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New insights on vanadium binding to human serum transferrin

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ABSTRACT

The knowledge on the binding of vanadium ions and complexes to serum proteins and how vanadium might be transported in blood and up-taken by cells has received much attention during the last decade, particularly as far as the transport of $V^{IV}O^{2+}$ is concerned. In this work we revise and discuss some relevant aspects of previous research, namely the two main types of binding proposed for transport of $V^{IV}O(carrier)_2$ complexes. New results, obtained by circular dichroism (CD), EPR and gel electrophoresis, regarding the binding of vanadium to hTF in the oxidation states +5 and +3 are also presented. Namely, evidences for the binding of V^{V}-species to diferric-transferrin, designated by $(Fe^{III})_2$hTF, as well as to $(Al^{III})_2$hTF, are presented and discussed, the possibility of up-take of vanadate by cells through $(Fe^{III})_2$hTF endocytosis being suggested. It is also confirmed that V^{III} binds strongly to hTF, forming di-vanadium(III)-transferrin, designated by $(V^{III})_2$hTF, and gel electrophoresis experiments indicate that $(V^{III})_2$hTF corresponds to a 'closed conformation' similar to $(Fe^{III})_2$hTF.

1. Introduction

Due to its possible physiological role as insulin-enhancer [1–3], anticancer [4–6,7], antiparasitic [8–11,12,13] as well as anti-tuberculosis [14] agents, much interest has been given to the prospective therapeutic use of vanadium compounds.

To have a physiological role the metal ion or vanadium complex must first be transported and up-taken by cells. This process is most likely conducted by plasma proteins such as human serum transferrin (hTF), human serum albumin (HSA) and immunoglobulin G (IgG) [15–28]. Many studies focused on how vanadium is transported in blood serum, namely a few reviews [15–17,20], and most publications agree in that hTF is the main vanadium transporter in serum. In this study, besides presenting a short critical review of several issues associated to the binding of vanadium to hTF, some new results are presented particularly focused on the possibility of transport of vanadium in the oxidation states +5 or +3.

Transferrin is the primarily transporter of Fe^{III} ions in the blood. It is a glycoprotein which contains around 630 amino acids arranged in two similar lobes, designated as the N-terminal (hTF_N) and C-terminal (hTF_C) lobes [29–31]. Each lobe can reversibly bind a Fe^{III} ion, but also other metal ions, such as Bi^{III}, Ga^{III}, In^{III}, Al^{III}, Cu^{II}, Mn^{II}, Zn^{II}, Ni^{II}, Ru^{III} [29–31]. Conformational changes occur in hTF upon binding or release of Fe^{III} ions: in the apo-form the protein is in the 'open conformation' while, upon binding two Fe^{III} ions forming what we designate as $(Fe)_2$hTF, the protein adopts a structure which corresponds to the 'closed conformation' of hTF. This conformation can be recognized by the hTF receptors located at the cell surface, and iron up-take occurs by internalization of transferrin in a receptor-mediated 'endocytosis' process.

Each Fe-binding site of hTF is located in clefts in the protein, one on the hTF_N, the other on the hTF_C lobe, each Fe^{III} being bound to one N-atom from His, three O-atoms from one Asp and two Tyr residues, as well as to O-atoms from the carbonate anion, usually designated by 'synergistic anion' [31,32].

Transferrin is present in human blood plasma in ~37 μM concentration. In normal serum, only about 30% of the total binding sites are occupied by iron [32,33]; thus there are still sites available for other metal ions, without need to replace the strongly bound Fe^{III}. Vanadium, in the form of $V^{IV}O^{2+}$, can bind hTF and evidences indicate that this binding occurs mainly in the Fe-binding sites, although binding to histidine residues at the protein surface has also been suggested [15,17–28,31,34–38]. Recently, [39] geometry optimization calculations were carried out for the binding of $V^{IV}O^{2+}$ to the hTF_N lobe, indicating that in the presence of CO_3^{2-} or HCO_3^-, V^{IV} is bound to five atoms of the Fe-binding site in a distorted geometry. The ^{51}V (and ^{14}N) *A* tensors were also calculated by DFT methods and compared with the experimental values.

