Journal of Inorganic Biochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

The effects of the glycation of transferrin on chromium binding and the transport and distribution of chromium *in vivo*

Ge Deng, Samantha L. Dyroff, Molly Lockart, Michael K. Bowman, John B. Vincent *

Department of Chemistry, The University of Alabama, Tuscaloosa, AL 35487-0336, USA

ARTICLE INFO

Article history:
Received 16 February 2016
Received in revised form 1 August 2016
Accepted 22 August 2016
Available online xxxx

Keywords:
Transferrin
Chromium
Kinetic
Transport
Distribution

ABSTRACT

Chromium (III) has been shown to act as a pharmacological agent improving insulin sensitivity in rodent models of obesity, insulin resistance, and diabetes. To act in beneficial fashion, chromium must reach insulin-sensitive tissues. Chromium is transported from the bloodstream to the tissues by the iron-transport protein transferrin. When blood concentrations of glucose are high (as in a diabetic subject), transferrin can be glycated, modifying its ability to bind and transport iron. However, the effects of glycation of transferrin on its ability to bind and transport Cr have not been examined previously. Storage of transferrin at 37 °C in the presence and absence of glucose has significant effects on the binding of Cr. Transferrin stored in the absence of glucose only binds one equivalent of Cr tightly, compared to the normal binding of two equivalents of Cr by transferrin. Glycated transferrin (stored in the presence of glucose) binds two equivalents of Cr but the changes in its extinction coefficient at 245 nm that accompany binding suggest that the Cr-bound transferrin possesses a conformation that deviates appreciably from untreated transferrin. These changes have dramatic effects, greatly reducing the ability of transferrin to transport Cr *in vivo* in rats. The results suggest that glycation of transferrin in subjects with high blood glucose concentrations should reduce the ability of Cr from pharmacological agents to enter tissues.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Pharmacologically relevant doses of Cr can generate improvements in insulin sensitivity and blood cholesterol levels in animals with stresses on the glucose and lipid metabolism systems, most notably in rodent models of type 2 diabetes (for a review, see Ref. [1]). The mechanism for these effects at a molecular level is not clearly established; but, a role for transferrin in Cr transport associated with insulin action has been proposed. Cr has been proposed to be an artificial second messenger in insulin action [2]. Increases in blood levels of insulin and subsequent activation of the insulin signaling pathway result in a movement of Cr from the bloodstream to tissues where the Cr in turn appears to increase insulin sensitivity by interacting with the insulin signaling machinery in cells [2].

Transferrins are a class of proteins (molar mass ~ 80 kDa) that reversibly bind two equivalents of metal ions [3]. Transferrins display selectivity for Fe^{3+} as the metal binding sites have evolved to bind metal ions with large charge-to-size ratios. Serum transferrins serve as the major iron transport protein in the bloodstream; conalbumin (egg white transferrin) and lactoferrin (milk transferrin) have been proposed to possess antibacterial roles, depriving bacteria of iron. The transferrin molecule is composed of two lobes with approximately 40% sequence homology; as a result, the three-dimensional structures of the lobes are nearly superimposable [3]. An iron-binding site is located in each lobe, so that the protein can tightly bind two metal ions. Ferric ion coordination is similar in each site, being distorted octahedral and comprised of two tyrosine residues, a histidine residue, an aspartate residue, and a chelating (bi)carbonate ion. The presence of the anion is essential for Fe binding. Ferric ions bind concomitantly with a synergistic anion, usually (bi)carbonate.

Transferrin can serve as a transport protein as its metal affinity varies greatly as a function of its environmental conditions, most notably pH. The transferrin molecule undergoes a significant conformational change when binding and releasing ferric ions. The apoprotein possesses a more open conformation; in the Fe-loaded conformation but not apoprotein conformation, transferrin binds tightly to transferrin receptor, a transmembrane protein of the cell membrane. Transferrin is carried into the cell by endocytosis. The subsequent acidification of the endosome releases the Fe^{3+}; then, fusion of the endosome with the cell membrane releases and recycles the apotransferrin. In humans, the protein is present at a concentration of approximately 3 mg/ml in serum and is normally about 30% saturated with Fe, providing it the potential to bind and transport other metal ions. For a review, see Ref. [3].

