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Study of the Binding of Iron and Indium to Human Serum Apo-Transferrin

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ABSTRACT

Indium is a heavy metal belonging to group III_a. It is believed that indium may interfere with iron metabolism from the sites of absorption, transportation, utilization and storage in the cells. The present investigation was established to study and compare the binding of iron and indium to human apo-transferrin (apo-tf). Pure human apo-tf was used and the binding activity of iron and indium, as a complex with citric acid (1:20), to apo-tf was studied. The binding of iron and indium to apo-tf in Tris-HCl buffer, pH 7.4 showed a maximum absorbance at 465 and 255 nm, respectively. The binding of both metals to apo-tf appears to be pH dependent. Using equilibrium dialysis technique, the binding of iron to apo-tf and the effect of indium on the binding process were also studied. Addition of indium to the outside of dialysis sac reduced iron uptake by 37%. The results indicate that indium competes with iron in binding to apo-tf. Although, the binding sites for these two ions seem to be similar, the binding of iron to apo-tf is more tightly than indium. *Iran. Biomed. J. 7 (2): 73-77, 2003*

Keywords: Iron , Indium, Transferrin, Spectrophotometry

INTRODUCTION

Indium is a group III non-essential heavy metals. Consumption of this metal has been increased in the past decade [1]. The primary uses for indium are coatings for glass, such as liquid crystal displays and heat-or height-reflective coatings, doping, and/or energy capture applications in semiconductors and solar cells [2]. The animal studies have shown that acute intravenous administration of indium trichloride ($InCl_3$) is extremely toxic to the liver and kidneys [1], and it is more reactive with biological membranes than cadmium and mercury [3]. Previous studies have shown that liver and kidney are targets of indium administered by either intra-peritoneal or intra-venous routes [4-5].

Transferrin with approximate molecular weight of 80 kDa is a serum iron transport β_1 glycoprotein with a blood concentration of about 2 to 4 mg/ml. The normal functions are to transport iron as ferric from the sites of absorption to the site of utilization and storage in the cells [6]. This protein is a single chain polypeptide comprising two lobes and each lobe is capable of binding with high affinity to one atom of iron. In addition to iron, transferrin also binds with high affinity to several other metal ions, especially the tripositives [6], such as aluminium [7-8], chromium [8] and gallium [9-10].

With regard to the chemical similarities between indium and iron, we studied the binding of iron and indium to apo-transferrin (apo-tf) using spectro-photometric titration and equilibrium dialysis techniques. The interaction of these metal ions with each other on the binding to apo-tf was also investigated.

MATERIALS AND METHODS

All chemicals used in this project were reagent grade and obtained from Sigma Chemical Company (Germany), except indium trichloride that was obtained from Merck Chemical Company (Darmstadt, Germany). Deionized water was used throughout this study for washing and preparing the solutions to minimize metal contamination.

Laboratory glassware were soaked overnight in 10% HNO_3 and then thoroughly rinsed with distilled and deionized water. Plastic ware was pre-washed with 10 mM EDTA followed by three washes each of distilled and deionized water.

Preparation of iron and indium citrate complexes. Separate stock standard solutions of 20 ml ferric chloride (3 mM) and 20 ml indium trichloride (3 mM) were prepared in deionized

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water and mixed with the equal volume of 60 mM citric acid solution. The solutions were adjusted to pH 7.4 with 1 N NaOH and made up to a final volume of 50 ml by deionized water [10].

Spectrophotometric titration technique for metal binding to apo-tf. The binding of iron and/or indium to apo-tf was carried out using spectrophotometric titration technique. This technique was performed at room temperature (25°C). To achieve spectrophotometric titration for binding of either iron or indium to apo-tf, 200 µl apo-tf (107.5 µM) in 50 mM Tris-HCL buffer, pH 7.4, 0.02M $NaHCO_3$, was added to a standard 1 cm pre-acid washed glass tubes. Aliquots of 1.2 mM iron-citrate (1:20) or 1.2 mM indium-citrate (1:20) complexes were then added to the tubes and the volumes made up to 2 ml with the same buffer, they mixed vigorously by vortexing. The tubes were capped and left for up to 2 h at room temperature. The absorbance of the test tubes was taken using Perkin-Elmer UV/Vis spectrophotometer model 551-S [8, 10].

