COMPARATIVE BINDING STUDIES OF ALUMINUM AND IRON TO SERUM TRANSFERRIN

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SUMMARY: The binding characteristics of aluminum and iron to serum transferrin were investigated and compared by using spectrophotometric titration technique. Both metal ions bound to serum transferrin and competed for the same binding sites on the transferrin.Aluminum transferrin (Al-tf) and iron transferrin (Fe-tf) complexes had maximum absorbances at 240 nm and 470 nm respectively. Bicarbonate was necessary for binding activities and citric acid facilitated the binding of both metals to transferrin. Tyrosyl residues were involved in iron binding but not in aluminum binding to transferrin.The complete saturation of transferrin with iron or aluminum was performed within 30 mins. Iron uptake by transferrin was reduced in the presence of aluminum. The relationship between aluminum and iron binding to this protein and the occurrence of anemia in hemodialyzed patients is discussed.

Key Words: Aluminum, iron, transferrin, hemodialysis.

INTRODUCTION

Recently, a number of publications indicating the presence of high concentration of aluminium in serum of chronic renal failure patients maintained on hemodialysis (1). The relationship between aluminium toxicity and the appearance of a number of pathophysiological disorders in these patients has been well documented. Aluminium has been reported to cause neurological disorders including encephalopathy and Alzheimer's in hemodialysis patients (2). The occurrence of vitamin D resistance osteomalacia (3) and hypochromic microcytic anemia (4) have been reported subsequently. The major sources of aluminium contamination is either water supply uses for the preparation of dialysis fluid (5) or aluminium phosphate binders (6) which prevent phosphate absorption in these patients.

Aluminium from these sources enters blood circulation and binds to serum proteins. Transferrin has been reported to be the major aluminium carrier protein in the plasma (7). It is a glycoprotein with a molecular weight of 80KD and transports iron from its site of absorption to the site of utilization in the cells (8). The binding of iron to this protein occurs under specific circumstances. Bicarbonate ion and citric acid are required for this process (9). Up to our knowledge the binding characteristics of aluminium to transferrin has not been well characterized. The major aim of this project was to study the binding characteristics of aluminium to serum transferrin in comparison to iron binding.

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MATERIALS AND METHODS

Preparation of apotransferrin

Human Transferrin (Hoechst) was dissolved in Earle's medium pH 7.4 (10) and successively dialyzed against 50 mmol/L EDTA in 50 mmol/L sodium citrate pH 5.2, 150 mmol/L NaCl, 20 mmol/L $NaHCO_3$ in 150 mmol/L NaCl, and 150 mmol/L NaCl to prepare apotransferrin (11). The homogeneity of the apo-transferrin was checked by SDS, electrophoresis technique as reported elsewhere (7). The iron and aluminium contents of the dialyzed protein was measured respectively by spectrophotometry and flameless atomic absorption spectrophotometry techniques (12, 13).

Preparation of iron and aluminium citrate complexes

Seperate standard solutions of ferric nitrate (3.75 mmol/L) and aluminium potassium sulphate (3.75 mmol/L) were prepared in deionized water containing 0.1 M HNO_3 and mixed with equal volumes of 75 mM citric acid.

The solutions were adjusted to pH 7.4 with 1 M NaOH and made up to a final concentration of 1.5 mM of iron or aluminium. The concentrations of iron and aluminium in the solutions were measured.

It should be noted that throughout this project plastic containers were pre-cleaned with 10 mM of EDTA, followed by three washes in deionized water. Glassware were soaked overnight in 3 M nitric acid and then thoroughly rinsed with deionized water to minimize metal contamination.

All chemicals were of reagent grade obtained from Sigma Chemical Company unless otherwise stated.

RESULTS

Spectra measurements of iron and aluminium transferrin complexes

The first experiment carried out was the measurement of the absorption spectrum of iron transferrin complex. This was done at room temperature using a Unciam SP8-300 recording spectrophotometer.

