Iranian Biomedical Journal 5 (4): 149-153 (October 2001)

Identification of Amino Acids Involve in Indium Binding To Serum Human Apo-Transferrin

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ABSTRACT

Indium is a heavy metal belonging to group IIIa. It is used as a radioimaging and chemotherapeutic agent in diagnosis and also in the treatment of cancers. It is believed that indium may interfere with iron metabolism and reduce cell growth in cancer tissue. The present report was established to study the binding of iron and indium to apo-transferrin (apo-tf) and to identify amino acids involved in this process. The pure human transferrin was used and iron and indium, as citrate complexes (1:20), were added to apo-tf. The binding constant was calculated using spectrofluorometric titration technique. Maximum wavelengths for excitation and emission of apo-tf were 300 and 335 nm, respectively. When apo-tf was complexed with iron, the emission was decreased 69%; whereas, the binding of indium to apo-tf increased the emission 29%. The approximate binding constant for iron-transferrin complexes were 1 ò 10^8 M^{-1} and 0.11 ò 10^8 M^{-1}, respectively. The pKa's of aspartate, histidine, tyrosine, lysine and arginin were identified. The data indicated that the 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 approximately 9 times more tightly than indium. *Iran. Biomed. J. 5 (4): 149-153, 2001*

Keywords: Iron, Indium, Transferrin

INTRODUCTION

Iron is an essential metal which involves in more cell activities in form of enzymes and/or other metaloproteins, including cytochromes, ribo-nuclease, myoglobin and hemoglobin [1]. Iron binds to a glycoprotein in the plasma namely apo-transferrin (apo-tf) [2]. This single chain glyco-protein containing two binding sites for iron has binding constants within the range of 10'23 M'-1 to 10'19 M'-1 [1, 2]. This protein with a molecular weight (MW) of approximately 80 kDa contains 679 amino acids [3]. In addition to iron, transferrin, with relatively high affinity, binds to several other metal ions, especially the trivalent ones [3], including aluminium [4, 5], chromium [5], gallium [6, 7] and indium [7, 8].

Indium is a non-essential heavy metal ion that is used in industry since 1940s. For making glass, graphite and cathode oscillographs and also for its prevention of corrosive and metal fatigue in alloys [9]. In recent years, various indium-containing compounds including indium arsenide (InAs) and indium phosphide (Inp) have been gaining greater attention from the semiconductor industry because of their electronic and optical properties [10]. Indium chloride (InCl3) is a substrate or by-product in process used to generate pure indium, in complex with arsenide and/or phosphorous [11].

The animal studies have shown that acute intravenous and intratracheal administration of InCl)3 is extremely toxic to the liver, kidneys [12] and lung [13]. Also, it is more reactive than cadmium and mercury with biological membranes [14]. With regard to the chemical similarities between indium and iron, the present report was established to study the binding of iron and indium to apo-tf spectrofluorometrically. The interaction of these metals with each other on the binding to apo-tf was also investigated.

MATERIALS AND METHODS

In this project, purified human apo-transferrin was used [4, 15]. All chemicals 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

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throughout this study for washing and buffer preparation. In order to minimize metal contaminations of the laboratory glassware, they 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.

Iron and indium ions were used as complexes with citric acid (1:20). Separate stock standard solutions of 20 ml $FeCl_3$ (3 mM) and 20 ml $InCl_3$ (3 mM) were prepared in deionized water and mixed with 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 [5, 15]. Each milliliter of these solutions contains 1.2 mM iron or indium.

Spectrofluorometric titration technique. The binding of iron and indium to human serum apo-tf and their interactions with each other were investigated using spectrofluorometric titration technique. This method was performed at room temperature. To achieve spectrofluorometric titration, 200 µl of prepared apo-tf (107.5 µM) in 50 mM Tris-HCl buffer, pH 7.4, 0.02 M $NaHCO_3$ was added to a pre-acid washed test tubes. Aliquots of 1.2 mM iron-citrate complex or 1.2 mM indium-citrate complex were added to the series of tubes and the volumes were made up to 2ml with the same buffer. The solutions were then mixed vigorously by vortexing. The tubes were capped and left for up to 2h at room temperature. The fluorescence intensity of the test tubes was taken using Perkin-Elmer luminescence spectrophotometer LS-3B [15]. The binding constant of iron and indium to apo-tf was calculated by the Stern-Volmer relationship (i.e. $F_0/ F = 1 + K [Q]$) and plot of F_0/F against [Q] [16]. Where F and F_0 are the fluorescence intensities in presence and absence of the ion (iron or indium) respectively, [Q] is the concentration of element (iron or indium) and K is the binding constant.

