-aluminium in the presence of 25 μg of HSF/ml was 96% of the control value and in the presence of 100 μM-Fe was 102% of the control value.

Colorimetric aluminium assay

The colorimetric assay used was adapted from ref. [16]. It depends on the production of a colour change when aluminium complexes with the dye Catechol Violet. It was designed to measure large volumes (35 ml) of dilute aluminium solutions, e.g. river water. For our purposes the assay was scaled down 50-fold.

The following stock solutions were made: (A) 1 M-HCl; (B) 1 g of 1,10-bathophenanthrolinesulphonate/l (Sigma); (C) 0.094 g of Catechol Violet (Sigma) in 250 ml of water; (D) 150 g of hexamethylenetetra-amine (Sigma) + 8.4 ml of ammonia solution (0.880 specific gravity) in 500 ml of water.

Milli-Q water and acid-washed glassware were used throughout. Samples were prepared in 100 mM-Mops buffer, pH 6.8–7.4, or other suitable buffer. It is important that the final pH of the assay is between 6.0 and 6.2; it was therefore necessary to predetermine the amount of 1 M-HCl needed to give a final pH of 6.1. To a sample containing 1–10 nmol of aluminium in 0.6 ml of 100 mM-Mops or other suitable buffer, 50–100 μl of 1 M-HCl (to give a pH of 6.1), 20 μl of reagent B, 80 μl of reagent C, 200 μl of reagent D were added, followed by Milli-Q water to 1000 μl. A standard curve was prepared from 100 mM-stock $AlK(SO_4)_2,12H_2O$ (ACS-grade reagent from Sigma) in 0.5 g of H_2SO_4/l, diluted in sample buffer. The absorbance of the solutions was read at 582 nm.

Iron determination

Iron concentrations were measured using modifications (C. M. Morris, personal communication) of methods described previously [17,18]. The method is based on spectrophotometric determination of an Fe(II) complex with ferrozine.

Reagents were made up in Milli-Q water in acid-washed glassware. Sample (300 μl), containing 0.5–3 μg of Fe, and 300 μl of freshly prepared ascorbic acid solution (20 g/l) were mixed and incubated at 42 °C for 1 h; 300 μl of $Na_4P_2O_7$ in water (40 g/l) and 300 μl of trichloroacetic acid solution (250 g/l) were then added and the mixture was placed in a boiling water bath for 10 min. The samples were cooled and centrifuged for 5 min at full speed in an MSE Microcentaur centrifuge. Traces of iron were removed from any precipitate by resuspending it in 100 μl volumes of the ascorbic acid, $Na_2P_4O_7$ and trichloroacetic acid solutions, with heating for 10 min before re-centrifugation. The supernatants from the two centrifugation steps were combined and the total volume measured. Up to 1 ml of the supernatant was mixed with 0.5 ml of ferrozine reagent, containing 3 g of ferrozine/l (Sigma) and 3 g of neocuproine/l (Sigma) in 5 mM-HCl. Water was added to make the volume up to 2 ml and the absorbance at 562 nm was read. A solution of 100 μg of $(NH_4)_2Fe(SO_4)_2,6H_2O$/ml in 0.5 g of H_2SO_4/l was used to prepare a standard curve.

Equilibrium dialysis

Equilibrium dialysis was carried out in a Dianorm R equilibrium dialyser (MSE) and Macro 1 type cells with a working half-cell volume of 1 ml. The apparatus, which holds 20 cells, can be immersed in a temperature-controlled water bath and the cells rotated to ensure rapid equilibration times. Different concentrations of aluminium citrate in 100 mM-Mops buffer, pH 6.8 or 7.4, were placed in compartment 1 (volume 1 ml) and were allowed to equilibrate for 18 h at 25°C across a semipermeable membrane into compartment 2 (volume 1 ml) containing Mops buffer only, or Mops buffer plus protein. At the end of the experiment, the free aluminium concentration in compartment 1 was measured. In a single experiment a range of five concentrations of aluminium citrate could be used (five samples of ferritin in duplicate and five pairs of protein-free controls).

