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On the Fate of Vanadate in Human Blood

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As a summary of recent activities at our research group, a model describing the distribution of pentavalent vanadium (referred to as vanadate throughout the present work) in human blood has been constructed based on our speciation studies performed in the physiological medium of 0.150 M Na(Cl) with various blood constituents. In addition, other data (most notably with the high mass serum constituents albumin and transferrin) have also been used to give as broad a view as possible. Two antidiabetic drug candidate ligands, picolinate and maltol, have also been included in order to investigate the stability of their vanadium complexes in the presence of blood constituents, i.e. to account for ligand-exchange possibilities. The model predicts transferrin to be the major carrier of vanadium in the bloodstream. It is capable of almost completely replacing the vanadium-bound picolinate even if the latter is in large excess. Maltol, on the other hand, can retain most of the vanadium when it is supplied in about a 5 mM concentration. The model still has certain limitations but it serves as a useful base for investigating structure, stability, relationship effects and provides insight into the fate of vanadate in human blood.

Introduction

Arguably the biggest contribution to the renewed interest in the bioinorganic chemistry of vanadium originates from the discovery of the insulin-enhancing properties of this transition metal and its compounds. *Diabetes mellitus*, with its explosively increasing incidence worldwide[1] and many global and societal implications,[2] is one of the most threatening and costly "epidemics" of our times. Currently insulin is used as the core treatment in both type I and type II diabetes. Although it is extremely important, there is need for substitutes, especially when dealing with type II diabetes. In addition, being destroyed in the stomach, insulin cannot be administered orally in mammals and the constant use of subcutaneous injections is inconvenient. Thus, the ideal substitute would be orally applicable and effective, particularly in the case of type II diabetes. It also should meet other criteria regarding its absorption, stability in body fluids, a desired high specificity and low toxicity.[3]

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Vanadium compounds have long been shown to exert insulin-like effects both in vitro,[4–8] and in vivo,[3,9,10,11–20] the first example being documented as early as 1899.[21] In all these tests, vanadium has been demonstrated to perform insulin-like actions in virtually all respects.[9,22,23] The amount of vanadium needed to induce the required metabolic effects has been found to be in the range of micromolar to millimolar concentrations,[4,6,7,8,24] depending not only on the nature of the compound but also on the time course of the management (generally lower doses are needed when long-term treatment is applied). It is worth noting that usually a longer time has been required during in vivo experiments to observe the desired insulin-like effects as compared to in vitro tests. On the other hand, the effects have mostly been long lasting, even after stopping the administration of the compounds. These indicate a possible accumulation of the active component(s) in the body,[9] as has been shown in the case of bones.[25,19,20] It is also important to point out that plasma insulin levels have not been increased during the treatments. Thus, the glucose-lowering effects are not due to a vanadium-induced increase in insulin secretion,[14,26] but rather they are realised by mechanisms that are (at least partly) different from that of insulin.[3,27] However, these mechanisms are not yet completely elucidated. In addition, different vanadium compounds operate along (partially) different pathways, further complicating the picture.[9,28,29]

MICROREVIEWS: This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

Unfortunately, mostly owing to the low adsorption and specificity of vanadium, problems with toxicity and side effects arise. On the other hand, these problems can in theory be overcome by applying the proper ligands. In fact a great advantage of vanadium compounds is that virtually all of their important features, such as stability, oral availability, as well as absorption, toxicity, etc. can be fine-tuned by means of different ligands. While investigating the effects of different vanadium complexes, it has also been suggested that it is always the uncomplexed vanadium that is the active component, irrespective of the introduced species.[30] In other words, the only role of the ligand could be to deliver vanadium as efficiently as possible, including translocation across the cell membrane. In opposition to this hypothesis, different mechanisms of action have been suggested for different vanadium complexes, depending on the nature of the compound.[9,28] Whatever the case may be, ligands may still play an important role in facilitating the uptake and/or transport of vanadium, thereby reducing toxicity and side effects and possibly increasing effectiveness.