A role for transferrin in Cr^{3+} transport *in vivo* has been well established. When rats are administered $^{51}CrCl_3$ by stomach tube, over ≥99% of the ^{51}Cr in blood is found to be associated with non-cellular components while 80% of the ^{51}Cr immunoprecipitates with transferrin

* Corresponding author at: Department of Chemistry, 250 Hackberry Lane, Box 870336, The University of Alabama, Tuscaloosa, AL 35487-0336, USA.
E-mail address: jvincent@bama.ua.edu (J.B. Vincent).

http://dx.doi.org/10.1016/j.jinorgbio.2016.08.008
0162-0134/© 2016 Elsevier Inc. All rights reserved.

Please cite this article as: G. Deng, et al., The effects of the glycation of transferrin on chromium binding and the transport and distribution of chromium *in vivo*, J. Inorg. Biochem. (2016), http://dx.doi.org/10.1016/j.jinorgbio.2016.08.008

[4]. Transferrin with two bound Cr^{3+} ions inhibits binding of Fe_2-transferrin to the surface of reticulocytes [5], presumably at transferrin receptor. Injection of $^{51}Cr_2$-labeled transferrin into the bloodstream results in a rapid and insulin-sensitive movement of Cr into the tissues as transferrin-bound Cr [4–6]; over half of the Cr is transported to the tissues within 30 min. Subsequent decreases in tissue chromium with time are mirrored by increases in urine Cr. Hence, transferrin, in an insulin-dependent fashion, can transfer Cr to tissues from which Cr is excreted in the urine [6–8].

Cr^{3+} binds to the two Fe^{3+}-binding sites of apotransferrin and in a similar fashion concomitantly binds two equivalents of (bi)carbonate as it does when binding ferric ions [9]. The binding of Cr is accompanied by intense changes in the protein's ultraviolet spectrum. The changes in the ultraviolet spectrum suggest that each chromic ion binds to two tyrosine residues, indicating that Cr presumably binds specifically in the two Fe-binding sites [9]. The role of tyrosine in ligating Cr has been confirmed by Raman spectroscopy [10]. In accord with the metal binding sites being shared by ferric and chromic ions, excess Fe^{3+} decreases the ability of Cr^{3+} to bind to transferrin, while binding of Fe^{3+} (at physiological concentrations) to transferrin is not affected by a physiologically relevant concentration of Cr^{3+} [11]. Displacement of a few percent of the Fe^{3+} with concomitant binding of Cr^{3+} results from Cr^{3+} being added to transferrin loaded 50% with Fe^{3+} (with the C-terminal lobe theoretically filled with Fe^{3+}) [11].

The rate at which Cr^{3+} binds to transferrin, the rate of the conformation changes associated with Cr binding, and the stability of Cr-transferrin have recently been investigated [12–15]. *In vitro* studies had suggested that the binding of Cr to transferrin was slow. For example, the generation of Cr_2-transferrin for spectroscopic studies generally used samples of transferrin that had been allowed to come to equilibrium with Cr^{3+} ions over the course of up to two weeks to guarantee a stoichiometric amount of Cr^{3+} binding. This brought about question as to how transferrin could be responsible for Cr^{3+} transport *in vivo* if Cr^{3+} binding actually requires days or weeks to achieve equilibrium given the half-life of transferrin in serum and that of transferrin-bound iron are on the order of hours [16,17]. However, in the presence of 25 mM (bi)carbonate, the concentration in human blood, and other potential Cr^{3+}-binding species present in blood plasma, two chromic ions bind rapidly and tightly to apotransferrin [15]. Yet, conformation changes associated with metal binding are slow for Cr binding compared to Fe binding [15].

Under conditions of high blood glucose concentration, as in subjects with diabetes, glucose can bind chemically and irreversibly (glycation) to several proteins in the bloodstream, including transferrin. This chemical modification can alter the properties and functions of these biomolecules. The effects of glycation of transferrin on iron transport have recently been a matter of concern [18–21]. Glycation of transferrin alters how tightly the protein binds iron [18, 19] and may alter the conformation of diferric transferrin, presumably changing its ability to deliver the iron to tissues [21]. The degree of glycation has been reported to increase from ~1 to 2% in healthy subjects to ~5% in diabetics [22], while this is reported to increase from ~4% in healthy children to ~11% in type 1 diabetic children [23]. Given that Cr(III) complexes have been proposed as drugs to increase insulin sensitivity, particularly in type 2 diabetic subjects [1], understanding the ability of glycated transferrin to bind and transport Cr is important in determining the appropriate amount of Cr necessary for potentially being used as a drug to treat insulin insensitivity and its symptoms. Herein are described studies examining the binding of Cr^{3+} to glycated serum transferrin and the transport of Cr *in vivo* by glycated transferrin.