Determination of the binding sites of Iron and Indium to apo-tf by spectrofluorometric method. Aliquots of 45 µl of iron, (1.2 mM) and/or indium (1.2 mM) as complexed with citric acid (1:20) were added to a series of pre-acid washed test tubes containing 200 µl apo-tf , in $NaHCO_3$ (0.02 M), with different pH (3 to 12.2) [17]. The tubes were treated as mentioned earlier. The fluorescence intensity of the test tubes was determined using spectrophotometer [18].

RESULTS

Spectrofluorometric titration. Initially, maximum emission (λ) and excitation (λ) for apo-tf were obtained (Figs. 1 A and B). According to these Figures, maximum λ_{ex} and λ_{em} for apo-tf were found to be 300 and 335 nm, respectively. The intensity of fluorescence was decreased by 69% when apo-tf was complexed with iron citrate (1:20). The addition of indium-citrate complex to apo-tf caused an elevation of emission (λ) by 39% (Fig. 1B). The interaction of indium with iron-transferrin complex was investigated fluorometrically. The results are presented in Figure 2A. The addition of increasing amounts of iron (0 to 55 µl) caused the increase of the intensity of fluorescence apo-tf, whereas the addition of indium (55 µl) to the reaction medium containing iron-transferrin complex caused a reduction of approximately 21% (Fig. 3A). When varying amounts of indium (0 to 55 µl) were added to apo-tf solutions, an enhancement of fluorescence intensity was observed. The addition of iron (55 µl) to indium-transferrin complex caused quenching of fluo-rescence intensity (Fig. 3B). The binding constants for iron and/or indium to apo-tf were also calculated using data shown in Figure 3. The approximate binding constants for iron-transferrin and indium-transferrin complexes were 1×10^8 M^{-1} and $0.11 \times 10^8 M^{-1}$, respectively (Fig. 3).

Determination of binding sites. The nature of iron and indium binding sites in apo-tf was investigated using method of Farzami *et al.* [18]. The fluorescence intensity of apo-tf in absence and presence of iron and indium at different pH (3 to 12.2) was determined (Fig. 4A). Then using data shown in Figure 4A, the difference of various fluorescence intensity (Δ F.I) of apo-tf in the absence and presence of these ions (iron and indium) against various pH (3 to 12.2) was obtained (Fig. 4B). The peaks (Fig. 4B) represent the PK_a's of amino acids that were linked to metal ions. The data show that the following amino acids were involved in the binding activities, and the obtained PK_a's are related to asparate (4 and 4.8), histidine (5.8 and 6.2), tyrosine (9.9 and 10.3), lysine (11.2) and arginin (11.8) which are the major sites for iron in binding activity. Also for indium binding to apo-tf, the following PK_a's are related to asparate (3.4, 4, 4 and 4), histidine (6), tyrosine (9.6), lysine (11.4) and arginin (12) (Fig. 4B).

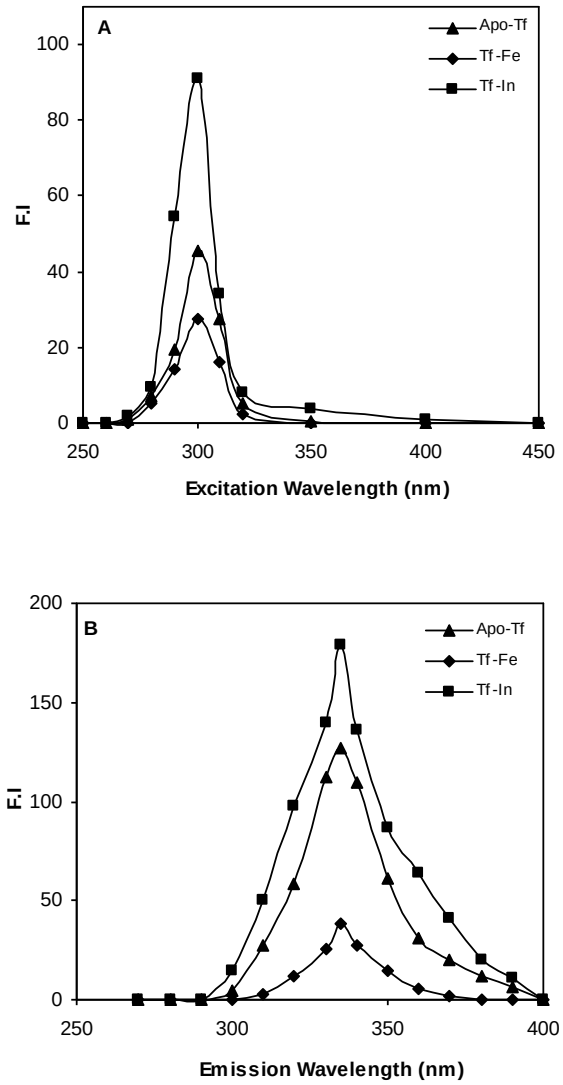


Fig. 1. The maximum of excitation (Fig. A) and emission (Fig. B) wavelengths of apo-transferrin (▲) and the effect of iron (◆) and indium (■) on the fluorescence intensity (F.I) of apo-transferrin at these wavelengths. ([Apo-tf] = 10.75 μM, $[Fe^{+3}] = [In^{+3}]$ = 27 μM, pH 7.4, t = 25°C).

