Inorganica Chimica Acta 420 (2014) 75–84

Contents lists available at ScienceDirect

Inorganica Chimica Acta

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

Uptake of potential anti-diabetic $V^{IV}O$ compounds of picolinate ligands by red blood cells

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ARTICLE INFO

Article history:
Available online 14 January 2014
Recent Advances in Vanadium Chemistry
Special Issue

Keywords:
Vanadium
Anti-diabetic compounds
Hemoglobin
Erythrocytes
Bioinorganic chemistry
EPR spectroscopy

ABSTRACT

The interaction of three potential anti-diabetic $V^{IV}O$ compounds formed by picolinate (pic), 3-methylpicolinate (3-mepic) and 6-methylpicolinate (6-mepic) with hemoglobin (Hb) and red blood cells was studied with the combined application of spectroscopic (EPR), spectrophotometric (UV–Vis) and computational (DFT methods) techniques. In the ternary systems with hemoglobin, pic and 3-mepic (L) form mixed species *cis*-VOL_2(Hb), with the equatorial binding of an accessible His residue, whereas 6-mepic forms VO(6-mepic)(OH)(Hb). The experiments about the uptake of $V^{IV}O$ complexes by red blood cells indicate that only [$VO(pic)_2(H_2O)$] penetrates the erythrocyte membrane in a significant amount, whereas for [$VO(3\text{-mepic})_2$] and [$VO(6\text{-mepic})_2$] the hydrolytic reactions at physiological pH hinder the diffusion in the intracellular medium. Inside the red blood cells, the biotransformations depend mainly on the strength of the ligand. Pic and 3-mepic form *cis*-VOL_2(Hb) and *cis*-$VOL_2(Cys\text{-}S^-)$ with the equatorial coordination of a thiolate-S^- stemming from GSH or a membrane protein. Instead, the less thermodynamically stable compound, [$VO(6\text{-mepic})_2$], loses the two ligands after the interaction with the membrane or inside the erythrocytes to give the same species formed by free $V^{IV}O^{2+}$ ion: $(VO)Hb^{\beta}$ and $(VO)Hb^{\gamma}$, with $V^{IV}O^{2+}$ coordinated to the sites β and γ of hemoglobin, and $VO(L^1,L^2)$ and $VO(L^3,L^4)$, where L^1, L^2, L^3 and L^4 are generic red blood cell bioligands, such as proteins (for example, Hb) or low molecular mass (l.m.m.) components. The distribution of an insulin-enhancing V compound between the serum and the red blood cells may influence the mechanism of action and the activity of a V drug and explain the different effectiveness observed in the literature.

1. Introduction

Vanadium compounds exhibit a wide variety of pharmacological properties, among which anti-parasitic, spermicidal, anti-viral, anti-HIV, anti-tuberculosis and anti-tumor action [1,2]. One of the most important applications of V derivatives in medicine is their potential use in the therapy of patients suffering from type II diabetes mellitus (DM) [3–8], which affects worldwide more than one hundred million people according to the estimates of World Health Organization [9]. A class of very promising complexes consists of neutral $V^{IV}O$ species with bidentate anionic ligands L^- (with L named also organic *carrier*) with composition VOL_2; for example, [$VO(maltolato)_2$] or BMOV is now the benchmark compound for the new molecules with anti-diabetic action [3,6,10,11]. $V^{IV}O$ complexes are more effective in lowering the glucose concentration in blood serum than the parent salt $VOSO_4$ and are well tolerated in all the animal models of diabetes. One derivative of BMOV, [$VO(ethylmaltolato)_2$] or BEOV, got to phase IIa of the clinical trials [12].