Equilibrium dialysis technique for metal binding to apo–tf. The binding of iron to human apo-tf and the effect of indium on iron binding activity were also investigated using equilibrium dialysis at 2-4°C. Two ml solution of apo-tf (21.5 µM) was placed in a dialysis sac opened to the atmosphere, which was immersed in a glass vessel contain 160 ml 50 mM Tris-HCl buffer, pH 7.4, 0.02 M $NaHCO_3$ [10]. Fifty µl of iron (1.2 mM) as ferric-citrate or indium or indium (1.2 mM) as indium-citrate solutions were added at time intervals to the buffer surrounding the dialysis sac with the aid of a magnetic stirrer. After 24 h, 100 µl of sample from inside and 100 µl from outside of the dialysis sac was removed and analyzed for iron. Iron concentration was determined using Brittenham method [11]. The binding constant of iron to apo-tf without indium was calculated using Scatchard relationship (i.e. $[B]/[F] = -1/K[B] + n[E]_T/K_S$) and plot of [B]/[F] against [B], [12]. Where [B] is concentration of bound ion to protein, [F] is concentration of free ion, n is number of binding site, $[E]_T$ is concentration of total protein and K_S is the dissociation constant.

The effect of pH on the binding of iron and indium to apo-tf. To a series of pre-acid washed test tubes containing 200 µl apo-tf (107.5 µM), 45 µl of iron solution (1.2 mM) or indium (1.2 mM) as complex with citric acid were added and the volumes made up to 2 ml by Tris-HCl buffer with varying pH within the range of 5.8 to 8. The solutions were vortexed and incubated for 2h at room temperature. The absorbance of the test tubes was measured by spectrophotometer.

Metal ions interactions with each other for binding to apo-tf. The binding of iron to apo-tf and the effect of indium on iron binding activity and vice versa, were also investigated by spectro-photometric titration. Initially, the effect of indium on iron binding to apo-tf was studied. Prepared apo-tf (107.5 µM) in 200 µl, in 50 mM Tris-HCl buffer, pH 7.4, 0.02M $NaHCO_3$ was added to pre-acid washed test tubes first. Then 55 µl of indium (1.2 mM), as complex with citrate (1:20) and 0-55 µl of iron (1.2 mM) as complex with citric acid were added to the series of tubes and the volumes were made up to 2 ml with the same buffer. The solutions were then mixed vigorously by vortexing and left for up to 2 h at room temperature. The absorbances of the test tubes were measured at 465 nm. The same experiment was also repeated without indium.

Next, the effect of iron on binding of indium to apo-tf was studied. As mentioned above 200 µl of apo-tf was added to a series of test tubes. Iron solutions (55 µl) and 0-55 µl of indium solution were added and the volumes were made up to 2 ml with Tris-HCl buffer, pH 7.4. The absorbance of the test tubes was measured at 255 nm. This study was also repeated in the absence of iron.

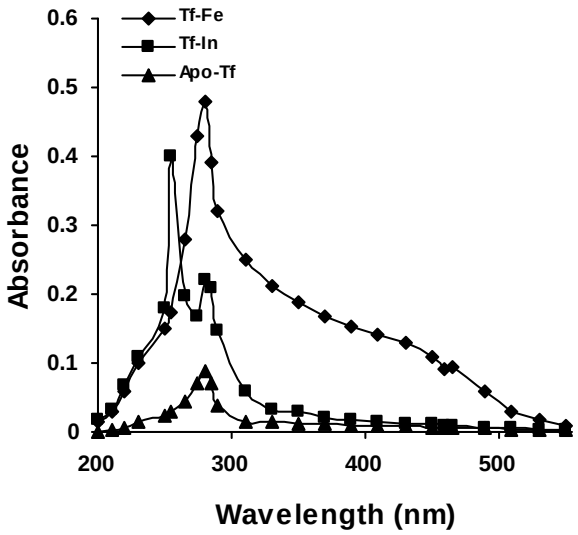

Fig. 1. Absorption spectrum (200-550 nm) of apo-tf, iron-transferrin and indium-transferrin. ([Apo-tf] = 10.75 µM, $[Fe^{+3}] = [In^{+3}]$= 27 µM, pH 7.4, t = 25°C).

