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Comparative Binding Study of Aluminum and Chromium to Human Transferrin

Effect of Iron

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ABSTRACT

The characteristics of aluminum and chromium binding to apo-transferrin (apo-tf) have been investigated and compared. Both metal ions were taken up by human transferrin forming complexes with the maximum absorbances at 405 nm for chromium-transferrin (cr-tf) and 240 nm for aluminum-transferrin (Al-tf). In the presence of citric acid, chromium binding to transferrin is five times more than aluminum. The binding of aluminum or chromium to apo-transferrin was reduced by 18 and 22% in the presence of 200 ng/mL of iron. The binding of both metals to apo-tf appears to be pH dependent. In acidic pHs, less chromium and more aluminum binding occurred.

Index Entries: Chromium-transferrin; iron-transferrin; hemodialysis; chelating agents.

INTRODUCTION

There is a considerable body of literature suggesting that the plasma concentration of aluminum is altered in chronic renal failure patients maintained on hemodialysis (*1*). Aluminum enters the blood stream via dialysis fluid (2) or by aluminum phosphate binder agents for the reduction of phosphate absorption through the gut (*3*). In the plasma, aluminum binds to transferrin (*4*) and interfers with a number of biochemical parameters. Vitamin D-resistance osteomalacia (*5*), hypochromic micro-

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cytic anemia (*6*), and neurological diseases (*7*) are the most prevalent pathophysiological disorders produced by aluminum toxicity in renal failure patients. Transferrin is a β_1-glycoprotein with a mol wt of approx 80 kDa (*8*). It is responsible for the transportation of iron from its site of absorption to the site of utilization.

Using different biochemical techniques, including spectrophotometric titration and gel filtration, it has been postulated that aluminum binds to serum transferrin (*9*). The binding of aluminum to transferrin has also been reported by other laboratories throughout the world (*10*).

It is now well evidenced that chromium enters blood circulation via dialysis fluid (*11*) and binds to serum transferrin (*12*). The major source of chromium is the dialysis apparatus, and not from the water supply as reported for aluminum (*11*). The binding of chromium to serum transferrin has already been reported (*12*). With regard to the chemical similarities between chromium, aluminum, and iron, the present project was established to compare the bindings of chromium and aluminum to serum transferrin and their effects on iron binding activity.

MATERIALS AND METHODS

Preparation of Apo-Transferrin

Human transferrin (6 mg/mL) was dissolved in Earle's medium, placed in prewashed visking sacs, and dialyzed twice against 100 vol of 50 m*M* acetate buffer, pH 5.2, first for 6 h and then 16 h. Protein solutions were then successively dialyzed against 100 vol of 0.15 m*M* NaCl, 0.02*M* $NaHCO_3$ in 0.15*M* NaCl, and finally Earle's medium, pH 7.4. The homogenity of prepared apo-transferrin was checked by SDS-polyacrylamide gel electrophoresis as reported elsewhere (*13*).

Preparation of Iron, Aluminum, and Chromium Citrate Complexes

Separate stock standard solutions of ferric nitrate (3.75 m*M*), aluminum potassium sulfate (3.75 m*M*), and chromium chloride (3.75 m*M*) were prepared in deionized water containing 0.1*M* HNO_3 and mixed with equal vol of 75 m*M* citric acid. The solutions were adjusted to pH 7.4 with 1*M* NaOH and made up to a final concentration of 1.5 m*M* iron chromium and aluminum.

Spectrophotometric Titration Technique of Metal Binding to Apo-Transferrin

Approximately 1.5 mL of prepared apo-Transferrin in Earle's medium was added to a standard 1-cm preacid washed glass cuvets. To the cuvets were added aliquots (10–100 μL) of 1.5 m*M* metal ion as the citrate complex. The cuvets were covered with parafilm, mixed thoroughly by

vortexing, and left for up to 2 h at room temperature. The absorbance of the cuvets was measured at appropriate wavelength. Throughout this project, all glassware was soaked overnight in 3*M* nitric acid, and then thoroughly rinsed with distilled and deionized water so as to minimize iron, chromium, and aluminum contaminations. Plasticware was prewashed with 10 m*M* EDTA followed by three washes each of distilled and deionized water.

RESULTS

Spectra measurements from chromium-transferrin, aluminum-transferrin, and iron-transferrin were taken using a Perkin-Elmer UV/Visible Spectrophotometer (Model 5515). This was done at room temperature 22 ± 1°C.