It should also be noted that the incidence of diabetes is increasing among pets, for similar reasons as in the case of humans, namely sedentary lifestyle and obesity.[31] Food supplies often contain nutrients in much higher amounts than the natural diet of the animal would do or require. For instance, there is a large amount of carbohydrates in certain commercial cat foods, despite the well-known fact that cats are strict carnivores, and hence their diet naturally is based mostly on protein and fat with very little carbohydrates.[32] This causes serious malnutrition as the high carbohydrate diet leads to decreased insulin sensitivity in cats.[33] However, applying regular injections in animal treatment is even more troublesome than in the case of humans. Thus, oral medication is also desired in veterinary practices, and vanadium compounds may represent one type of solution.

Finally, it has to be pointed out that, although research in this field involves vanadium in its +4 and +5 oxidation states (as well as +3, to a much lesser extent), in the present article we focus solely on the +5 oxidation state. Thus, from here on, vanadium in the text refers to vanadium(V), unless otherwise specified.

Speciation Studies and Modelling

Numerous drugs are known to be bound to plasma proteins when entering the bloodstream. The extent and nature of this interaction has a profound effect on the distribution of the drug into other compartments and on its therapeutic as well as toxic effects.[34–36]

Complete speciation studies are of great value in the fundamental research of this field. By the pH-independent formation constants obtained from such studies modelling of physiological conditions can be done, which is of fundamental interest in revealing vanadium interactions in humans as well as in drug design and production. Once vanadium speciation is established with all the major components in blood, including mixed ligand species, modelling can be used to determine the fate of any particular vanadium complex under physiological conditions, provided that

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the speciation with the given ligand in the complex is also known. This paper represents such an attempt. Although some of the constituents of human blood had to be excluded (see below), our ongoing series of investigations,[37–39] have provided us with enough data to form a base for model calculations that are of interest to researchers in this field.

Albumin is found in approximately 630 μM concentration (Table 1) in human blood, and it (together with α_1-acid glycoprotein) is one of the most common nonspecific binding proteins. Transferrin is another binding protein that is important in the case of metal transportation[40,41] (especially with iron, hence the name). It is present in human blood in about a 37 μM concentration (Table 1), and has two binding sites per protein to accommodate metal ions.[42] In normal serum, only about 30% of the total binding sites are occupied by iron.[43] This means that there are still sites available for other metal ions, without needing to replace the tightly bound iron. It has to be noted that there is a bidentate carbonate in the active site, usually referred to as the synergistic anion,[44,45] without which practically no iron–transferrin binding occurs.

Since both albumin and transferrin play important roles in the distribution and transportation of different compounds, they cannot be ignored when evaluating the interactions of any introduced vanadium-containing drugs in human blood. Indeed, it has been demonstrated that vanadium binds to both of these proteins,[46–54] although the binding is approximately 1000-times stronger to transferrin than to albumin.[55] Interestingly, vanadium has been found to be bound to transferrin even in the absence of HCO_3^-.[56]

Beside these two proteins, other constituents should also be included in a model that attempts to elucidate the fate of vanadium in human blood. Table 1 lists five additional low mass bioligands: glycine, lactate, phosphate, citrate and histidine, with their respective concentrations in human blood. Some constituents, such as carbonate, sulfate, cysteine, etc. have been excluded owing to the lack of (reliable) formation constants for their vanadate complexes. Of these excluded ligands, only hydrogen carbonate should be of considerable importance, because of its high concentration in blood (almost 25 mM).

In addition to the physiological concentrations of the selected blood constituents, Table 1 also lists the complexes they form with vanadate. These species have been included in the model calculation. Unfortunately, some of the formation constants given in Table 1 have not been determined in the physiological medium, which introduces an error in the model calculations. Moreover, in the case of transferrin, another simplification has been made. As explained earlier, transferrin is capable of binding two metals per protein, which could lead to the formation of a V_2Trf-type complex with vanadium. On the other hand, iron is present and competes with vanadium for the binding sites under physiological conditions. What is more, the binding of iron is favoured over other metal ions by transferrin. Since no iron is included in the model calculation, this kind of competitive binding has to be included in another way. It has been achieved by performing the calculation with VTrf-type complexes only. The other binding site can then be occupied by iron, as is most probably the case. Thus, the complex VTrf can in fact be considered as FeVTrf. Although this is a rather arbitrary simplification, it should give satisfactorily accurate results, since the amount of vacant binding sites of transferrin is estimated to be around 40 μM in human serum in the presence of iron.[42]

Table 1. Vanadate complexes with selected constituents in human blood. The concentrations of the ligands in healthy human subjects are given according to ref.[57] Maltol and picolinate are included to represent drug candidate ligands. The simplified notations for the complexes represent only their nuclearities and charges. Whenever charge is not indicated, it has not been determined in the study. Asterisks denote complexes with the same composition but different structures. The higher number of asterisks, the less dominating the species is. Formation constants for the complexes are shown at 25 °C, and the ionic media in which they have been determined are also noted.