$V^{IV}O$ species with $VO(N_2O_2)$ coordination appeared to be very active in the treatment of insulin dependent diabetes mellitus [13,14]. In 1995 [$VO(picolinato)_2(H_2O)$] was found to be a strong inhibitor of fatty acid mobilization and effective in the treatment of rats affected by diabetes induced with streptozotocin (STZ) [15]. Subsequently, it was shown that the complex formed by the methyl derivative of picolinate, [$VO(6\text{-methylpicolinato})_2$], exhibits better *in vitro* insulin-enhancing and *in vivo* hypoglycaemic effect in STZ-rats than parent [$VO(picolinato)_2(H_2O)$] [16,17]. Subsequently, several $V^{IV}O$ complexes formed by picolinate derivatives were tested, such as [$VO(2\text{-quinolinecarboxylato})_2$] [13], [$VO(3\text{-methylpicolinato})_2$] [18], [$VO(5\text{-iodopicolinato})_2$] [19], *trans*-[$VO(6\text{-ethylpicolinato})_2(H_2O)$] [20], *cis*-[$VO(3\text{-hydroxypicolinato})_2(H_2O)$] [21], *cis*-[$VO(5\text{-carbomethoxypicolinato})_2(H_2O)$] [22] and the bis-chelated *cis*-octahedral species formed by 2,5-dipicol-

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http://dx.doi.org/10.1016/j.ica.2013.12.038
0020-1693/

inic acid and its monoesters [23]. The results suggest that not only the electronic features of the substituent, but also its position on the pyridine ring can alter the biological activity of the complex. $V^{IV}O$ compounds with potential pharmacological activity were recently divided into three classes (named A, B and C): the ligands belonging to class A (for example, 3-methylpicolinic acid) form square pyramidal complexes in aqueous solution and in the solid state, the ligands belonging to class B (picolinic acid) form *cis*-octahedral species in which the two ligands adopt an (*equatorial–equatorial*) and an (*equatorial–axial*) arrangement and one water molecule occupies the *cis* position with respect to the V=O bond, whereas the class C ligands (6-methylpicolinic acid is one of them) yield bis-chelated species which, in aqueous solution, are in equilibrium between the square pyramidal and *trans*-octahedral form with one water in *trans* to the V=O group [24].

The biotransformation of the anti-diabetic complexes in the blood is an important aspect of the drug metabolism, but the mechanism with which vanadium is transported to the target organs and the *in vivo* form are not fully known and have been recently reviewed [4,5,8,25–27]. However, the attention of all of these studies has been devoted mainly to the plasma, where the interaction with the proteins plays a key role; in particular, the binding of $V^{IV}O^{2+}$ ion and vanadium anti-diabetic drugs to transferrin (hTf), albumin (HSA) and immunoglobulin G (IgG) was demonstrated in the literature [28–42]. There is a general consensus that, when the arrangement of V compound in aqueous solution at pH 7.4 is *cis*-octahedral and specific sites are not available for $V^{IV}O^{2+}$, species with stoichiometry *cis*-VOL_2(Proteins) are formed through the coordination of an accessible His-N that replaces the water molecule occupying the fourth equatorial position [29,33–39,42].

The participation of erythrocytes in the transport of potential anti-diabetic VOL_2 compounds has been considered previously but never described in detail. However, in the light of the recent experimental evidences that revealed no correlation between total serum V concentration and the insulin-enhancing action and that V pools other than the serum may be related to its pharmacological effects [43], it could be very important. Indeed, the uptake of vanadium complexes by the erythrocytes, the biotransformation owing to the interaction with the red blood cell bioligands, the possible excretion under the appropriate conditions or the confinement inside the erythrocytes are processes that may influence and be related to the anti-diabetic activity of V compounds. Yang et al. proved that $[VO(ma)_2]$ and $[VO(acac)_2]$, where ma is maltolate and acac is acetylacetonate, enter the cells through passive diffusion and have an absorption kinetically quicker than $NaVO_3$ [44], which instead is transported through the anion channels. The distribution of vanadium between blood serum and erythrocytes remains unknown, depending on the specific compound under examination, on the redox reactions between V^{V} and V^{IV}, on the binding of V^{IV} and V^{V} to the available bioligands and on the rate of influx and efflux across the cell membrane [45]. Electrophoresis suggested that about 77% of vanadium is associated with the serum fraction, regardless of the chemical form initially injected [45], but a general analysis of the data available in the literature suggested that in mammals the ratio between V in the plasma and erythrocytes is in the range 90:10–95:5 [46–48].

