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Binding of vanadium to human serum transferrin - voltammetric and spectrometric studies

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ABSTRACT

Previous studies generally agree that in the blood serum vanadium is transported mainly by human serum transferrin (hTF). In this work through the combined use of electrochemical techniques, matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry and small-angle X-ray scattering (SAXS) data it is confirmed that both V^{IV} and V^{V} bind to apo-hTF and holo-hTF. The electrochemical behavior of solutions containing vanadate(V) solutions at pH = 7.0, analyzed by using two different voltammetric techniques, with different time windows, at a mercury electrode, Differential Pulse Polarography (DPP) and Cyclic Voltammetry (CV), is consistent with a stepwise reduction of $V^{V} \rightarrow V^{IV}$ and $V^{IV} \rightarrow V^{II}$. Globally the voltammetric data are consistent with the formation of 2:1 complexes in the case of the system V^{V}-apo-hTF and both 1:1 and 2:1 complexes in the case of V^{V}-holo-hTF; the corresponding conditional formation constants were estimated. MALDI-TOF mass spectrometric data carried out with samples of $V^{IV}OSO_4$ and apo-hTF and of $NH_4V^{V}O_3$ with both apo-hTF and holo-hTF with V:hTF ratios of 3:1 are consistent with the binding of vanadium to the proteins. Additionally the SAXS data suggest that both $V^{IV}OSO_4$ and $NaV^{V}O_3$ can effectively interact with human apo-transferrin, but for holo-hTF no clear evidence was obtained supporting the existence or the absence of protein-ligand interactions. This latter data suggest that the conformation of holo-hTF does not change in the presence of either $V^{IV}OSO_4$ or $NH_4V^{V}O_3$. Therefore, it is anticipated that V^{IV} or V^{V} bound to holo-hTF may be efficiently uptaken by the cells through receptor-mediated endocytosis of hTF.

1. Introduction

Human serum transferrin (hTF) is the primarily transporter of Fe^{III} ions in the blood. It contains around 630 amino acids arranged in two similar lobes: the N-terminal (hTF_N) and the C-terminal (hTF_C) lobes. Each lobe can reversibly bind a Fe^{III} ion, but also other metal ions [1] [2] [3]. Conformational changes take place upon binding or release of Fe^{III} ions: in the apo-form (apo-hTF) the protein is in the 'open conformation', while upon binding two Fe^{III} ions, forming what we may designate as $(Fe)_2$hTF (holo-hTF), the protein adopts a structure which is often designated by 'closed conformation'. This conformation is recognized by the hTF receptors located at the surface of cells, iron uptake occurring by internalization of transferrin through a receptor-mediated 'endocytosis' process.

The ability of transferrin to bind Fe^{III} and other metal ions depends on the pH [1] [4] and previous studies globally agree that in the blood serum vanadium is transported mainly bound to transferrin [5] [6] [7] [8] [9] [10] [11] [12]. In the whole blood, for relatively high amounts of vanadium a significant part may be bound to erythrocytes (red blood cells) [13] [14] [15], most probably as V^{IV} [16] [17]; however, for the concentrations normally found in the blood of humans treated with vanadium compounds (e.g. $V^{IV}O(carrier)_n$, where carrier is the ligand present in the V-compound), ca. up to 1–5 μM [18] [19], vanadium is bound to hTF not including the carrier ligand [6] [12] [20] [21] [22] [23] [24]. In aqueous solutions, there is a global tendency for vanadium to be in the V^{V} oxidation state. For example, Chasteen et al. [25] reported that oxidovanadium(IV) ($V^{IV}O^{2+}$) is very susceptible to aerobic oxidation, and the pH-sensitive half-life for the oxidation is estimated