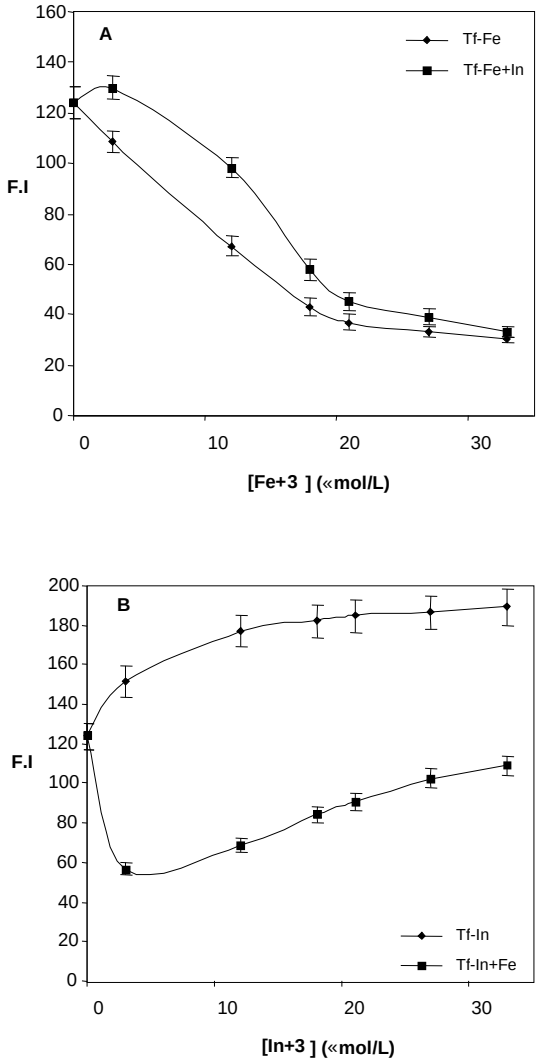


Fig. 2. Fluorometric titration of apo-transferrin with different concentrations of iron (0 to 33 μM) in the presence (■) and absence (◆) of indium (26 μM) (Fig. A) and also fluorometric titration of apo-transferrin with different concentrations of indium (0 to 33 μM) in presence (■) and absence (◆) of iron (26 μM) (Fig. B). Each point is the mean of three separate experiments. ([Apo-tf] = 10.75 μM, pH 7.4, t = 25°C, λ_{ex} = 300 nm, λ_{em} = 335 nm).

DISCCUSSION

The binding of iron to human serum apo-transferrin has been studied using different biochemical techniques including spectro-photometry [15], equilibrium dialysis [4, 5] and electrophoresis [4]. Previous reports suggest that due to biochemical similarities of few cations with iron, they may interfere with iron metabolism and cause some disorders. Among them are aluminum and chromium that could bind to serum transferrin and cause hypochromic microcytic anemia [5, 19]. In this study, we used spectro-fluorometric technique to investigate the iron binding to serum apo-transferrin and also the influence of indium in the binding activity. The

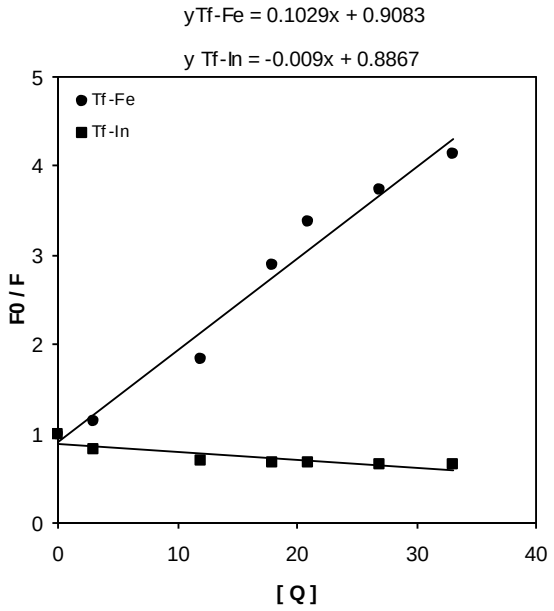
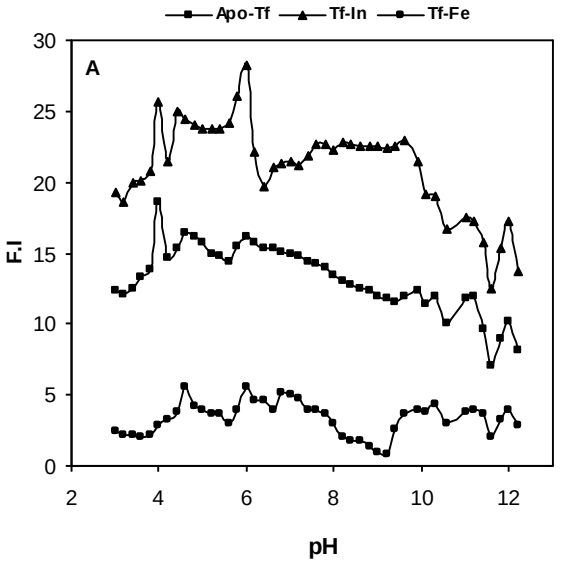