Abbreviations: hTF, human serum transferrin; Holo-hTF, holo-transferrin or di-ferric-hTF; Apo-hTF, apo form of hTF; hTF_N, N-terminal lobe of hTF; hTF_C, C-terminal lobe of hTF; CD, circular dichroism; ICP, inductively coupled plasma; MS, mass spectrometry.

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Globally, of all calculated $V^{IV}O$–hTF structures, the one that yielded lower calculated heats of formation and better agreement with the EPR data was the structure that includes CO_3^{2-} as synergistic anion (although the one with HCO_3^- cannot be ruled out). In this structure the V=O bond length is ~1.6 Å, and the V atom is also coordinated by the phenolate–O atom of Tyr188 (at ~1.9 Å), the aspartate-O of Asp63 (at ~1.9 Å), the His249 Nτ (at ~2.1 Å), and an $O_{carbonate}$ (at ~1.8 Å). The Tyr95 phenolic-O atom is at a long distance (~3.3 Å) from the V^{IV} center. All of the O atoms are able to establish dipolar interactions with groups of the protein.

It was demonstrated that when vanadium is administered in the form of a complex, *e.g.* $V^{IV}O(carrier)_2$, where carrier is an organic compound acting as a bidentate or tridentate ligand, often the doses needed to achieve the same therapeutic insulin-enhancing effect are significantly lower. Among the several $V^{IV}O$-complexes exhibiting insulin enhancing action, $V^{IV}O(maltolato)_2$ and $V^{IV}O(ethylmaltolato)_2$ have been extensively studied [18,40–44], as well as several $V^{IV}O(picolinato)_2$ and V^{V}-dipicolinato compounds [45]. The pyridinone, 1,2-dimethyl-3-hydroxy-4-pyridinone (Hdhp) has been used in the treatment of β-thalassaemia [42,46] and its insulin-like properties studied as well [47–51]. Long term *in vivo* insulin-enhancing properties of $VO(acac)_2$ (acac = acetylacetonato) and of acac derivatives in streptozotocin-induced diabetic Wistar rats were also reported [52].

Vanadium was found mostly to be bound to hTF even in the absence of carbonate [20,53], and either if it is supplied as $V^{IV}OSO_4$ or as a $V^{IV}O(carrier)_2$ complex, most evidences have been given to support that most of the vanadium in the serum is bound to hTF [15,17,18,22–28,34–36,53–55]. In particular, HPLC-ICP-MS studies made with blood serum samples indicated that vanadium is associated to the hTF fraction, irrespective of being introduced as $V^{IV}OSO_4$ or as a $V^{IV}O(carrier)_2$ complex [15].

In the case of $V^{IV}OSO_4$ most reports indicate that two vanadium ions are bound to apo-hTF at the Fe^{III} binding sites. The binding of $V^{IV}O$ to apo-hTF most certainly involves several amino acid residues of the Fe-binding site, and as concluded by urea gel electrophoresis experiments, the formation of $(V^{IV}O)_2$hTF species may occur with the closing of the hTF conformation as is the case in $(Fe^{III})_2$hTF [38], which is an essential feature for recognition by the transferrin receptor. If vanadium is introduced in blood in the form of a $V^{IV}O(carrier)_2$ complex, these carrier ligands and/or low molecular mass (lmm) bioligands may also participate in the transport of vanadium in blood [15,18,21–34,36,54–57], and two main types of binding have been proposed:

Type 1: the formulation $(V^{IV}O)$(hTF)(carrier) when the carrier is a synergistic anion, *cis*-$V^{IV}OL_2$(hTF) when it is not a synergistic anion and the V complex predominates in the octahedral *cis*-[$(V^{IV}O)$ $(carrier)_2(H_2O)$] form at pH ~7.4 [22,24–26,56]. The formation of *cis*-$V^{IV}O(carrier)_2$(hTF) was explained with the replacement of the equatorial water molecule by an imidazole-N of an accessible His, or carboxylate-O^- of accessible Asp or Glu residues (presumably on the protein surface) with a nonspecific coordination similar to that of other proteins such as HSA and IgG (Fig. 1).

