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Biochimica et Biophysica Acta 1770 (2007) 1212–1218
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbagen

Reduction of vanadium(V) to vanadium(IV) by NADPH, and vanadium(IV) to vanadium(III) by cysteine methyl ester in the presence of biologically relevant ligands

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Received 5 January 2007; received in revised form 18 April 2007; accepted 9 May 2007
Available online 21 May 2007

Abstract

To better understand the mechanism of vanadium reduction in ascidians, we examined the reduction of vanadium(V) to vanadium(IV) by NADPH and the reduction of vanadium(IV) to vanadium(III) by L-cysteine methyl ester (CysME). UV-vis and electron paramagnetic resonance spectroscopic studies indicated that in the presence of several biologically relevant ligands vanadium(V) and vanadium(IV) were reduced by NADPH and CysME, respectively. Specifically, NADPH directly reduced vanadium(V) to vanadium(IV) with the assistance of ligands that have a formation constant with vanadium(IV) of greater than 7. Also, glycylhistidine and glycylaspartic acid were found to assist the reduction of vanadium(IV) to vanadium(III) by CysME.

Keywords: Vanadium; Ascidian; NADPH; Biologically relevant ligands; Reduction

1. Introduction

Vanadium compounds with oxidation states from III to V have been found in living organisms and play various important roles in these organisms [1]. Haloperoxidases from marine algae and terrestrial fungi are vanadium(V) (V^V)-dependent enzymes that catalyze the halogenation of organic substrates by H_2O_2 and halide [2]. Amavadin, a natural vanadium(IV) (V^{IV}) complex isolated from the mushroom *Amanita muscaria* [3] is a unique non-oxo or "bare" V^{IV} complex with two chelating ligands (*S,S*)-2,2′-(hydroxyimino)dipropionate [4]. V^{IV} species are also regarded as potential therapeutic agents for diabetes mellitus [5,6].

In addition to functioning in biological systems described above, vanadium also accumulates as vanadium(III) (V^{III}) in the blood cells of certain ascidians (also known as sea squirts or tunicates). Ascidians of the suborder Phlebobranchia accumulate V^V from seawater and store it in their blood cells, which are known as vanadocytes, as V^{III} species [7,8]. During the accumulation process, V^V is reduced to V^{III} via V^{IV}, but the underlying reduction mechanism remains unknown. V^V can be reduced to V^{IV} without difficulty due to the adequate redox potential (VO_2^+/VO^{2+}: 1.00 V in a strongly acidic solution [9]). In fact, a number of biologically relevant reducing agents, including ascorbate [10–12], cysteine [13], norepinephrine [14], glutathione [12,15], oxalic acid [16] and tunichromes, blood pigments isolated from a tunicate [17,18], have been found to reduce V^V to V^{IV}.

Recently, the vanadocytes of vanadium-rich ascidians were found to express enzymes of the pentose-phosphate pathway [19,20]. Since the pentose-phosphate pathway supplies the reducing agent NADPH, NADPH may play a role as a reducing agent in ascidians. Shi et al. have shown that V^V is reduced by

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doi:10.1016/j.bbagen.2007.05.003

glutathione reductase/NADPH [21] and flavoenzymes/NADPH [22]. Furthermore, we previously showed that NADPH could reduce V^V to V^{IV} in the absence of enzyme catalysis when EDTA, which has a large stability constant for VO^{2+}(log$K$= 18.63: K=[complex]/[VO^{2+}][EDTA] [23]), is also present in the reaction mixture [24]. In contrast, NADPH can only partially reduce V^V in the absence of EDTA [24].

Reduction of V^{IV} to V^{III}, which has a rather low redox potential (V^{IV}/V^{III}: 0.337 V [9]), does not usually require the biologically relevant reducing agents shown above. Tunichromes, a class of hydroxy-dopa containing tripeptides, have been isolated from some ascidian species [17,18,25,26]. Since tunichromes contain pyrogallol and cathecol moieties that can have a reducing functionality, it was proposed that tunichromes might participate in the reduction of vanadium in ascidians [27]. In fact, pyrogallol (a model compound of tunichromes) reduces V^{IV} to V^{III} in tetrahydrofuran [28]. However, whether tunichromes directly participate in the reduction of vanadium in ascidians is not clear [29–31].

Frank et al. reported that large quantities of intracellular sulfate as well as aliphatic sulfonic acids such as cysteic acid, are present in ascidian blood cells [32,33]. Since cysteic acid is an oxidation product of cysteine, cysteine might be the reducing agent responsible for the reduction of V^{IV} to V^{III} in ascidians. Although cysteine itself cannot reduce V^{IV} to V^{III}, we have shown that cysteine methyl ester (CysME) can reduce V^{IV} to V^{III} with the assistance of EDTA and EDTA-like ligands [34]. Therefore, if cysteine does work as a reducing agent in ascidians, some biogenic ligands promoting the reduction of V^{IV} are expected to participate in the reaction.