Apotransferrin was saturated with iron as a complex with citric acid (1:20) pH 7.4. For this purpose 300 μl of 1.5 mM $FeCl_3$ in 30 mM citric acid pH 7.4 was added to a solution of apotransferrin (6 mg/ml) in Earle's medium and the volume made up to 2.5 ml with the same buffer and vortexed well. The solution was then incubated for 4 hrs at room temperature. The color of the solution was deep orange which was characteristic of the iron transferrin complex. The absorption spectrum of the iron transferrin complex showed a broad peak in the region 460-480 nm (Figure 1). Aluminium citrate complex was next added to the another portion of apotransferrin solution and the reaction mixture was treated as mentioned above, no color was seen. Taking a spectrum from this solution showed that, aluminium citrate complex had a maximum absorbances in the region of 220-240 nm (Figure 1). When bicarbonate was eliminated from Earle's medium no aluminium or iron bindings occurred (Data not shown).

Figure 1: Visible absorption spectrum of Fe-tf (---) and and Al-tf (—).

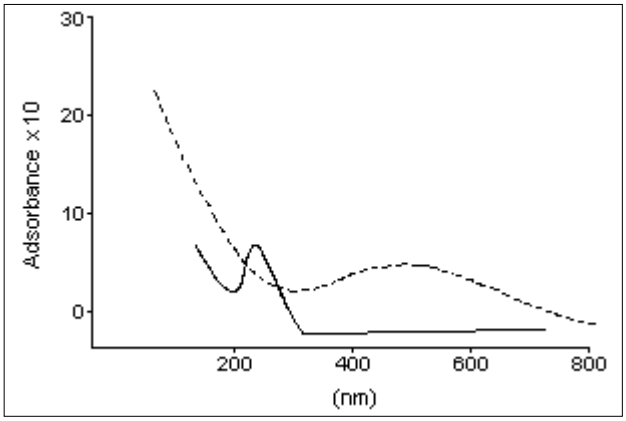
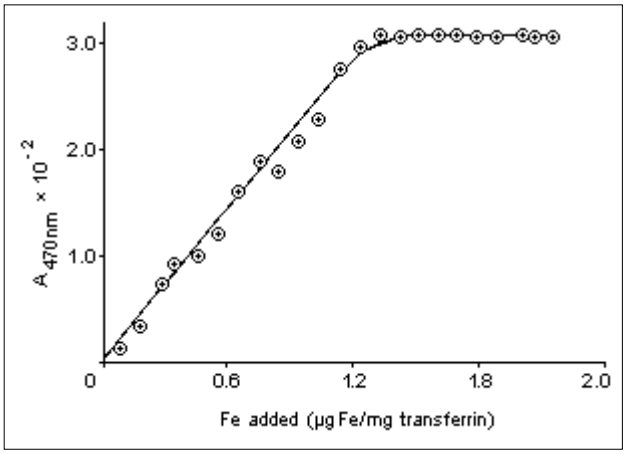


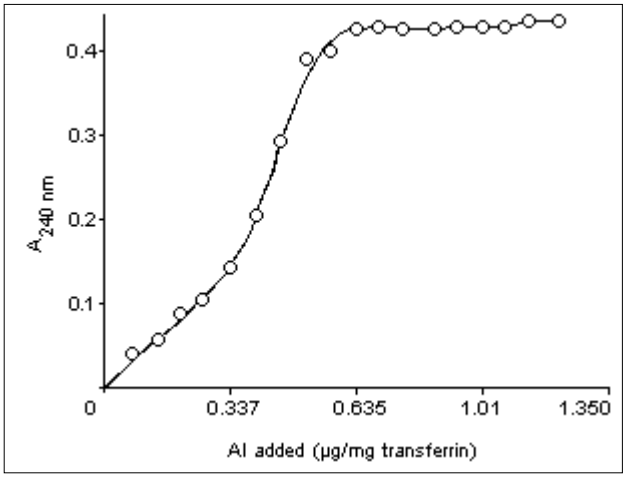
Figure 2: Spectrophotometric titration of human apotransferrin with iron in Earle's medium at pH 7.4. Each point is the mean of six observations.