Type 2: in the systems containing $VO(carrier)_2$ (carrier = maltolate, 1-2-dimethyl-3-hydroxy-4(1*H*)-pyridinonato, picolinato, and pyrimidinonate derivatives) and hTF the formation of $(V^{IV}O)$ (hTF)(carrier), $(V^{IV}O)_2$(hTF)(carrier) and $(V^{IV}O)_2$(hTF)$(carrier)_2$, not depending on the particular features of these carrier ligand (Fig. 1).

Concerning Type 1 binding, very probably it occurs. In fact some of us recently reported the characterization by X-ray diffraction of such a binding in the formation of $VO(picolinato)_2$-lysozime adducts [58]. In this complex, a carboxylate donor atom from the side group of Asp52 of lysozyme binds to V^{IV} and Asn46 interacts with the O_{oxido}, through an hydrogen bond.

Additionally, Sanna et al. [57] recently demonstrated, by EPR measurements, the interaction of $V^{IV}O^{2+}$ and of several insulin-enhancing $V^{IV}O(carrier)_2$ compounds with holo-hTF. It was shown that $V^{IV}O^{2+}$ can interact with e.g. surface sites of the protein, probably via the coordination of His-N, Asp-COO^- and Glu-COO^- donors; the residues of His-289, His-349, His-473, and His-606 were considered the most probable candidates for the complexation of the *cis*-$V^{IV}O(carrier)_2$ moieties studied. Since holo-hTF is recognized by the transferrin receptors, the formation of these complexes with holo-hTF may also be a way to transport vanadium compounds inside the cells.

However, it is questionable that this type of binding of a $V^{IV}O(carrier)_2$ to hTF justifies the high apparent binding constants determined for these type of species [20,25,26], even assuming that dipolar or hydrogen bond interactions of several atoms of $V^{IV}O(carrier)_2$ may be established with the protein. Moreover:

(i) It is normally considered that hTF binds two $V^{IV}O^{2+}$ ions [38,59–61]. If the relevant binding takes place at imidazole-N of surface His residues, as there are at least 12 histidines [62], there is no clear reason why hTF does not bind a significantly higher number of $V^{IV}O^{2+}$ ions.
(ii) If the binding is of type 1, it would be expected that hTF, HSA and IgG would all correspond to similar types of binding. However, although EPR spectra do not differ much, UV–Vis and circular dichroism spectra differ significantly. It is also known that HSA can bind more than two V^{IV}-centers in solutions containing vanadium-maltolato complexes [18,63,64], so why not also hTF?
(iii) The concentration of hTF in blood is ca. 37 μM, while that of HSA is ca. 630 μM and of IgG ca. 85 μM, and there are other proteins present. All these proteins have available N-imidazole atoms of His residues. However, it was found by HPLC-ICP-MS that in human serum blood samples the vanadium only binds to hTF [21,34]. Thus, the following question should be answered: 'what the His residues of hTF have so special that the $V^{IV}O^{2+}$ only binds to them, despite being present in much lower amounts than those of HSA or IgG'?

Thus, the issue of how V^{IV} is transported in the form of $V^{IV}O$-carrier complexes is not fully understood, as well as how relevant this is for up-take of vanadium by target cells. Additionally, vanadium in oxidation states +5 and +3 can also bind hTF [34,53,65,66], the binding of V^{III} to hTF being quite strong, probably almost as strong as that of Fe^{III} [20,34,53,66–68]. Not much attention has been given to the possibility of transport of vanadium in these oxidation states; thus, in this work we discuss aspects related to transport of vanadium in the oxidation states +5 and also +3.

2. Experimental

2.1. Buffer solutions

All measurements were carried out in aqueous buffered media. In some cases we included the major low molecular weight blood plasma constituents (e.g. citrate and lactate) in the buffer solutions used.

2.1.1. Hepes buffer

The composition of the Hepes buffer used is 50 mM Hepes (Sigma) and 25 mM carbonate added as $NaHCO_3$ (Sigma). This buffer system was adjusted to a pH of 7.4 using concentrated KOH. In some experiments $NaHCO_3$ was not added.

2.1.2. Hepes-S buffer

The composition of the Hepes-S buffer used is 50 mM Hepes (Sigma), 25 mM carbonate added as $NaHCO_3$ (Sigma), 1 mM phosphate added as $NaH_2PO_4{\cdot}H_2O$ (Merck) and 0.20 mM KCl (Merck).