Reconstitution of ferritin

Horse spleen apoferritin was reconstituted by adding an N_2-saturated solution of ammonium ferrous sulphate, to give a final concentration of 800 μM-Fe, to 0.25 μM-apoferritin in 100 mM-Mops buffer, pH 7.0, containing 100 μM-aluminium citrate prepared by method B (see above). The mixture was incubated at 4°C overnight. Another apoferritin sample was reconstituted in the absence of aluminium and after reconstitution it was incubated overnight with 100 μM-aluminium citrate. Apoferritin was also incubated with 100 μM-aluminium in the absence of iron. After reconstitution and incubation with aluminium the samples were passed through Bio-Gel P-100 desalting columns (1.2 cm × 7.5 cm) equilibrated with 100 mM-ammonium hydrogen carbonate containing 0.02% sodium azide to separate unbound aluminium and iron from ferritin. Fractions of 0.5 ml were collected under gravity flow, and a portion of each fraction was taken for protein determination. The remainder was freeze-dried and subsequently analysed for aluminium by graphite furnace atomic absorption spectroscopy. The amount of aluminium recovered in the protein-containing fractions was used to calculate the amount of aluminium bound. In a preliminary

experiment, ferritin was reconstituted in the presence and absence of aluminium citrate and the gel-filtration fractions were analysed for protein and iron. Virtually all recoverable iron was eluted with the protein peak.

RESULTS AND DISCUSSION

Equilibration of aluminium solutions across two different dialysis membranes

Equilibrium-dialysis experiments were carried out to investigate the nature of the three aluminium citrate solutions after dilution to 50 μM-aluminium in 100 mM-Mops buffer, pH 7.4. The two compartments were separated by a regenerated cellulose dialysis membrane with apparent pore-size limit of either 3500 Da (Spectrapor 3) or 12000–14000 Da (Spectrapor 2). After equilibration against an equal volume of aluminium-free buffer, the final aluminium concentration was measured in both compartments by graphite furnace atomic absorption spectroscopy. The results are shown in Table 1 and demonstrate that all three aluminium solutions equilibrate fully across the Spectrapor 2 membrane. With the Spectrapor 3 membrane, however, more than 50% of the aluminium recovered was in compartment 1, indicating that some aluminium did not equilibrate, and was too large to pass through the membrane. The recovery of aluminium citrate prepared by method A was approximately 90% with both membranes. Recovery was lower for the other two aluminium solutions, suggesting that they bound more readily to the membrane.

Interaction of ferritin with different aluminium citrate solutions studied by equilibrium dialysis

A Spectrapor 2 membrane was used for equilibrium-dialysis experiments with ferritin because there was full equilibration of aluminium solutions at both pH 6.8 and pH 7.4. In these experiments different concentrations of aluminium citrate were allowed to equilibrate with ferritin in aluminium-free Mops buffer. The free aluminium concentration (compartment 1) after correction for aluminium binding to the membrane showed a linear relationship with the initial aluminium concentration. The differences between the slopes of the best-fit lines of the protein-containing samples and protein-free controls were used to calculate the aluminium bound to the ferritin, expressed as a proportion (%) of the aluminium added. Figs. 1(*a*)–1(*c*) compare the binding of the three aluminium citrate solutions to HSF at pH 7.4. It can be seen that HSF binds aluminium from solutions B and C (37.0% and 41.4% of added aluminium bound respectively) more readily than from solution A (13.8% of aluminium added bound). AF (Fig. 1*d*) bound 19.5% of aluminium added from solution C which is approximately half that bound by holoferritin under the same conditions (Fig. 1*c*). Interestingly, the observed pattern of binding to HSF is similar to the binding to the dialysis membrane, as reflected in the recovery rates for the different aluminium solutions shown in Table 1. The two preparations that had higher binding to the membrane (B and C) also gave higher binding to HSF.