Ligand (Abbreviation)	Concentration in human blood	Complex notation	logβ	Medium
Glycine (Gly)	2.3 mM	VGly	1.8[a,i]	1.0 M KCl, HEPES buffer
Lactate (Lac^-)	1.51 mM	$VLac^{2-}$	0.88[b]	0.15 M Na(Cl)
		$VLac^-$	6.92[b]	
Phosphate (P^-)	1.1 mM	VP^{3-}	–5.68[c]	0.15 M Na(Cl)
		VP^{2-}	1.51[c]	
		VP_2^{4-}	–3.94[c]	
		VP_2^{3-}	2.36[c]	
Citrate (Cit^{3-})	99 μM	$VCit^{2-}$	14.19[d]	0.15 M Na(Cl)
Histidine (His)	77 μM	VHis	0.2[e,i]	0.15 M NaCl
		*VHis	–0.2[e,i]	
Albumin (Alb)	630 μM	VAlb	3.0[a,i]	1.0 M KCl, HEPES buffer
Transferrin (Trf)	37 μM	VTrf	6.5[f,i]	0.1 M HEPES buffer
Picolinate (Pi^-)	–	VPi_2^-	18.92[g]	0.15 M Na(Cl)
		*VPi_2^-	18.77[g]	
		**VPi_2^-	18.24[g]	
		VPi^-	9.31[g]	
		*VPi^-	8.70[g]	
Maltol (Ma)	–	VMa^-	2.66[h]	0.15 M Na(Cl)
		VMa^{2-}	–7.37[h]	
		VMa_2^-	7.02[h]	

[a] Ref.[51] [b] Ref.[37] [c] Ref.[38] [d] Ref.[39] [e] Ref.[58] [f] Ref.[48] [g] Ref.[59] [h] Ref.[60] [i] Constants are log K values, not log β.

In all cases, only those mononuclear complexes that may be relevant at the pH of human blood have been listed. Higher nuclearity species have been omitted completely, as they are extremely unlikely to be present at very low total concentrations of vanadium. For the present modelling $[V]_{tot}$ = 1 μM has been chosen, since vanadate species have shown insulin-like effects at this concentration, but no toxicity.[8] For a more complete list of vanadate species formed with a given ligand at various pH values and total concentrations, consult the references.

In order to represent an introduced vanadium-containing drug, either vanadate–picolinate or vanadate–maltol complexes (Table 1) have also been included in the model. This has been done to investigate whether the carrier ligand gets replaced by any of the blood constituents. Picolinate and maltol have been chosen since they form very stable mononuclear complexes with vanadium.[59,60] Extensive clinical research and in vitro tests have been carried out on complexes of vanadium and either of these ligands (or derivatives).[15,61,62,63–66] In addition, both ligands bind to vanadium over a wide pH range, covering conditions from acidic (stomach) to nearly neutral and slightly alkaline (blood, small intestines). This is of importance when taking a designed drug orally.

The calculations presented here have been performed using WINSGW,[67] a programme package based on the SOLGASWATER algorithm.[68] For the species formed in the H^+–$H_2VO_4^-$ binary system, formation constants have been taken from ref.[69] In cases of the different ternary H^+–$H_2VO_4^-$ ligand systems, only the complexes listed in Table 1 have been taken into consideration with the formation constants given there. For the model, $[V]_{tot}$ = 1 μM and pH = 7.4 have been used, with $[Ligand]_{tot}$ set to values given in Table 1 for each of the ligands. Thus, the matrix consisted of inorganic vanadates together with all the vanadate–ligand complexes given in Table 1.