2. Materials and methods

2.1. Materials

Iron-free human serum transferrin and insulin (bovine, zinc) were obtained from Aldrich (St. Louis, MO). Iron-free rat serum transferrin was obtained from Lee Biosolutions (Maryland Heights, MO). $^{51}CrCl_3$ was obtained from Perkin Elmer (Waltham, MA). Doubly deionized water was used throughout. All reagents were used as received unless otherwise noted. Plasticware was used whenever possible. All Cr-binding studies were performed in HEPES buffer, 0.1 M, pH 7.4 in polypropylene centrifuge tubes. Buffer pH was adjusted by the addition of NaOH to the free acid form of HEPES. For solutions containing $KHCO_3$, pH was readjusted to 7.4 after addition of $KHCO_3$ by addition of HCl. Cr^{3+} solutions were prepared by using $CrCl_3 \cdot 6H_2O$. Apotransferrin and apoconalbumin concentrations were determined using the extinction coefficient ($\varepsilon = 9.12 \times 10^4\ M^{-1}\ cm^{-1}$) at 280 nm [24]. Solutions containing added bicarbonate where prepared immediately before use. All kinetics results are presented as the average of at least triplicate experiments. Error bars in figures represent standard deviation.

2.2. Instrumentation

Ultraviolet-visible spectra were obtained by using a Cary (Agilent, Santa Clara, CA) 100 or Beckman Coulter (Brea, CA) DU800 UV–visible spectrophotometer. Electrospray ionization (ESI) mass spectral studies were performed on a Bruker (Billerica, MA, USA) HCT Ultra high capacity ion trap mass spectrometer. Gamma counting was performed on a Packard Cobra II auto-gamma counter (Meriden, CT, USA). Continuous wave (CW) EPR were measured on a Bruker (Billerica, MA) ELEXSYS E540 X-band spectrometer with an ER 4102 ST resonator. CW spectra were measured at ~9.43 GHz using 100 kHz magnetic field modulation with an amplitude of 30 gauss with a microwave power of 21.1 mW. After the appropriate binding time, glycerol was added to the samples to 15% v/v, and the solutions were transferred into 4 mm quartz EPR tubes and frozen in liquid nitrogen. EPR measurements were made with samples immersed in liquid nitrogen in a quartz insertion dewar.

2.3. Chromium-binding to transferrin

Chromium-binding studies were performed as described previously [15] and were performed at least in triplicate. Binding of Cr^{3+} to transferrin was monitored at 245 nm. Studies were initiated by the addition of Cr^{3+}. Solutions were continuously stirred by using a Starna (Atascadero, CA) "Spinette" electronic cell stirrer. Data analysis, calculation of averages and standard deviations and fitting of curves to the appropriate equations, was performed by using SigmaPlot 11 (SPSS, Inc., Chicago, IL). The iterative curve fitting algorithm of SigmaPlot 11 uses the Marquardt-Levenberg algorithm to find the parameters of the independent variables that provide the best fit between the data and the equation.

2.4. Glycation of transferrin

Glycation of transferrin was performed following the procedure of van Campenhout et al. [19]. Briefly, human or rat transferrin at a concentration of ~5 g/L was incubated in sodium phosphate buffer (0.1 M, pH 7.4) containing 1.0 M glucose for 14 days at 37 °C. As a control for any effects from incubation for 14 days at 37 °C, a sample of transferrin was incubated in a similar fashion using buffer without added glucose. The glucose was removed from the transferrin by cycles of dilution with buffer lacking glucose, followed by concentration *via* ultrafiltration using a Millipore (Billerica, MA) Biomax 30 kDa membrane.

For ESI mass spectral studies, the solutions of human serum transferrin that had been kept at 37 °C for two weeks in the presence and absence of glucose were desalted by cycles of dilution with doubly deionized water followed by concentration by ultrafiltration using a Biomax 30 kDa membrane.

The extent of glycation of human serum transferrin was measured colorimetrically by the nitroblue tetrazolium method (which assays fructosamine content) using a kit from Pointe Scientific (Canton, MI).

Please cite this article as: G. Deng, et al., The effects of the glycation of transferrin on chromium binding and the transport and distribution of chromium *in vivo*, J. Inorg. Biochem. (2016), http://dx.doi.org/10.1016/j.jinorgbio.2016.08.008

Download English Version:

https://daneshyari.com/en/article/5152557

Download Persian Version:

https://daneshyari.com/article/5152557

Daneshyari.com