Scatchard plots were not suitable because of the small number of data points and the finding that the amount of aluminium bound to ferritin was proportional to the initial concentration of aluminium. Thus Scatchard plots gave lines which were parallel to the horizontal axis, implying the existence of an infinite number of binding sites. In studying binding of Al^{3+}, and other metals, to animal and plant ferritins and synthetic iron cores using a micropartition method, Sczekan & Joshi [12] observed two types of binding, which were consistent with the existence of high-affinity ($K_d = 10^{-5}$–10^{-7} M) metal-binding sites on the core and low-affinity binding sites attributed to aggregation and autoprecipitation on the core surface. This latter type of binding is similar to that reported here. Scezekan & Joshi [12] report a K_d of 4.5×10^{-5} M for binding of aluminium chloride solutions to HSF; however, at the pH used (pH 6.0), these dissociation constants would have to be lowered by a factor of 16 if $Al(H_2O)_6^{3+}$ was the species bound [14]. Martin also pointed out that K_d values that are close to or exceed the concentration of free Al^{3+} (1 μM) allowed by the solubility of $Al(OH)_3$ are unreliable. The stability constant for the 1:1 aluminium citrate complex is approx. 10^8 M^{-1} [15] which is equivalent to a K_d of 1×10^{-8} M. It is therefore clear that citrate will compete strongly with the high-affinity sites of the ferritin core for Al^{3+}. The presence of citrate in the aluminium solutions may therefore have prevented the binding of aluminium to ferritin. The observed binding may have been due to the aggregation of poly(hydroxyaluminium) and citrate-containing species small enough to traverse the 3-fold or 4-fold channels in the protein shell (0.3–0.4 nm), and bind to the iron core and, to a lesser extent, to the protein. Binding of aluminium to the dialysis membrane and to AF was observed, although this was always less than that to HSF under the same conditions.

The solution chemistry of aluminium is complex. At pH < 5, Al^{3+} exists as the octahedral $Al(H_2O)_6^{3+}$, but as the pH is raised successive deprotonations of water ligands lead to the precipitation of $Al(OH)_3$ which redissolves at alkali pH as a result of the formation of $Al(OH)_4^-$. The insolubility of $Al(OH)_3$ limits the total free mononuclear Al^{3+} concentration in aqueous solution to 8 μM at pH 7.4. At this pH most of the aluminium will be in the $Al(OH)_4^-$ form and the concentration of $Al(H_2O)_6^{3+}$ will not exceed 3×10^{-12} M. Since citrate binds free $Al(H_2O)_6^{3+}$, the concentration of this species will be even less in the presence of citrate. Once the solubility limit is exceeded, polynuclear species of aluminium will form in a time-dependent manner [19], even in the presence of citrate [20]. An equimolar solution of Al^{3+} (of more than a few micromolar) and citrate at pH 6.8–7.4 may therefore contain various concentrations of mononuclear aluminium species, principally $Al(OH)_4^-$ and the 1:1 aluminium citrate complex (in various states of protonation) together with polynuclear hydroxyaluminium and aluminium citrate complexes. Equilibrium dialysis of aluminium citrate solutions

Table 1. Equilibrium dialysis of aluminium citrate solutions using Spectrapor 2 and Spectrapor 3 membranes

Aluminium citrate (50 μM) in 100 mM-Mops buffer, pH 7.4, prepared as described in the Materials and methods section was placed in compartment 1 (volume 1 ml) and allowed to equilibrate for 18 h at 25 °C against 1 ml of aluminium-free buffer in compartment 2. Spectrapor 3 has a molecular-mass cut-off of 3500 Da and Spectrapor 2 has a cut-off of 12000–14000 Da. The aluminium concentration was determined in both compartments by graphite furnace atomic absorption spectroscopy. The amount recovered in compartment 1 is expressed as a percentage of total aluminium recovered from both compartments; a value of 50% indicates complete equilibration.