Aliquots of Cr-citrate, Al-citrate, and Fe-citrate, pH 7.4, were added to solutions of apo-transferrin in Earle's medium, pH 7.4, the volumes made up to 2.5 mL with the same buffer, and vortexed well. The solutions were then incubated for 2 h at room temperature. The absorption spectra of Cr-, Al-, and Fe-transferrin complexes were measured separately. The results obtained showed that the absorption spectrum of the chromium-transferrin complex showed two peaks at 405 nm and 295 nm, respectively. Al-transferrin showed a broad peak in the region of 220–240 nm and Fe-transferrin at 465 nm.

The effect of pH on the binding of Fe, Al, and/or chromium was studied next. To examine this, to a series of plastic tubes containing 2.5 mL of tris buffer, which had been adjusted with acetic acid to varying pHs within the range of 3.4–8.8 and containing transferrin (3 g/L), aliquots of Cr-transferrin, Al-transferrin, and Fe-transferrin were added. The tubes were treated as mentioned above. The A_{465} of the iron-containing solutions showed a marked increase above pH 6.0, whereas the absorbance of chromium-containing solution markedly increased above pH 6. The A_{240} of aluminum-transferrin showed minimal level at pH 6, but was increased when pH fell to 4 or rose to 8 (Fig. 1).

The effect of aluminum and/or chromium as a complex with citric acid on iron uptake by apo-transferrin was studied spectrophotometrically. One hundred microliter aliquots of 1.5 m*M* $FeCl_3$ in 30 m*M* citric acid were added to 2.5 mL apo-transferrin (3 mg/mL) in Earle's medium, pH 7.4, and incubated at room temperature for 2 h. The A_{465} nm was measured and corrected for the volume of the solution, an increase in the A_{465} nm indicating the binding of iron to apo-transferrin.

When the apo-transferrin was titrated with iron in the presence of either 500 μg/L of aluminum or 1 μg/L of chromium as the complex with citric acid, the increases in A_{465} were much smaller (Fig. 2). It is clear that chromium is a much more potent inhibitor of iron uptake by apo-transferrin in comparison to aluminum.

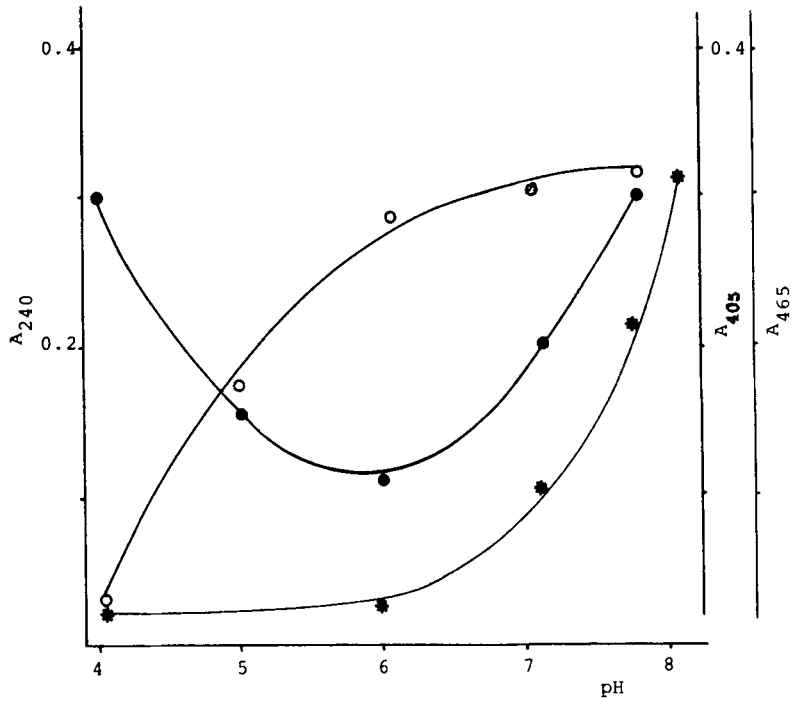

Fig. 1. Effect of pH on aluminum (●), chromium (○), and iron (★) binding to apo-transferrin. Each point is the mean of three separate experiments. For details, *see* text.

The next experiment carried out was the study of the effect of iron on chromium or aluminum uptake by apo-transferrin. It was found that the addition of 200 μg/L of Fe(III) as Fe-citrate in the medium led to the reduction of aluminum or chromium uptake by 18 and 22%, respectively (Fig. 3).