RESULTS

The absorption of apo-tf, iron-transferrin and indium-transferrin complexes was measured first. The results in Figure 1 show that the absorption spectrum of the indium-transferrin complex has two peaks at 280 and 255 nm. Iron-transferrin showed a broad peak at 465 and 280 nm and apo-tf at 280 nm.

The effect of different pH within the range of 5.8 to 8 on the binding of iron and indium to apo-tf were studied. It showed that both iron and indium bind to apo–tf mostly at pH 7.4. The lower the pH, the less binding of these metals to apo-tf (Fig. 2).

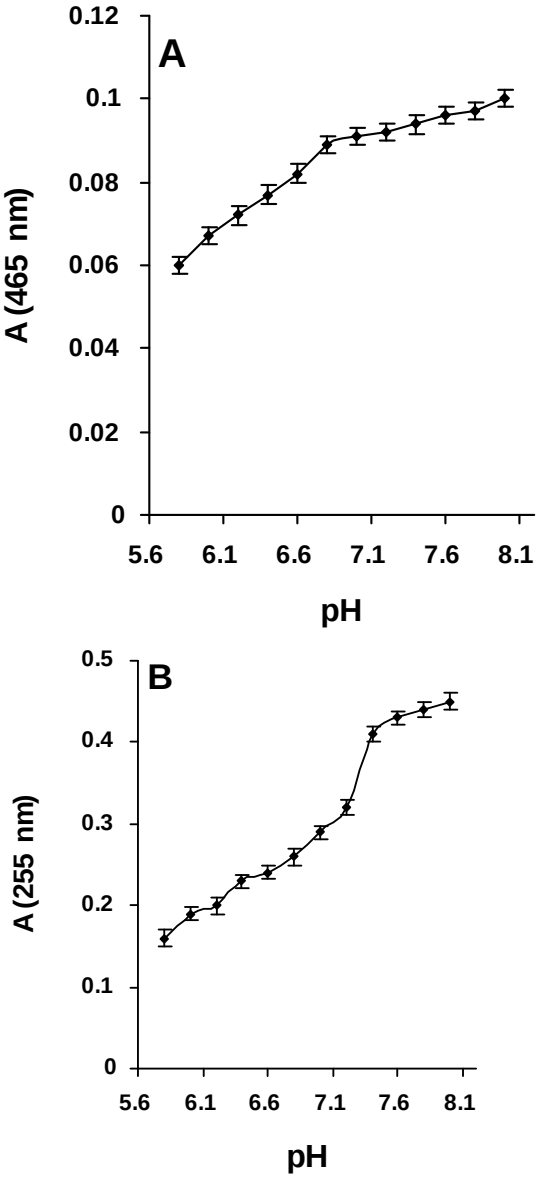

Fig. 2. Effect of different pH (5.8 to 8) on iron binding (A) and (8.5 to 8) no ignidnib nor (A) and indium binding (B) to apo-tf. Each point is the mean of three separate experiments. ([Apo-tf] = 10.75 µM, [Fe^{+}] = [In^{+3}] = 27 µM, t = 25°C).