Dialysis membrane	Aluminium citrate solution	Percentage of recovered aluminium in compartment 1	Percentage of total aluminium recovered
Spectrapor 3	A	62.1±0.4	90.9±3.2
Spectrapor 3	B	63.9±1.3	69.0±2.4
Spectrapor 3	C	59.2±2.3	61.3±8.9
Spectrapor 2	A	49.6±3.5	90.3±4.0
Spectrapor 2	B	49.8±0.5	74.0±1.6
Spectrapor 2	C	49.6±0.4	74.1±4.0

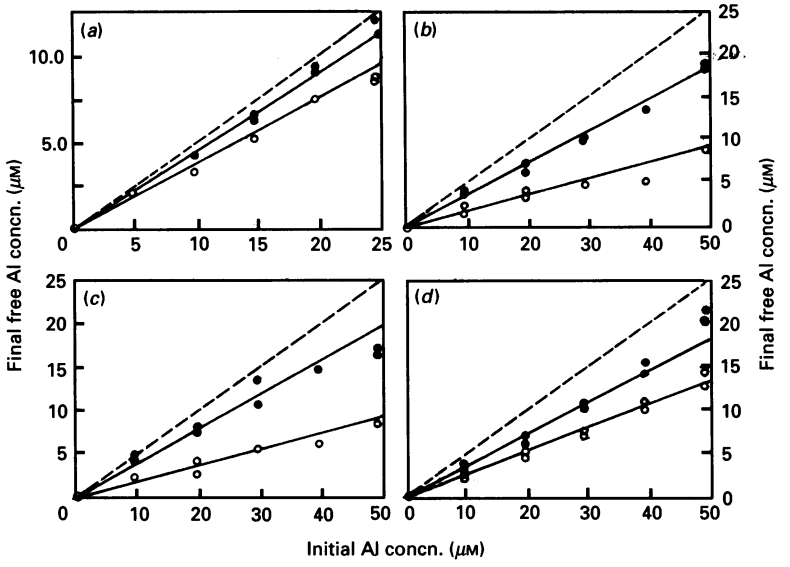

Fig. 1. Equilibrium dialysis of HSF and AF against aluminium citrate

Mops buffer (100 mM; 1 ml) at pH 6.8 or 7.4 containing aluminium citrate in the range of concentrations shown (Initial Al concn.) was placed in compartment 1 and allowed to equilibrate for 18 h at 25 °C against 1 ml of aluminium-free buffer containing HSF (*a–c*) or AF (*d*) (○) or against buffer only (●) in compartment 2. The membrane used was Spectrapor 2 with a molecular-mass cut-off of 12000–14000 Da. After equilibration for 18 h at 25 °C the free aluminium concentration (in compartment 1) was measured and plotted against initial aluminium concentration. The proportion of aluminium bound by the protein was then determined. The broken line (----) represents the expected final free aluminium concentration if there is complete equilibration of the solutions between both compartments and complete recovery. Different aluminium solutions were used by: (*a*) 2 μM-HSF, aluminium solution A, pH 7.4; (*b*) 2 μM-HSF, aluminium solution B, pH 7.4; (*c*) 2 μM-HSF, aluminium solution C, pH 7.4; (*d*) 2 μM-AF, aluminium solution C, pH 7.4.