The last experiment was the comparison of the influences of citric acid and/or oxalic acid on the binding of aluminum and chromium to apo-transferrin. Both chelating agents enhanced binding activities, but citric acid was much more potent than oxalic acid in the binding processes (Fig. 4).

DISCUSSION

The data that has been presented in this article elucidated the probable mechanism by which Al and Cr bind to apo-tf. Absorption spectra obtained from aluminum and or chromium-apo-tf complexes indicated two maximum absorbances at 240 and 405 nm, respectively. Earlier reports have shown the maximum absorbances for Al-tf complex in the

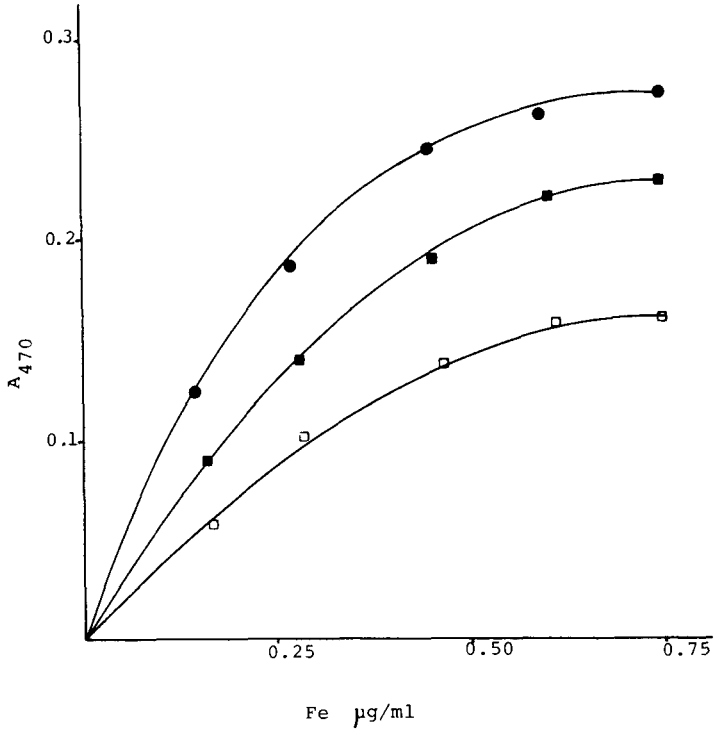

Fig. 2. Effect of aluminum (■) and chromium (□) on iron binding to apo-transferrin (●). Each point is the mean of three separate experiments. For details, *see* text.

region of 242–290 nm (*9*) and two weak bands at 615 and 414 nm for cr-tf complex (*12*). It is worth mentioning that the differences seen in the maximum absorbances might be partly owing to the composition of the buffer used for the experiments. When the effects of pH on the binding of aluminum to apo-tf were studied, it was found that the minimal binding of aluminum occurred at neutral pH with a gradual increase in both sides of pH 7 (Fig. 1). This binding activity of aluminum at different pHs is very close to the pH-dependent solubility curve of aluminum in aqueous solutions (*14*). On the other hand, when the effect of pH on the binding of chromium was studied, it was observed that the binding activity of chromium was markedly elevated at pHs above 6. Lastas (1976) showed that the binding of Fe to apo-tf was higher at alkaline pH than at acidic pHs (*15*). The binding activity of chromium seems to be more similar to that of iron than that of aluminum. A chelating agent is necessary for the binding of iron to apo-tf (*16*). The chemical similarities between aluminum and chromium with iron are thought to follow the same mechanism for binding to transferrin. Results of the present study showed that, in the absence of bicarbonate or citric acid (or oxalic acid),