Spectrophotometric titration of apotransferrin with iron or aluminium

To a series of tubes containing apotransferrin (0.27 mg/ml) in Earle's medium pH 7.4 increasing amounts of 1.5 mM $FeCl_3$ in 30 mM citric acid was added and the volumes made up to 2.5 ml with Earle's buffer. The tubes were capped, mixed rapidly and allowed to stand of 4h at room temperature. Their absorbances ware read at 470 nm (Figure 2). Maximum absorbance was achieved at approximately 1.4 μg iron mg protein. Assuming a molecular weight for transferrin of 80KD. This is equivalent to 2 moles Fe/mol transferrin.

Figure 3: Spectrophotometric titration of human apotransferrin with aluminium in Earle's medium at pH 7.4. Each point is the mean of three observations.



To another series of tubes containing apotransferrin (0.72 mg/ml) in Earle's medium pH 7.4 increasing amounts of 1.5 mM AIK $(SO_4)_2$ in 30 mM citric acid was added and the volumes made up to 2.5 ml with the buffer. The tubes were treated as described for Fe-transferrin.

The binding of aluminium to apotransferrin was accompanied by an increase in absorbance at 240 nm (Figure 3) maximum absorbance was reached at approximately 0.65 μg per mg protein. This is equivalent to 2 moles aluminium per mole transferrin with a molecular weight of 80KD for transferrin.

Time course for iron and aluminium uptake by apotransferrin

In this study aliquots of $FeCl_3$ (1.5 mM) or AIK $(SO_4)_2$ solutions were added to apotransferrin (1.0 mg/ml) in Earle's medium. The reaction mixtures were mixed rapidly and the absorbance readings at 240 nm and 280 nm for aluminium, 280 nm and 470 nm for iron were taken at intervals of 0-45 mins. The results are shown in (Figures 4 and 5). It was found that binding of iron to apotransferrin was accompanied by a marked increase in the A_{280} nm and A_{470} nm. For binding of aluminium there was an increase in A_{240} nm but not at the A_{280} nm, maximum absorbance was found at 30 mins (Figures 4 and 5).

Effect of aluminium on binding of iron to apotransferrin

Attempt was made to study the effect of aluminium on iron uptake by transferrin. To series of solutions of apotransferrin (6 mg/ml) in Earle's medium was added either 1.8 μM AIK $(SO_4)_2$ and 36 μM citric acid or 3.0 mM AIK $(SO_4)_2$ and 60 μM citric acid. After leaving for 10 min at room temperature increasing volumes of a solution of 1.5 μm $FeCl_3$ in 0.3 μm citric acid were added. The pH of the tubes were adjusted 7.4 and the volumes were made up to 2.5 ml. The absorbance of each solution at 470 nm was measured after a further incubation for 30 mins at room temperature. It is clear that in the presence of 81 μg/l of aluminium less iron binds to transferrin (Figure 6).

Figure 4: Time course for binding iron and aluminium to human apotransferrin in Earle's medium at pH 7.4. Each point is the mean of three observations.

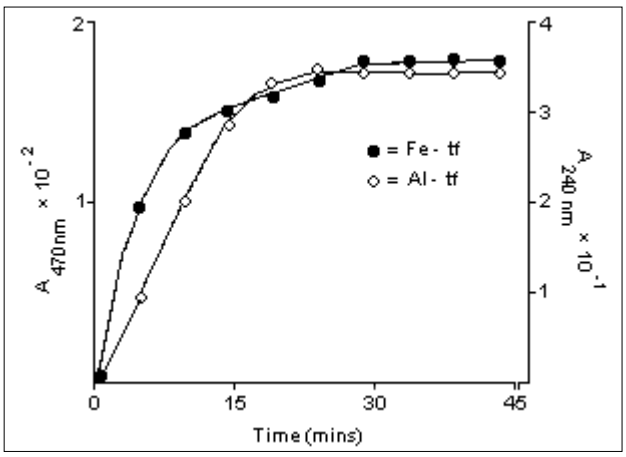
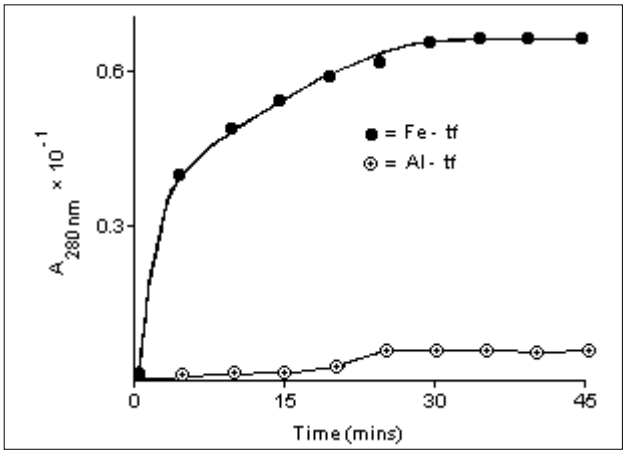