Fig. 3. The plot of F_0/F against [Q] that was obtained from Fig. 2A data and Fig. 2B. The binding constant of apo-transferrin to iron (•) and indium (■) by F_0/F = 1+K [Q] relationship was calculated.

existence of some cyclic amino acids (Tyrosine, phenylalanine and etc.) in apo-transferrin cause its fluorescence property [16]. Maximum wavelengths for excitation (λ_{ex}) and emission ((λ_{em}) were found to be 300 and 335 nm, respectively. According to the previous reports, the maximum of excitation and emission for apo-tf is 295 and 330 nm, respectively [20], which is close to our results. Since excitation at 295 nm is specific for tryptophan residues, tryptophan but not tyrosine must be responsible for the change in fluorescence intensity [20]. The addition of iron to apo-tf caused quenching of the fluorescence, whereas the addition of indium caused enhancement of fluorescence intensity.

Although, indium is a trivalent cation, due to physicochemical differences with iron, they show different characteristics in fluorescence intensity and they produced different binding constant (1×10^8 M^{-1} vs. 0.11×10^8 M^{-1}). Therefore it seems that binding of iron to apo-tf is approximately 9 times more tightly than indium. Using different experimental conditions, $\log K_1$ and $\log K_2$ for binding constant of iron and indium to apo-tf, reported 21.44 (K_1), 20.34 (K_2) and 18.3 (K_1), 16.44 (K_2), respectively [21]. We believe that such discrepancy is due to the nature of techniques used. Results obtained from pH study showed that the obtained peaks are related to PK_a's value of



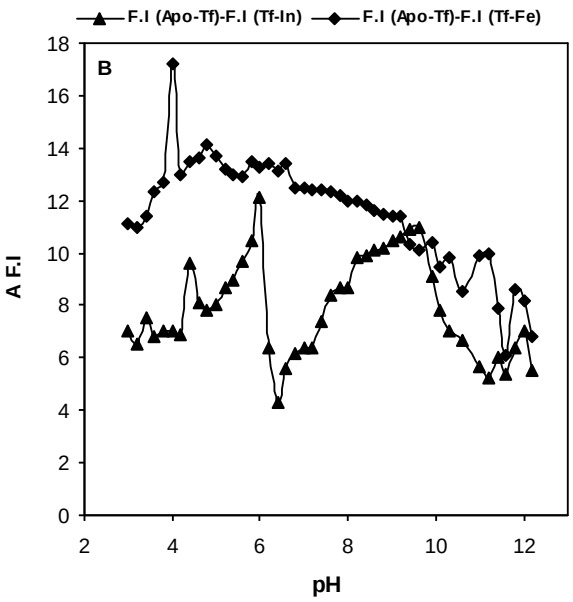


Fig. 4. The Changes of fluorescence intensity, Apo-transferrin (■) with iron (•) and indium (▲) in different pH (3 to 12.2) (Fig. A). Difference fluorescence intensity against various pH, apo-transferrin in absence and presence of iron (◆) and also apo-transferrin in absence and presence of indium (▲) (Fig. B) were obtained by Fig. A data. ([Apo-tf] = 10.75 μM, $[Fe^{+3}] = [In^{+3}]$ = 27 μM, pH 7.4, t = 25°C, (λ_{ex} = 300 nm, λ_{em} = 335 nm).

aspartate, histidine, tyrosine, lysine and arginin. These findings are in agreement with previous work of Farzami *et al.* [18], concerning the binding of Ca^{+2} to calmodulin and the involvement of various amino acids in binding activity.

Overall, it seems that both iron and indium bind to and complete for sites of the transferrin and more investigations are needed to elucidate these discrepancies.

ACKNOWLEDGMENTS

This project (No 79182) was granted by vice-chancellor in research affairs of Isfahan-Medical Science University. We thank Mrs. Monsefypour for typing the manuscript.

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