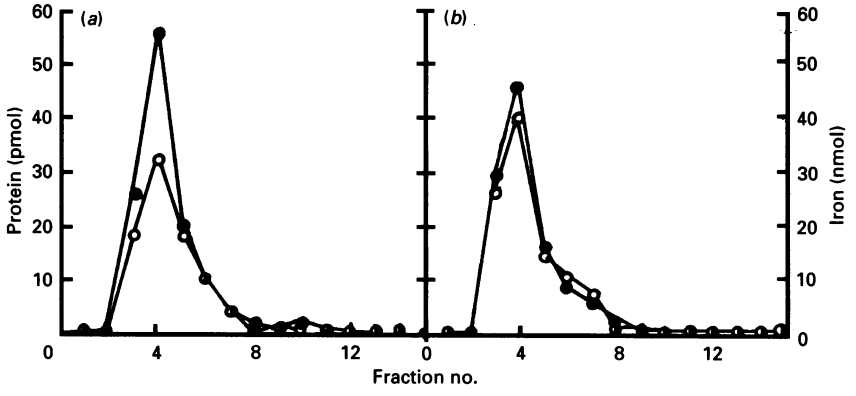

Fig. 2. Reconstitution of ferritin in the presence and absence of aluminium citrate

Ferritin was reconstituted by adding ferrous ammonium sulphate (final concentration 500 μM) to 0.25 μM-AF (0.5 ml in 100 mM-Mops buffer, pH 7.0) in the presence (*a*) or absence (*b*) of 25 μM-aluminium citrate prepared by method B (see the Materials and methods section). After 18 h at 4 °C the samples were fractionated on Bio-Gel P-100 desalting columns and the first 15 × 0.5 ml fractions were analysed for iron (●) (right scale) and protein (○) (left scale).

suggests that different preparation methods may affect the species distribution in those solutions. In method A, the addition of sodium salts of citric acid and Mops to an acidified $AlK(SO_4)_2,12H_2O$ solution raises the pH to around 8, favouring the formation of $Al(OH)_4^-$ which will tend to polymerize, as has been shown with Fe(III)–citrate solutions [21]. Polynuclear species may be stabilized by citrate and not redissolve even when the pH is lowered to 7.4 or 6.8. By contrast, in methods B and C, aluminium solutions were kept at low pH (< 4) until required. $Al(H_2O)_6^{3+}$ is the predominant free form of aluminium below pH 5 and polynuclear species formed at low pH may be different from those formed from $Al(OH)_4^-$. If polynuclear species are bound by ferritin, then differences in species distribution of aluminium prepared by different methods would influence the amount of binding. Binding would also show some pH-dependence since the pH will affect the relative proportions of different species in the solution [14,21]. Solutions B and C may show more binding to the core because they contain a higher proportion of species which can assess the ferritin core.

Interaction of ferritin with aluminium citrate during reconstitution

Fig. 2 shows the elution pattern of ferritin reconstituted in the presence and absence of 25 μM-aluminium citrate prepared by method B. Virtually all the recoverable iron was associated with the protein peak in both cases. Ferritin was also reconstituted by adding Fe(II) to a final molar excess of 3200-fold over apoferritin in the presence of 100 μM-aluminium citrate (a 400-fold molar

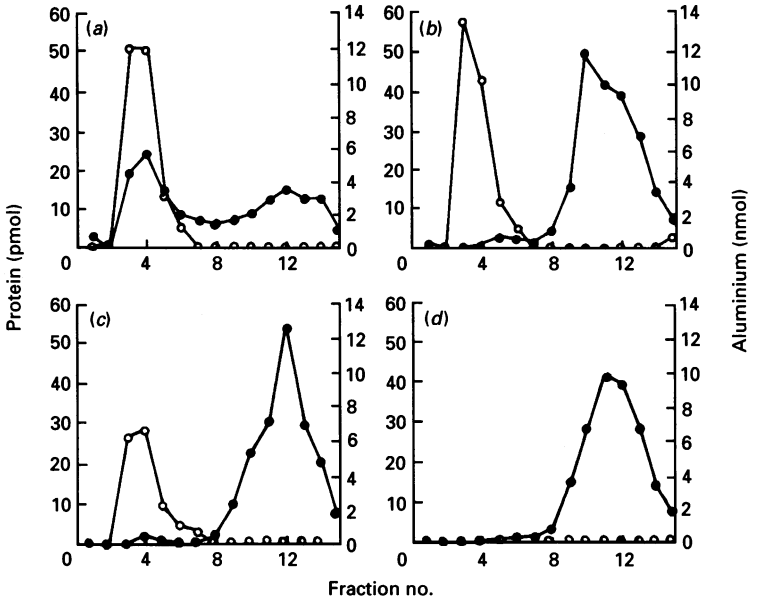

Fig. 3. Reconstitution of ferritin from apoferritin in the presence and absence of aluminium citrate

