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Interactions of vanadium(V)–citrate complexes with the sarcoplasmic reticulum calcium pump

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Abstract

Among the biotargets interacting with vanadium is the calcium pump from the sarcoplasmic reticulum (SR). To this end, initial research efforts were launched with two vanadium(V)–citrate complexes, namely $(NH_4)_6[V_2O_4(C_6H_4O_7)_2]\cdot 6H_2O$ and $(NH_4)_6[V_2O_2(O_2)_2(C_6H_4O_7)_2]\cdot 4H_2O$, potentially capable of interacting with the SR calcium pump by combining kinetic studies with ^{51}V NMR spectroscopy. Upon dissolution in the reaction medium (concentration range: 4–0.5 mM), both vanadium(V):citrate (VC) and peroxovanadium(V):citrate (PVC) complexes are partially converted into vanadate oligomers. A 1 mM solution of the PVC complex, containing 184 μM of the PVC complex, 94 μM oxoperoxovanadium(V) (PV) species, 222 μM monomeric (V1), 43 μM dimeric (V2) and 53 μM tetrameric (V4) species, inhibits Ca^{2+} accumulation by 75 %, whereas a solution of the VC complex of the same vanadium concentration, containing 98 μM of the VC complex, 263 μM monomeric (V1), 64 μM dimeric (V2) and 92 μM tetrameric (V4) species inhibits the calcium pump activity by 33 %. In contrast, a 1 mM metavanadate solution, containing 460 μM monomeric (V1), 90.2 μM dimeric (V2) and 80 μM tetrameric (V4) species, has no effect on Ca^{2+} accumulation. The NMR signals from the VC complex (−548.0 ppm), PVC complex (−551.5 ppm) and PV (−611.1 ppm) are broadened upon SR vesicle addition (2.5 mg/ml total protein). The relative order for the half width line broadening of the NMR signals, which reflect the interaction with the protein, was found to be V4 > PVC > VC > PV > V2 = V1 = 1, with no effect observed for the V1 and V2 signals. Putting it all together the effects of two vanadium(V)–citrate complexes on the modulation of calcium accumulation and ATP hydrolysis by the SR calcium pump reflected the observed variable reactivity into the nature of key species forming upon dissolution of the title complexes in the reaction media.

Keywords: Vanadium–citrate complex; Calcium pump

1. Introduction

Almost 30 years after the discovery that vanadium was a muscle inhibiting factor and a Na^+/K^+-ATPase inhibitor present in commercially available ATP [1–3], it still remains to be clarified whether or not vanadium has a physiological role in the muscle. It is believed that the complex chemistry of vanadium itself, its interactions with small molecules of biological interest, and vanadate similarities with phosphate constitute the rudiments of research emerging from the increased interest in vanadium in biological systems. Among the effects attributed to vanadium salts and complexes, the insulin mimetic action stands prominent [4,5]. However, vanadium toxicity and poor absorption in the gastrointestinal tract is still a major obstacle in the development of therapeutic applications [6]. The challenge of

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overcoming these obstacles has spurred considerable research activity, targeting the interaction of vanadium with biomolecules and organic ligands, in order to enhance absorption and efficiency while concurrently reducing toxicity. To that end, a number of ligands, such as citric acid, have been employed as potential ligand binders of vanadium in biological systems [7–9].

It is known that vanadium ions and vanadium complexes inhibit or stimulate the activity of various enzymes. In the case of the membrane bound E1–E2 Ca^{2+}-ATPase (calcium pump) from sarcoplasmic reticulum (SR), the inhibition is promoted by orthovanadate binding to the E2 conformation of the protein [10]. Moreover, the use of vanadate is related to the catalytic site of Ca^{2+} ATPase, which contains an aspartyl that is phosphorylated during the catalytic cycle thereby forming an acyl phosphate anhydride [11]. In addition to monomeric vanadate species, it has been reported that other vanadate oligomers interact with the SR calcium pump [12]. It has been demonstrated that some of the interactions, e.g. decameric species, disrupt the energy coupling and enzyme turnover. Other interactions of vanadium, e.g. monomeric species, may have no implicit effect or even improve the coupling to the Ca^{2+} pump [13]. The SR calcium pump was reported to have a decreased activity in some diabetic conditions, which could result in impaired cardiac relaxation [14]. Several studies have been carried out using vanadium(V) for characterization of SR calcium pump [13,15]. Indeed, SR calcium pump has proven to be an excellent model to study Ca^{2+} homeostasis affected by acute and cronic exposure of nitrosative stress [16].