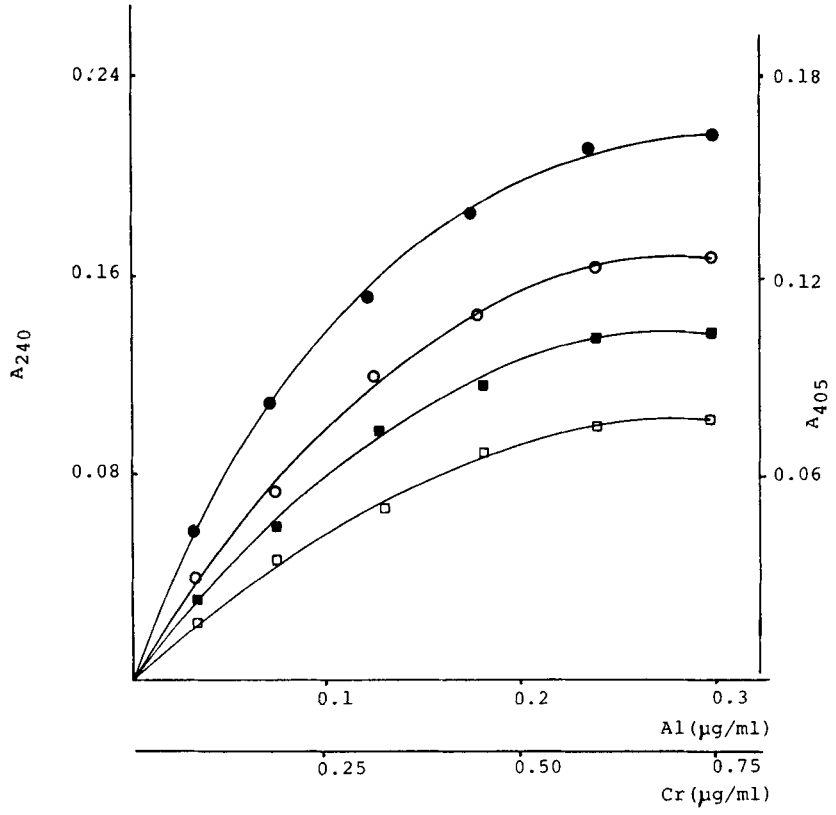

Fig. 3. Effect of iron on aluminum and chromium binding to apo-tf (Al-tf ●, Cr-tf ■, Fe-Al-tf ○, Fe-Cr-tf □). For more details, *see* text.

no significant binding of either aluminum or chromium to transferrin occurred (Fig. 4). The reduction in iron uptake by either aluminum or chromium on one hand and the competition of iron with aluminum or chromium suggest that these three metal ions compete for the same binding site on the transferrin. However, still more investigations should be done to clarify the exact mechanism by which these processes occur.

SUMMARY

The maximum absorbance of Al-tf and Cr-tf complexes appeared to be different. The binding of both metal ions to apo-transferrin was pH dependent. Citric acid was a potent chelating agent and necessary for binding activities. Iron might compete with aluminum and chromium for binding to apo-transferrin.

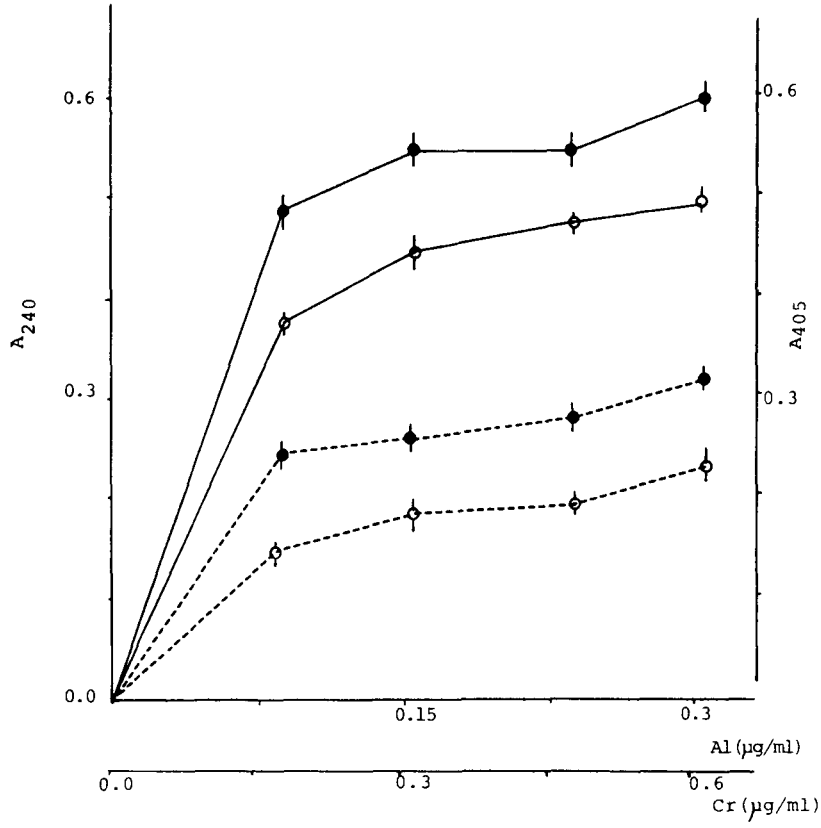

Fig. 4. The effect of chelators citrate (●), and oxalate (○) on the aluminum (——) and chromium (------) binding to apo-transferrin.

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