Ferritin was reconstituted by adding ferrous ammonium sulphate (final concentration 800 μM) to 0.25 μM-AF (0.5 ml in 100 mM-Mops buffer, pH 7.0). Reconstitution was carried out in the presence of 100 mM-aluminium citrate prepared by method B (see the Materials and methods section) (*a*); alternatively ferritin was reconstituted and subsequently incubated with 100 μM-aluminium citrate (*b*). Controls containing AF and aluminium (*c*) or aluminium-containing buffer only (*d*) were also treated in the same manner. Unbound iron and aluminium were removed by gel filtration on Bio-Gel P-100 columns equilibrated with 100 mM-ammonium hydrogen carbonate/0.2 g of sodium azide/l and 15 × 0.5 ml fractions were analysed for protein (○) (left scale) and aluminium (●) (right scale).

excess over protein). Reconstituted ferritin and apoferritin were also incubated with 100 μM-aluminium citrate and these samples were fractionated on Bio-Rad P-100 columns as described and the fractions analysed for protein and aluminium content. The results are shown in Fig. 3. It was found that ferritin reconstituted in the presence of aluminium citrate contained, on average, 120 aluminium atoms/molecule. In contrast, apoferritin and reconstituted ferritin only bound 7.6 and 9.5 aluminium atoms respectively on incubation with 100 μM-aluminium citrate for 18 h. Thus it is obvious that aluminium can be incorporated into a growing iron core. However, there is no need to postulate that the binding in this case is different from the non-specific binding seen in equilibrium-dialysis experiments, but once bound and covered by the growing core, aluminium is trapped and cannot dissociate freely unlike surface-bound aluminium.

Implications for the binding of aluminium to ferritin *in vivo*

Details of the interaction between ferritin and aluminium *in vivo* remain largely unknown. Studies *in vitro* have led to the formation of binding constants [12] but these are of limited value because they fail to take into account the concentrations of different aluminium species in the solution. Another group [13] reported the binding of aluminium after gel filtration in buffered 30 μM-aluminium citrate at pH 7.4: up to 98 aluminium atoms/ferritin molecule were bound after incubation, at 37 °C, with aluminium citrate at a concentration above 200 μM. We have observed the binding of only 10 aluminium atoms/ferritin molecule during equilibrium dialysis with an initial concentration of 50 μM-aluminium citrate at 25 °C, pH 7.4 (Fig. 1*c*) and 50 aluminium atoms/molecule with an initial concentration of aluminium of 500 μM (pH 7.4) (results not shown). Our study indicates that this binding is dependent to some extent on the method of preparation of the aluminium citrate solution. In addition, the duration and temperature of incubation may also be important [13].

The concentration of aluminium in biological tissues is generally very low. In the cerebral cortex, total aluminium concentrations are normally less than 3 μg/g of total protein, although this may be increased in renal-dialysis patients [22]. Nevertheless, there will be an excess of small ligands such as citrate and phosphate groups that bind aluminium strongly and may prevent the accumulation of aluminium by ferritin unless the ferritin is turning over rapidly enough to allow its accumulation into growing iron cores. We have recently studied ferritin isolated from the cerebral cortex of normal, Alzheimer's-disease and renal-dialysis patients and found no significant change in the aluminium concentrations associated with Alzheimer's-disease and renal-dialysis patients [22]. On the basis of our results obtained, *in vitro*, we conclude that, in the presence of other aluminium chelators which bind the metal efficiently, ferritin will not protect cells from the toxic effects of aluminium by sequestering large amounts of the metal in the iron core.