The effect of different amounts of iron (0 to 33 µM) on binding of apo-tf and the effect of indium (33 µM) on iron up-take by apo-tf were investigated. Initially, it was found that each molecule of apo-tf was saturated with approximately 2 atoms of iron, corresponding to 1.4 µg iron per mg transferrin (Fig. 3A, curve Tf-Fe), when indium (33 µM) was added, the absorbance of transferrin-iron at 465 nm was reduced by approximately 16% (Fig. 3A, curve Tf-Fe+In). The binding effect of different amounts of indium (o to 33 µM) as a complex with citric acid and the effect of iron on indium binding to apo-tf were also investigated. The results show that each molecule of apo-tf was also saturated with approximately 2 atoms of indium, corresponding to 2.85 µg indium per mg transferrin (Fig. 3B, curve Tf-In). The presence of iron (33 µM) in the reaction mixtures reduced the absorbance of transferrin-indium complex at 255 nm by approximately 48% (Fig. 3B, curve Tf-In+Fe).

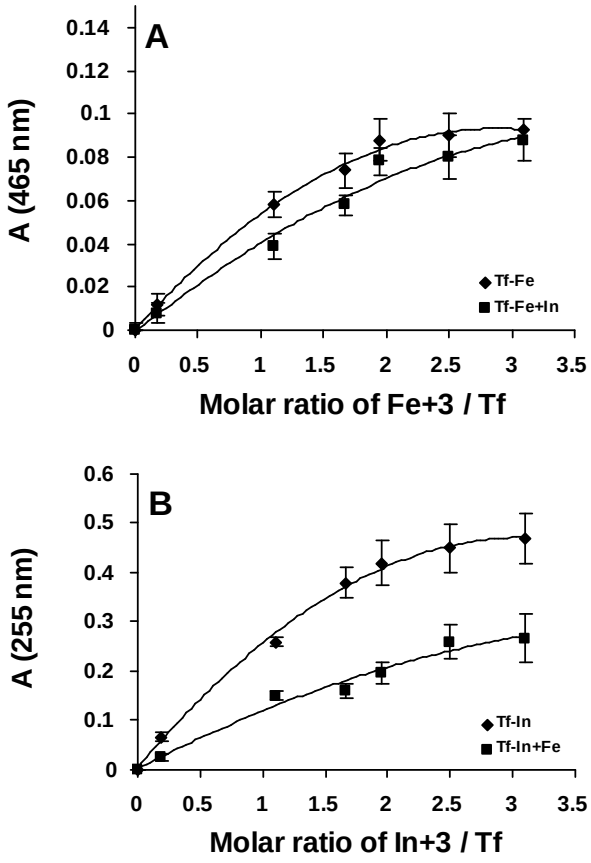

Fig. 3. Spectrophotometric titration of human apo-tf with different concentration of iron (0 to 33 µM) in the presence and absence of indium (33µM) (A) and also spectrophotometric titration of human apo-tf with different concentrations of indium (0–33 µM) in the presences and the absence of iron (33 µM) (B). Each point is the mean of three experiments. ([Apo-tf] = 10.75 µM, pH 7.4, t =25°C).

In order to confirm the binding of iron and/or indium to apo-tf, equilibrium dialysis technique was used (Fig. 4A). The binding of iron to apo-tf and the effect of indium on the binding activity presented in Figure 4A. In the presence of indium as a complex with citric acid, approximately 37% reduction in iron binding to apo-tf was seen (Fig. 4A). Using Scatchard plot analysis and the data in Figure 4A, the binding constant for iron binding to apo-tf in the absence of indium was calculated (Fig. 4B). The approximate binding constant for iron-transferrin complex was 8.7×10^6 M^{-1} (Fig. 4B).