Figure 1 shows the results of one of the model calculations. As can be seen, when there is no carrier ligand introduced with vanadium and the high mass serum constituents (HMS), albumin and transferrin, are excluded from the model, most of the metal is present as inorganic monovanadate, $H_2VO_4^-$ and HVO_4^{2-} (Figure 1, a). Approximately 12% of vanadium is bound to glycine, and about 1–2% to other constituents (mostly phosphate). When the proteins are also included, however, more than 98% of the total vanadium is bound to transferrin (Figure 1, b). To illustrate the strength of picolinate itself as a carrier ligand in the absence of HMS, the distribution is also shown for that case (Figure 1, c). The diagram shows that a substantial fraction of vanadium (about 90%) is bound to picolinate when neither of the proteins is present. However, when HMS are included the transferrin binds almost all of the vanadium (approximately 90%), despite the high concentration of picolinate (20 mM, Figure 1, d). This is in accordance with the results obtained with canine blood,[47] where it has been shown that almost 80% of vanadium is bound to transferrin, regardless of what kind of inorganic vanadium species has been injected originally. It is also in accordance with the fact that introduced vanadium seems to be targeted towards iron-rich cells,[70] i.e. the transport route of vanadium follows that of iron.

Interesting results are found when doing the same kind of modelling with maltol, instead of picolinate (Figure 2). Using the same concentration for maltol as for picolinate in the previous calculation (20 mM), it is obvious how much stronger this ligand binds to vanadate at physiological pH (Figure 2, a). Even in the presence of HMS, almost 97% of the total vanadium is bound as VMa_2^- (Figure 2, b). When lowering the total concentration of maltol to 5 mM, more vanadium is bound to transferrin, but still not more than approximately 34% (Figure 2, c). This means that although transferrin binds vanadium very strongly, a considerable fraction of the metal can still be bound to its original carrier if an appropriate ligand is applied. Certainly the ligand needs to be in excess, but it does not have to be at extreme concentrations to be able to effectively compete with the HMS. In contrast, recent findings with vanadium(IV)–bismaltolato complexes suggest that transferrin is capable of

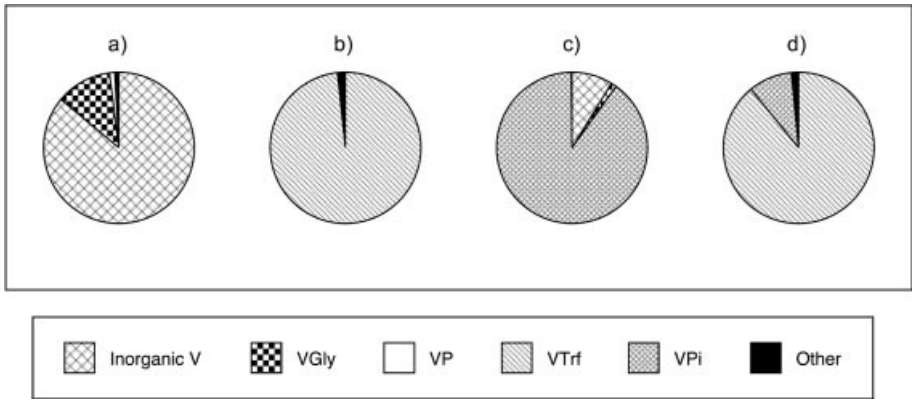

Figure 1. Distribution of vanadate in the presence of different blood constituents at pH = 7.4. $[V]_{tot}$ = 1 μM in all cases, concentrations of the constituents are according to Table 1. HMS is short for the high mass serum constituents, albumin and transferrin. Gly stands for glycine, P for phosphate, Trf for transferrin and Pi for picolinate in the legend. From left to right: (a) No HMS, no Pi (b) No Pi, HMS included (c) No HMS, $[Pi]_{tot}$ = 20 mM (d) $[Pi]_{tot}$ = 20 mM, HMS included.