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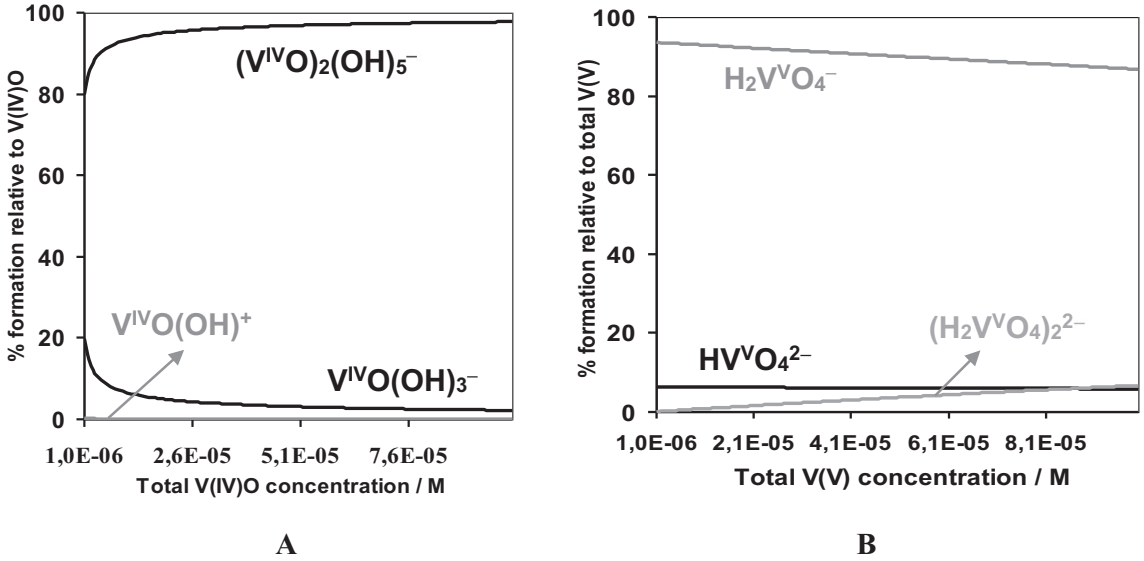

Fig. 1. Concentration distribution diagram for the hydrolysis of (A) oxidovanadium(IV) and (B) dioxidovanadium(V) at pH = 7.0, calculated using the HySS computer program [30], in the concentration range (total) of V^{IV} and V^{V} between 1×10^{-6} and 1×10^{-4} M. The stability constants values for V^{IV} correspond to Eq. 1 and for V^{V} to Eq. 2.

between 5 and 13 min. Notwithstanding, the + IV state can be significantly stabilized when a suitable organic ligand is present, or a bioligand (such as a protein), this probably extending significantly the lifetime of $V^{IV}O$-species.

Vanadate(V) can be present in several chemical forms, oligomeric or monomeric, this depending on the pH, ionic strength and total vanadium concentration [21] [26] [27] [28]. If V^{IV} is present the species that predominate also depend on pH and total vanadium concentration [21] [29]. Fig. 1 depicts concentration distribution diagrams for the hydrolysis of $V^{IV}O^{2+}$ and $V^{V}O_2^{+}$, calculated using the HySS computer program [30], in the concentration range of V^{IV} and V^{V} between 1×10^{-6} and 1×10^{-4} M (total concentrations), the range expected to be relevant in most biological systems, as well as in most electrochemical experiments. For the oxidovanadium(IV) system the stability constants are defined according to Eq. 1 [21] [29], and for the dioxidovanadium(V) system (or vanadate(V) system) they are defined here based on $H_2V^{V}O_4^{-}$ according to Eq. 2 [26].

$$p\ V^{IV}O^{2+} + r\ H^{+} \leftrightarrows (V^{IV}O)_p(H)_r \quad (1)$$

$$p\ H_2V^{V}O_4^{-} + r\ H^{+} \leftrightarrows (H_2V^{V}O_4)_p(H)_r \quad (2)$$