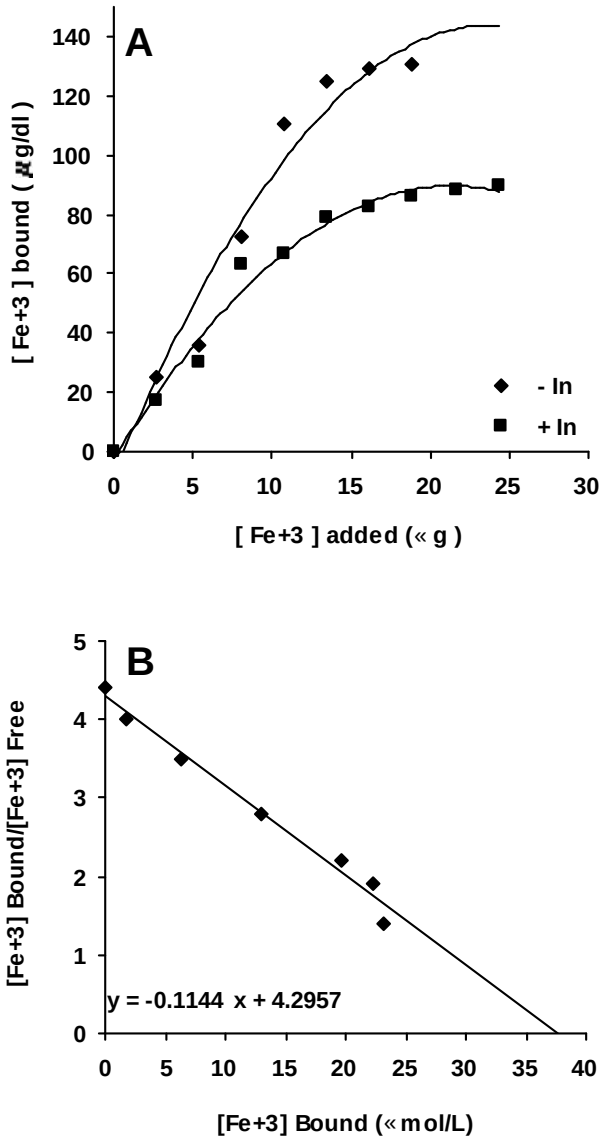

Fig. 4. The study binding of iron to apo-tf in the absence and the presence of indium by equilibrium dialysis (A) and Scatchard plot for the binding constant of iron to apo-tf (B). Each point is the mean three experiments. ([Apo-tf] = 21.5 µM, pH 7.4, t = 0-4°C) .

DISCUSSION

Previous reports suggest that due to biochemical similarities of few cations with iron, they may interfere with iron metabolism and cause some disorders [7, 13]. Among them are aluminum and chromium that could bind to serum transferrin and cause hypochromic microcytic anemia. In this study, we used spectrophotometry technique to investigate the iron binding to serum apo-tf and the influence of indium in the binding activity. Absorption spectra obtained from iron and/or indium-transferrin complexes indicated two maximum absorbencies at 465 and 255 nm, respectively. According to the previous reports, the maximum absorbance for iron-transferrin complex is 465 nm [10] and/or 470 nm [14-15] and for indium-transferrin complex is 250 nm [16] and/or 245 nm [17], which is comparable to our findings.

When the effect of pH on the binding of iron and indium to apo-tf was studied, it was found that the optimal binding activity of these two metals occurred at pH 7.4. When pH was decreased to acidic pH, release of these two ions from transferrin occurred. The release of indium was more than iron. This might be due to the affinity of indium to apo-tf, which is less tightly than iron. The observations of Lastas [18] and Ponka [19] are in agreement with our results binding of iron and indium to apo-tf are higher at alkaline pH (pH 7.4) that at acidic pH. The reduction in iron up-take by indium and vice versa, and the competition of iron with indium on the other hand suggest that these two metal ions may compete for the same binding site on the apo-tf. The same results were obtained when other trivalent elements were added to the human apo-tf [8, 15, 17]. These competitive binding activities were also verified by equilibrium dialysis. In the indium outside of the dialysis sac, reduced binding of iron to apo-tf about 37%. Using this technique and Scatchard plot it was found that the binding constant for iron to apo-tf was 8.7×10^6 M^{-1}. The difference between obtained binding constant for iron to apo-tf could be due to the discrepancies in the methods of binding and the factors such as composition of buffer etc. Overall, it seems that both iron and indium bind to and complete for the same sites of transferrin. The occurrence of microcytic anemia in patients with indium overload may, however, be due to the competition of indium with iron in iron metabolism. More investigations should be done to elucidate the

exact mechanism of these competitions particularly at the cellular levels.

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