In the present study, the effects of two vanadium(V)–citrate complexes namely $(NH_4)_6[V_2O_4(C_6H_4O_7)_2]\cdot 6H_2O$ and $(NH_4)_6[V_2O_2(O_2)_2(C_6H_4O_7)_2]\cdot 4H_2O$, on the modulation of calcium accumulation and ATP hydrolysis by the SR calcium pump were evaluated. The results demonstrated that it is of primary importance to precisely characterize the aqueous behaviour of the vanadium(V) species and their interactions with the investigated biosystem prior to attempting to understand the promoted effects. Both vanadium(V)–citrate complexes interact and affect the activity of the SR calcium pump, with the peroxovanadium(V):citrate complex solutions being the most potent inhibitor.

2. Materials and methods

2.1. Reagents

Chemicals used to prepare buffers were of reagent grade. Ammonium metavanadate and citric acid were purchased from Riedel-de Haën. ATP (vanadium free) was supplied by Sigma. All other reagents were of biochemical analysis grade from BDH, Merck or Sigma.

The synthesis of the vanadium(V) complexes $(NH_4)_6[V_2O_4(C_6H_4O_7)_2]\cdot 6H_2O$ and $(NH_4)_6[V_2O_2(O_2)_2(C_6H_4O_7)_2]\cdot 4H_2O$ was achieved according to published literature procedures [17,18]. In these articles, the characterization of the complexes was carried out with analytical, spectroscopic techniques (UV–Visible, ^{13}C NMR (in the case of the ternary vanadium(V)–peroxo-citrate complex), FT-IR) and X-ray crystallographic techniques. Therefore, the structures of the two complexes are known along with their chemical reactivity. The ^{51}V NMR, however, is not available for the two species and thus cannot be included in this manuscript.

Vanadium–citrate complex stock solutions (9 mM) used in the studies were prepared from vanadium(V)–citrate $(NH_4)_6[V_2O_4(C_6H_4O_7)_2]\cdot 6H_2O$ and from vanadium(V)–peroxo-citrate $(NH_4)_6[V_2O_2(O_2)_2(C_6H_4O_7)_2]\cdot 4H_2O$ salts in a medium containing 25 mM Hepes (2-(4-(2-hydroxyethyl)-1-piperazinyl)ethanesulphonic acid), pH 7.0, and 0.1 M KCl.

2.2. Preparation of sarcoplasmic reticulum vesicles

Sarcoplasmic reticulum vesicles (SRV) were prepared from white skeletal rabbit muscles as described elsewhere [13]. Isolated SRV suspended in KCl 0.1 M, tris(hydroxymethyl)aminometane (Tris) 10 mM (pH 7.0) were diluted 1:1 with sucrose 2 M and frozen in liquid nitrogen prior to storage at −80 °C. For SRV preparations used in NMR studies, no sucrose was added, once sucrose forms complexes with vanadium and in the ^{51}V NMR appears two signals at −540 and −550 ppm as described previously [13]. Protein concentration was determined by the biuret method, using bovine serum albumin as a standard [19]. The percentage of each protein present in the SRV preparations was determined trough densitometry analysis of sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis (7.5% acrylamide). The SR Ca^{2+}-ATPase analysed by SDS–polyacrylamide gel electrophoresis was at least 70% of the total protein in SRV.

2.3. NMR measurements

^{51}V Nuclear magnetic resonance spectroscopy measurements on vanadium(V)–citrate complex solutions were taken on a Bruker AM-400 MHz, by using a 90° pulse Fourier transform technique. Chemical shift values are given with reference to $VOCl_3$ (0 ppm) as a standard. The relative areas of several free and bound vanadate resonances were integrated and the line widths were obtained by subtracting the value (20 Hz) used in line broadening. The concentrations of vanadate oligomers V_x were calculated from the fractions of the total integrated areas observed in the recorded spectra as described in the equation below. The symbol A corresponds to the area measured for the x vanadium(V) species (V_x) with n being the aggregation number (number of vanadium atoms), $A(V_t)$, the sum of the measured area and $[V_t]$ the total vanadium(V) concentration

$$[V_x] = A(V_x)\cdot\frac{1}{A(V_t)}\cdot\frac{[V_t]}{n(V_x)}.$$

The spectra of vanadium(V)–citrate complex solutions (VC and PVC complexes) were obtained at several concentrations in the reaction medium, containing KCl 0.1 M, $MgCl_2$ 5 mM, Hepes 25 mM (pH 7.0), $CaCl_2$ 50 μM and ATP 500 μM in Hepes 25 mM (pH 7.0). Spectra of 2 mM vanadium(V)–citrate complex solutions were also obtained in the same reaction medium, in the absence and presence of 2.5 mg/ml of SR preparations. The spectra were acquired at room temperature, spectral with 40,355 Hz, accumulation time 0.05 s, number of transients between 12,000 and 25,000 and relaxation delay 0.01 s. For quantitative measurements all of the spectral parameters were kept constant [12].