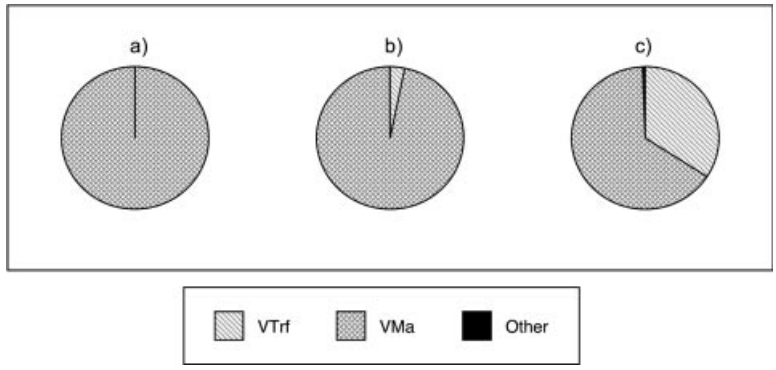

Figure 2. Distribution of vanadate in the presence of blood constituents at pH = 7.4. $[V]_{tot}$ = 1 μM in all cases, concentrations of blood constituents are according to Table 1. HMS is short for high mass serum constituents (albumin and transferrin). Trf stands for transferrin and Ma for maltol in the legend. From left to right: (a) No HMS, $[Ma]_{tot}$ = 20 mM (b) HMS included, $[Ma]_{tot}$ = 20 mM (c) HMS included, $[Ma]_{tot}$ = 5 mM.

replacing the maltol ligand(s) of the introduced complex at certain concentration ratios.[71] However, it is worth noting that vanadium(IV) has a higher affinity to transferrin than does vanadium(V).[56] In other words, vanadium(V) complexes generally have a better chance to avoid ligand replacement by transferrin than the corresponding ones with vanadium(IV). This is especially interesting when comparing the vanadium–bismaltolato complexes in each oxidation state. Vanadium(IV)–maltol (or derivative) complexes have been extensively studied,[61–65] and have shown promising insulin-enhancing properties. However, the corresponding vanadium(V) species seems to be considerably less active in this regard.[72] Why that is so is hard to explain by our speciation model alone, especially when taking into account the ease by which vanadium(V) and (IV) could interconvert under physiological conditions. Our model indicates that vanadium(V) forms very strong complexes with maltol (Figure 2), but says nothing about the kinetic lability of the formed species. A too inert species may effectively remove vanadium from the system by binding it too tightly and thereby prohibiting its interactions with other compounds. Additionally, it could be a charge effect. The bismaltolato complex of vanadium(IV) is neutral, whereas the corresponding vanadium(V) species is –1 charged. This certainly affects the way the complex can enter the cells and might be at least one of the reasons why they show different insulin-enhancing activities.

It has to be mentioned that limitations do apply to the models presented above. First of all, the formation constants used are from different media and a considerable simplification has been made when considering only VTrf but not V_2Trf in the model. In addition, mixed ligand vanadate complexes so far have only been determined with lactate and citrate[39] out of all the bioligands shown in Table 1. Although these are extremely weak complexes, others (with phosphate, for instance) might not necessarily be. In addition, recent data on the interactions of the vanadium(IV)–bismaltolato complex with HMS indicated that albumin may take part in the formation of such mixed-ligand complexes (in that case a vanadium(IV)–maltol–albumin species).[71] While no such species have yet been reported with vanadium(V) complexes, their existence cannot be ruled out. Nevertheless, these are not likely to cause major changes in the overall picture, and the model should be valid for all the major features and trends.

Concluding Remarks

Speciation studies are very useful for the bioinorganic and medicinal chemistry of vanadium compounds in many respects. First of all, they provide insight to the complex formation patterns and preferences of vanadate in the presence of various biologically important or potential drug candidate ligands. This is extremely valuable, since it helps in tailoring the properties of the complex and also casts light on the fate of vanadium in the presence of e.g. blood constituents. In addition, by means of the formation constants obtained from such studies, parameters can be optimised for crystallisation studies or in general to obtain a maximum yield in the synthesis of a given complex. Moreover, physiological conditions can also be modelled (with certain restrictions) to predict what happens to any introduced vanadium compound when it enters e.g. the stomach, bloodstream, etc. Naturally, care must be taken to use the proper ionic medium for the studies (e.g. 0.150 M Na(Cl), to represent the ionic strength of human blood). A series of speciation studies with pentavalent vanadium has been carried out with different blood constituents in this context.[37–39] As incomplete as this series is, it forms a solid base for model calculations aiming to elucidate the fate of vanadate (compounds) in human blood. Although certain limitations apply to the model presented in this paper, the major features should be valid nonetheless, providing a useful overview and practical guidelines.

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