We thank G. A. Taylor of the MRC Neurochemical Pathology Unit, Newcastle-upon-Tyne, for performing atomic-absorption spectroscopic analyses on ferritin samples and Dr. J. Candy for help in preparation of the manuscript. We thank the Wellcome Trust for financial support.

REFERENCES

1. Perl, D. P. & Brody, A. R. (1980) Science **208**, 297–299
2. Candy, J. M., Klinowsky, J., Perry, R. H., Perry, E. K., Fairbairn, A., Oakley, A. E., Carpenter, T. A., Atack, J. R., Blessed, G. & Edwardson, J. A. (1986) Lancet **i**, 354–356
3. Kerr, D. N. S. & Ward, M. K. (1988) in Metal Ions in Biological Systems (Sigel, A. & Sigel, H., eds.), vol. 24, pp. 217–258, Marcel Dekker, New York
4. Martin, R. B., Savory, J., Brown, S., Bertholf, R. L. & Wills, M. R. (1987) Clin. Chem. **33**, 405–407
5. Martin, R. B. (1986) J. Inorg. Biochem. **28**, 181–187
6. Morris, C. M., Candy, J. M., Court, J. A., Whitford, C. A. & Edwardson, J. A. (1987) Biochem. Soc. Trans. **15**, 498

7. Candy, J. M., Morris, C. M., Oakley, A. E., Taylor, G. A., Mountfort, S. A., Chalker, P. R., Bishop, H. E., Ward, M. K., Bloxham, C. A. & Edwardson, J. A. (1987) Biochem. Soc. Trans. **17**, 669–670
8. Van Renswoude, J., Bridges, K. R., Harford, J. B. & Klausner, R. D. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 6186–6190
9. Bali, P. K., Zak, O. & Aisen, P. (1991) Biochemistry **30**, 324–328
10. Crichton, R. R. (1984) Trends. Biochem. Sci. **9**, 283–286
11. Harrison, P. M., Artymiuk, P. J., Ford, G. C., Lawson, D. M., Smith, J. M. A., Treffry, A. & White, J. L. (1989) in Biomineralisation: Chemical and Biochemical Perspectives (Mann, S., Webb, J. & Williams, R. J. P., eds.), pp. 257–294, VCH Verlag, Weinheim
12. Sczekan, S. R. & Joshi, J. G. (1989) Biochim. Biophys. Acta **990**, 8–14
13. Cochran, M. & Chawtur, V. (1988) Clin. Chim. Acta **178**, 79–84
14. Martin, R. B. (1986) Clin. Chem. **32**, 1797–1806
15. Martin, R. B. (1988) in Metal Ions in Biological Systems (Sigel, A. & Sigel, H., eds.), vol. 24, pp. 1–58, Marcel Dekker, New York
16. Dougan, W. K. & Wilson, A. L. (1974) Analyst **99**, 413–430
17. Foy, A. L., Williams, H. L., Cortell, S. & Conrad, M. E. (1967) Anal. Biochem. **18**, 559–563
18. Carter, P. (1971) Anal. Biochem. **40**, 450–458
19. Smith, R. W. (1971) in ACS Advances in Chemistry Series, vol. 106, pp. 250–279, American Chemical Society, Washington
20. Ohman, L. & Sjoberg, S. (1983) J. Chem. Soc. Dalton Trans. 2513–2517
21. Birchall, J. D. & Chappell, J. S. (1988) Clin. Chem. **34**, 265–267
22. Dedman, D. J., Treffry, A., Candy, J. M., Taylor, G. A., Morris, C. M., Bloxham, C. A., Perry, R. H., Edwardson, J. A. & Harrison, P. M. (1992) Biochem. J. **287**, 509–514

Received 13 March 1992; accepted 9 April 1992