2.4. ATP hydrolysis by calcium pump

ATP hydrolysis was measured by colorimetric determination of inorganic phosphate [20]. Kinetic experiments were performed at 25 °C in a reaction medium containing KCl 0.1 M, Hepes 25 mM (pH 7.0), $MgCl_2$ 5 mM, $CaCl_2$ 50 μM, 0.285 g/ml of SRV preparations, in the absence or presence of 1 mM vanadate, vanadium(V) complexes or citrate, according to the required experimental conditions. Vanadate, vanadium–citrate complexes or citrate solutions were added to the medium prior to protein addition. The reaction was initiated by adding Mg-ATP 500 μM and was subsequently monitored for up to 20 min.

2.5. Ca^{2+} uptake experiments

Ca^{2+} uptake experiments were performed as described elsewhere [12]. The experiments were carried out at 25 °C in a 4 ml assay medium, containing KCl 0.1 M, $MgCl_2$ 5 mM, Hepes 25 mM (pH 7.0), $CaCl_2$ 20 μM, and 0.1 mg/ml SR preparations. The reaction was initiated by adding ATP 500 μM in Hepes 25 mM (pH 7.0). At the end of each experiment, an internal calibration was performed by addition of standard $CaCl_2$ solutions. Vanadium(V)–citrate complex or metavanadate solutions at several concentrations were added to the medium prior to ATP addition.

3. Results and discussion

3.1. Spectroscopic characterization of the complexes

The ^{51}V NMR spectra of 9 mM solutions of the vanadium(V):citrate complex (VC) in water (pH 6.6) exhibit resonances at −546 and at −549 ppm (Fig. 1). The relative areas of the resonances reflect a 1:1 ratio, with the half line width of these signals being very similar (180 ± 10 Hz). When the VC complex solution was prepared in the reaction medium, another NMR signal was detected at −578 ppm. Upon dissolution, of the VC complex, in the reaction medium at lower concentrations (from 4 to 0.5 mM), the VC complex is partially converted into vanadate oligomers (Fig. 2). NMR signals ascribed to mono

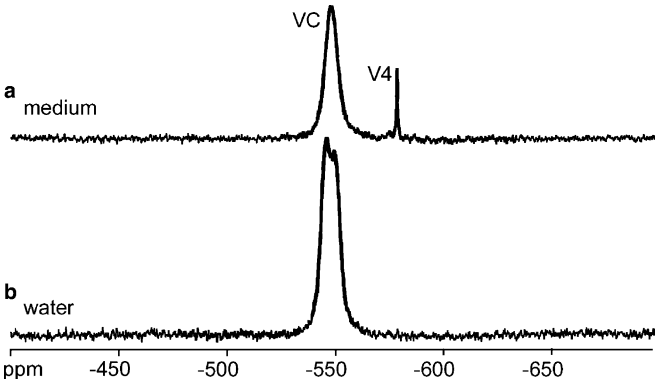

Fig. 1. 105.2 MHz ^{51}V NMR spectra, at 22 °C, of a 9 mM vanadium(V)–citrate complex solution prepared in water (a) or in the reaction medium (b). VC and V4 stands for vanadium(V)–citrate and tetrameric vanadate species, respectively.