In the red blood cells the most important bioligand appears to be hemoglobin (Hb), whose concentration is about 5.1 mM [49]. In many papers it has been affirmed that $V^{IV}O^{2+}$ ion is bound mainly to Hb [50–52]; for example, the amount of $V^{IV}O^{2+}$ bound to hemoglobin was diminished by addition of ATP or EDTA, two ligands than can compete for the metal complexation [51]. An unambiguous demonstration of $V^{IV}O^{2+}$ binding to Hb *in vitro* has been given very recently in the literature [53].

In this work, the uptake by the red blood cells of three potential anti-diabetic VOL_2 compounds formed by picolinate derivatives, $[VO(pic)_2(H_2O)]$, $[VO(3\text{-mepic})_2]$ and $[VO(6\text{-mepic})_2]$, with pic, 3-mepic and 6-mepic indicating picolinate, 3-methylpicolinate and 6-methylpicolinate (Scheme 1) was studied through the combined application of spectroscopic (EPR and UV–Vis spectroscopy) and computational (DFT methods) techniques. The systems with hemoglobin were examined to determine the biotransformation of the three complexes inside the erythrocytes. The results can provide new information about the transport of pharmacologically active V compounds toward the target organs; in particular, it is important to know if these species can flow in and out of the erythrocytes or, in contrast, remain confined in an inactive form inside the red blood cells.

2. Experimental and Computational Section

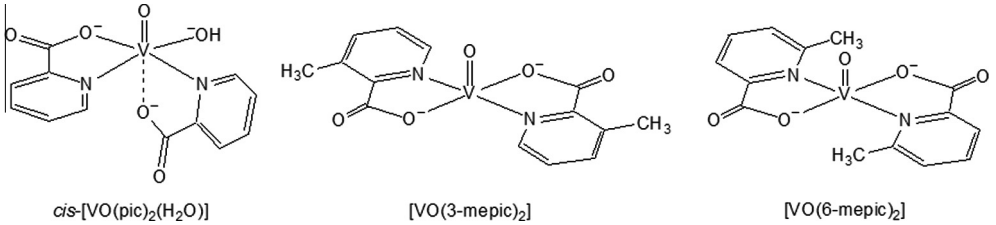
2.1. Chemicals

Water was deionized prior to use through the purification system Millipore MilliQ Academic. $V^{IV}O^{2+}$ solutions were prepared from $VOSO_4{\cdot}3H_2O$ following literature methods. Hemoglobin (Hb) was purchased from Sigma (H7379) and has a molecular mass of 64.5 kDa. The product specification sheet accompanying Hb states that this contains 0.31% of iron, which corresponds to a saturation of the iron sites of about 95%. Other chemicals, i.e. picolinic, 3-methylpicolinic and 6-methylpicolininic acid, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 1-methyimidazole (1-MeIm), GSH, 3-mercaptopropionyl sulfonic acid (3-mps), 4-(2-pyridylazo)-resorcinol (PAR), EDTA, NaCl, NaH_2PO_4 and glucose were Aldrich products of the highest grade available and used as received.

2.2. Preparation of the solutions for EPR measurements

The solutions were prepared dissolving in ultra-pure water $VOSO_4{\cdot}3H_2O$. Argon was bubbled through the solutions to ensure the absence of oxygen and avoid the oxidation of $V^{IV}O^{2+}$ ion. To the solution containing the metal ion, HEPES as a buffer (1.0×10^{-1} M) and an appropriate amount of the ligand (pic, 3-mepic and 6-mepic) were added to have a ratio between the organic *carrier* and $V^{IV}O^{2+}$ ion of 2.

Subsequently, pH was raised to *ca.* 5.5 and to 1 mL of the solution, again carefully purged with argon, Hb was added to obtain a concentration of 3.1×10^{-4} M. Finally, pH was adjusted to *ca.* 7.4



Scheme 1. Structures of the three anti-diabetic V compounds studied.