Vanadium is believed to be incorporated into the tissues of ascidians by binding to macromolecules such as proteins [35]. Several vanadium-binding proteins (known as Vanabins) have been isolated from vanadium-rich ascidians [36–38]. All Vanabins are rich in cysteine residues, but these cysteine residues exist entirely in the oxidized form as disulfide bonds in the structurally characterized compounds [39]. Vanabins in their oxidized forms cannot work as reducing agents. However, if reduced Vanabins exist in ascidians, they may act as reducing agents. In addition, the amino acid residues in Vanabin proteins may provide the functionalities that promote the reduction of V^{IV} [40].

In general, chelating ligands (chelators) that stabilize a metal complex in a lower oxidation state can promote the reduction of the metal. One such example is the strong metal chelator EDTA (log$K$ for VO_2^+ is 15.5 [41]) is the case as shown in our previous paper [24]. Porphyrins and siderophores are strong naturally occurring chelators of iron, but similar strong chelators of vanadium have not been found in ascidians. Therefore, proteins or peptides, or other generally occurring chelating compounds, must participate in the reduction of vanadium in ascidians. The goal of the present study was to determine the minimal value of the stability constant for VO^{2+} required for a biogenic ligand to promote the reduction of V^V to V^{IV} by NADPH. In addition, we have examined whether CysME reduces V^{IV} to V^{III} using biogenic ligands such as amino acids and small peptides that are moderately weak chelators compared to EDTA.

2. Experimental

2.1. Reagents

Reagent grade chemicals were obtained from Wako Pure Chemicals or Aldrich. All reagents were used without further purification.

2.2. Sample preparation for UV-vis spectroscopy

Reduction of V^V: Typically, sodium orthovanadate(V) Na_3VO_4 (0.0147 g: 0.08 mmol) and an appropriate amount of ligand were dissolved in 4.0 ml of buffer (50 mM Tris–HCl, pH 7.0) to yield a 20 mM solution of vanadium. The resulting solution was stirred for more than 24 h until it became colorless, and was then transferred to Tube A of a mixing chamber (Scheme 1). The solution was deaerated, and then saturated with argon. The vanadate solution was transferred to Tube B, which already contained 10 equivalents of solid NADPH. After the NADPH was dissolved, the mixture was put back to Tube A and the pH of the solution was readjusted to ~7, if necessary. The solution was returned to Tube B and transferred to a cuvette equipped with stopcocks using a syringe.

Reduction of V^{IV}: The procedure for the glycyl-L-histidine (GlyHis) system is shown below as a representative method. Vanadyl sulfate $VOSO_4 \cdot 3H_2O$ (0.0543 g: 0.25 mmol) and GlyHis (0.0531 g: 0.25 mmol) were dissolved in 20 ml of buffer (100 mM NaOH in CH_3COOH, pH 4.4) to make a 12.5 mM vanadium solution. The mixture was stirred for 24 h to allow it to reach equilibrium. At this stage, the reaction mixture was generally light blue. The solution was then transferred to Tube A of the mixing chamber. Then 5 equivalents of L-cysteine methyl ester (CysME) hydrochloride (0.215 g: 1.25 mmol) were placed in Tube B. After the mixing chamber had been deaerated and saturated with argon, CysME was dissolved in the vanadium solution. The mixing chamber was immediately deaerated and refilled with argon. The resulting mixture was returned to Tube A and 1 M NaOH was added to adjust the pH of the solution 4.4. The solution was put back into Tube B and an appropriate volume of the solution was transferred to a cuvette for analysis.

2.3. Measurements

UV-vis absorption spectra were recorded on a SHIMADZU UV-3100PC spectrometer. All spectra were blanked against water since a solution containing only ligands and NADPH or CysME did not give any absorption bands in the visible region even after standing for a long period. During subsequent spectral measurements, the reaction solution was not stirred. The reaction was conducted at ambient temperature (20±2 °C). Electron paramagnetic resonance (EPR) spectra were measured on a JEOL JES-RE1X spectrometer under anaerobic conditions with argon gas continuously flowing through the solution.

3. Results and discussion

3.1. Reduction of V^V to V^{IV} by NADPH in the presence of aminopolycarboxylates

In our previous report, we described the reduction behavior of a V^V/EDTA/NADPH system at pH 7.0; in this system, EDTA significantly enhanced the reduction of V^V [24]. In the present work, we examined whether several biologically relevant ligands such as amino acids and small peptides, and the related aminopolycarboxylates other than EDTA such as iminodiacetate (IDA), nitrilotriacetate (NTA), *N*-hydroxyethyliminodiacetate (HEIDA) and *N*-pyridylmethyliminodiacetate (PMIDA)