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Binding affinity of aluminium to human serum transferrin and effects of carbohydrate chain modification as studied by HPLC/high-resolution ICP-MS —Speciation of aluminium in human serum—

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Abstract

Aluminium (Al) in the blood is bound to transferrin (Tf), a glycoprotein of about 80 kDa that is characterized by its need for a synergistic anion. In this focused review, the binding affinity of Al to Tf is surveyed in the context of our recent studies using on-line high-performance liquid chromatography/high-resolution inductively coupled plasma mass spectrometry (HPLC/HR-ICP-MS). Al in human serum without any in vitro Al-spikes was present in a form bound to the N-lobe site of Tf. The influences of sialic acid in the carbohydrate chain of human serum Tf (hTf) were studied using asialo-hTf, obtained by treatment with sialidase. The binding affinity of Fe was similar between asialo-hTf and native-hTf, while that of Al for asialo-hTf was larger than that for native-hTf, especially in the presence of oxalate, a synergistic anion. The above findings are discussed in relation to diseases in which the serum concentrations of carbohydrate-deficient Tf and oxalate are augmented.
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Keywords: Aluminium; Transferrin; Sialic acid; Sialidase; ICP-MS

1. Introduction

In human blood, aluminium (Al) is bound to transferrin and transferred to receptors. Transferrins (Tfs) are a group of iron (Fe)-binding glycoproteins that require carbonate anions for metal binding [1]. Tfs have two metal-binding sites: the N-lobe site and the C-lobe site [2–4]. These lobes are homologous but distinct [5]. A striking difference between the two lobes is the presence of a carbohydrate chain with sialic acid residues in the C-lobe. The carbohydrate chain can differ in its degree of branching; this difference is known as microheterogeneity [6,7]. Carbohydrate-deficient Tfs (CDTs) have fewer sialic acid residues, and CDT levels are elevated in several diseases [8–13].

This focused review surveys research on the binding affinity of Al to Tf in the context of our recent studies, focusing on how the sialic acid present in the carbohydrate chain of the C-lobe influences the metal binding (the principal role) of Tf. In addition, the effects of small co-existing compounds that have elevated concentrations in some diseases are also surveyed.

2. Chemical forms of metals bound to Tf

2.1. Analytical techniques

The binding of Fe to Tf has been widely studied using various techniques, including equilibrium dialysis, ESR

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and NMR [14]. Regarding the binding of Al to hTf, many reports have considered the uptake of Al into human serum transferrin (hTf) from a thermodynamic aspect [15,16]. Al forms stable complexes with citrate and even more stable complexes with hTf [15,17]. Therefore, Tf is the predominant Al carrier in serum and through the blood brain barrier [18].

On the other hand, hyphenated techniques have been applied to speciate the chemical forms of Al in human blood. Most reports have dealt with Al-spiked serum [19–21] or serum from patients receiving continuous ambulatory peritoneal dialysis [22,23]. High-performance liquid chromatography/high-resolution inductively coupled plasma mass spectrometry (HPLC/HR-ICP-MS) is one such hyphenated technique, combining HPLC as a separation method and HR-ICP-MS as a metal detection method [24]. If the chemical forms of hTf could be separated into four peaks (for example, apo-, two monoferric and diferric hTfs) and if their Fe-content could be directly quantified, the preferential Fe-binding lobe and the need for synergistic anions could be clarified. In this regard, the binding of metals (Fe and Al) to hTf was analyzed using an HPLC instrument connected directly to an HR-ICP-MS instrument [25].

The operating conditions for the hyphenated HPLC and HR-ICP-MS system have been previously reported [25]. Briefly, an HPLC apparatus (LC-10Ai; Shimadzu, Kyoto, Japan) equipped with an anion-exchange column (Mono-Q; Amershem BioSciences, Sweden) was connected directly with an ELEMENT 1 HR-ICP-MS instrument (Finnigan MAT, Bremen, Germany). A 0.1-ml sample was injected into the system, and the eluate was transferred to a UV detector and then introduced to the nebulizer of the HR-ICP-MS instrument. The levels of sulfur (S), Al and Fe were continuously monitored. The ^{32}S level was used to monitor the protein levels in the HPLC eluate.

New gradient conditions were established to separate all four chemical forms of metals bound to hTf. To analyze an asialo-Tf solution, another gradient condition was established, since asialo-hTf has a sialic acid-free sugar chain and behaves differently in anion-exchange experiments [27]. For Al-speciation, a thorough clean-up procedure was necessary to obtain a detection limit of 0.1 μg L^{-1} ($S/N = 3$). Ultra-pure water was bubbled with nitrogen gas to remove oxygen and bicarbonate. Bicarbonate acts as a synergistic anion in metal-hTf binding [14]. After preparing each sample solution, all the tubes and bottles were sealed under a nitrogen gas atmosphere.

2.2. Assignment of Fe(Al)-peaks as Fe bound to hTf

Typical chromatograms obtained using HPLC/HR-ICP-MS are shown in Fig. 1. Four UV-peaks were detected, and three ^{56}Fe-peaks were observed. Iron in the form of iron citrate was added to the hTf solution. Since peak C did not show an Fe peak, it was assigned to apo-hTf. The iron-peak B was 1.8 times larger, relative to the UV-peak. Therefore, this peak was assigned to Fe_2-hTf (two Fe atoms bound to hTf). The remaining two peaks were ascribed to monoferric hTfs. To assign the two monoferric hTfs, DFO (desferrioxamine) was used.

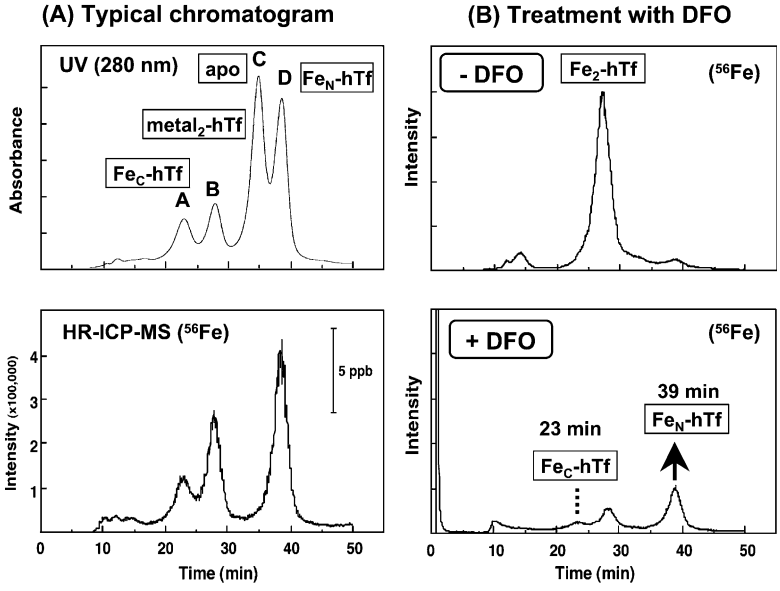

Fig. 1. Assignment of all four chemical forms of metals bound to hTf. (A) Typical HPLC/HR-ICP-MS chromatograms for apo-hTf partly saturated with Fe^{3+} (Fe:hTf = 0.5:1) in the presence of bicarbonate. (B) HPLC/HR-ICP-MS chromatograms (^{56}Fe level) for Fe-saturated hTf-solution before (upper) and after (lower) the addition of desferrioxamine (DFO).

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