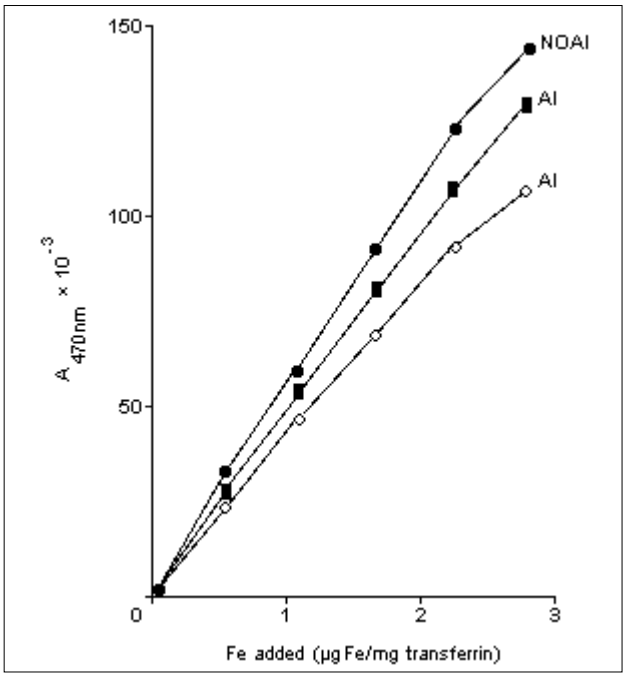
Figure 5: A_{280} of human transferrin solutions after addition of iron-citrate and aluminium-citrate complexes. Each point is the mean of three observations.



DISCUSSION

The chemical similarities between aluminium and iron may lead to the disturbances of iron metabolism in chronic renal failure patients with aluminium overload. The data that have been presented in this paper showed that aluminium bound to transferrin and had a maximum absorbance at 240 nm whereas the complex of iron transferrin had a maximum absorbance at 465 nm (Figure 1). The involvement of tyrosyl residues of transferrin iron binding sites was evidenced for iron but not for aluminium confirming earlier observations of the binding of iron to tyrosine and aluminium to other amino acids located in iron binding site of transferrin (14). Observation of trapp in 1983, showed that aluminium bound to one of the iron binding sites and interfered with iron metabolism (15). Tomimatsu and Donovan (16) in 1977 reported that 3-5 molecules of aluminium bound to transferrin nonspecifically. In the present study transferrin was first saturated with aluminium and then dialyzed against Earle's medium to remove unbound aluminium in the solution. Measurement of aluminium bound transferrin showed that only 0.67 μg of aluminium was bound to a molecule of transferrin. Conforming the binding of two molecules of

Figure 6: Spectrophotometric titration of human apotransferrin with in Earle's medium at pH 7.4. The effect of adding aluminium.



aluminium per molecule of transferrin. These findings are in good agreement with the earlier observations reported by others (17).

When the effect of aluminium on iron uptake by transferrin was studied, it was found that iron uptake by transferrin was reduced by 30 percent. Earlier observations using equilibrium dialysis technique showed that aluminium competed with iron to bind to transferrin (18).

In conclusion, aluminium competes with iron in order to bind to transferrin and my disturb iron metabolism by interference with iron uptake by the cells (19) or make damage to the heme synthesis (20).

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