Because apo-hTF does not absorb visible radiation and as vanadium (V) has no d-electrons, in solutions containing vanadate(V) and apo-hTF at pH 7.4 no electronic bands show up for $\lambda > 400$ nm; only bands due to e.g. charge-transfer transitions may be recorded if intense enough. Upon additions of a $NaV^{V}O_3$ solution to hTF the circular dichroism (CD) spectra in the 200–350 nm range show significant changes [31]. Although it is not clear why these changes occur, they are the result of interaction of V^{V}-species with chiral centers of apo-hTF. The bands at ca. 300–350 nm are probably due to phenolate-O^{-} to V^{V} charge transfer transitions. Harris [32] proposed the binding of V^{V} close to the iron hTF binding sites. It was also reported that apo-hTF is able to bind two equivalents of V^{V} in the presence or absence of the synergistic carbonate anion, which is necessary for the coordination of other metal ions [8], but not for V^{V} ions.

Several distinct techniques have been used to study the interaction of V^{V} with apo-hTF [8] (and refs therein) [25] [33] [34], and conditional binding constants (K′) have been reported. The constants obtained from calorimetric [34] and ultrafiltration [35] data are at least one order of magnitude lower than those derived from difference UV measurements [33]. More recently, from ^{51}V NMR spectroscopy Jakusch et al. [8] determined log (K'_1) = 6.0 and log ($K'_1K'_2$) = 11.5 for (V^{V})(apo-hTF) and (V^{V})$_2$(apo-hTF), respectively, these being consistent with data from ultrafiltration experiments. The binding constants K'_1 and K'_2 correspond to conditional stability constants, valid in the experimental conditions of the medium used (in the above cases at pH = 7.4), defined according to Eq. 3:

$$K'_p.\ :apo - hTF + pV^{V} \leftrightarrows [(V^{V})_p(apo - hTF)] \quad (3)$$

In the ^{51}V NMR spectra of solutions of vanadate(V) and apo-hTF at pH ~7.4 two distinct ^{51}V NMR chemical shifts are detected at $\delta_V = -529$ and -531 ppm (shoulder, often not clearly visible), and these were assigned to binding at residues of the C- and N-terminal sites [36]. In a previous study [31] some of us discussed several aspects concerning the probable relevance of binding of V^{V} to apo-hTF and holo-hTF; the binding of $V^{IV}O^{2+}$ to holo-hTF has also been addressed [37]. It is possible that a small amount of vanadate may act as a synergistic anion or bind close to the Fe-binding site; it was suggested that besides the possibility of uptake of vanadate by cells through phosphate channels, its uptake through holo-hTF endocytosis cannot be ruled out.

It was also confirmed that V^{III} binds strongly to apo-hTF [10] [31] [38] [39] and that (V^{III})$_2$(apo-hTF) corresponds to a 'closed conformation' similarly to holo-hTF [31]. The possibility of formation of V^{III}-species in blood has been proposed by several other authors [15] [40] [41]; whether (V^{III})$_2$hTF or (Fe^{III}/V^{III})hTF complexes [31] may form or not in blood serum, thus having the possibility of being uptaken by receptor-mediated endocytosis, is a subject that needs and deserves further research to be fully clarified.

Based on EPR measurements made immediately after the preparation of the solutions at pH 7.4, in the absence of dioxygen it was proposed that $V^{IV}O$-species may bind to holo-hTF [37], but it is not clear how fast these $V^{IV}O$-species may oxidize in blood serum conditions. By UV difference spectroscopic experiments Harris and Carrano [33] obtained evidence for the binding of a small amount of V^{V} to holo-hTF (up to ca. 2:0.14 (Fe:V molar ratio)) and analyzing the V and Fe content in desalted samples containing holo-hTF and vanadate(V) at pH 7.4 by ICP-AES, it was shown that V^{V} binds to holo-hTF (ca. 2:0.29 (Fe:V molar ratio)) [31]. The binding of V^{V} to holo-hTF may thus be relevant for the up-take of vanadium by cells and in this work we evaluate the binding by electrochemical techniques, matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry and small-angle X-ray scattering (SAXS).

Voltammetric methods have been widely used to investigate metal ion complexation taking advantage of the electro activity of the metal