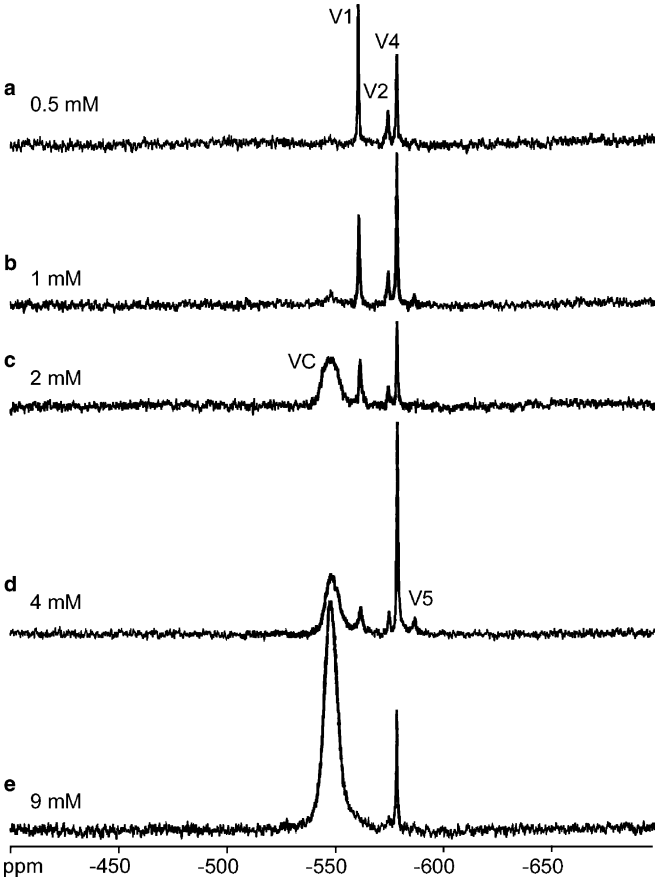

Fig. 2. 105.2 MHz ^{51}V NMR spectra, at 22 °C, of different concentrations, namely 0.5 mM (a); 1 mM (b); 2 mM (c); 4 mM (d) and 9 mM (e) total vanadium, of a vanadium(V)–citrate (VC) complex in a medium containing 25 mM Hepes, pH 7.0, and 0.1 M KCl. V1 and V2 NMR signals correspond, respectively, to monomeric ($H_2VO_4^-$) and dimeric ($HV_2O_7^{3-}$ and $H_2V_2O_7^{2-}$) vanadate species irrespective of the protonation state, whereas V4 and V5 correspond to cyclic tetrameric ($V_4O_{12}^{4-}$) and pentameric ($V_5O_{15}^{5-}$) vanadate species.

(V1) at −561 ppm, dimeric (V2) at −574 ppm and tetrameric (V4) at −578 ppm vanadate species were observed, whereas the VC NMR resonance at −546 ppm

almost disappeared at concentrations as low as 1 mM in total vanadium (Fig. 2).

Three NMR signals are observed for the peroxovanadium(V):citrate (PVC) complex dissolved in water (pH 4.4) or in the reaction medium (9 mM total vanadium). The signals appear at −542 ppm (PVC1), −551 ppm (PVC2) and −609 ppm (PV), with the half line widths of these signals being about 200, 150 and 100 Hz, respectively (Fig. 3). The relative areas show a first signal twice as large as the second, probably reflecting conformers of the same complex. The NMR signal observed at −609 ppm (PV, Fig. 3) is due to an oxoperoxovanadium(V) species, indicating that the PVC complex dissociates as well. Furthermore, upon dissolution of the PVC complex in the reaction medium, at lower concentrations (from 4 to 0.5 mM), it is partially converted into vanadate oligomers (Fig. 4), a behaviour that was also observed above for VC complex. The two complexes as discrete chemical species possess their own chemical properties reflected in unique pattern of degradation. To this end, the PVC complex appears to be more stable toward ultimate vanadate oligomer degradation than the VC complex. That can be easily attested by ^{51}V NMR, showing that the vanadate oligomers signals are solely and clearly observed for concentrations $[V^{V}_{total}] \leqslant 1$ mM (Fig. 4). In fact, the species composition of the VC and PVC complex solutions varies with the total vanadium concentration (Fig. 5). At vanadium concentrations up to 2 mM, different species occur simultaneously for both the VC and PVC complexes, whereas at higher vanadium(V) concentrations the VC and PVC complexes are favoured. Even for solutions 9 mM in total vanadium, only about 4.2 and 3.6 mM of VC and PVC complexes are present, respectively (Fig. 5). Therefore, it was difficult to obtain a solution containing exclusively a single vanadium(V) species, and even when that was achieved, other species appeared when the solution was diluted in the reaction medium. Consequently,

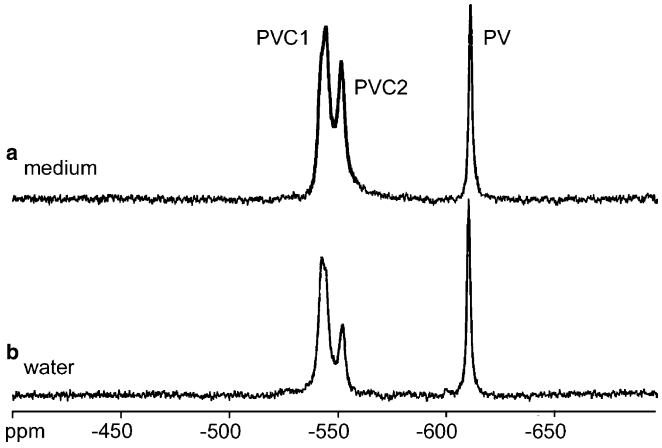

Fig. 3. 105.2 MHz ^{51}V NMR spectra, at 22 °C, of a 9 mM peroxovanadium(V)–citrate complex solution prepared in water (a) or in the reaction medium (b). PVC1 and PVC2 stands for peroxovanadium(V)–citrate species, while PV stands for oxoperoxovanadium(V) species.

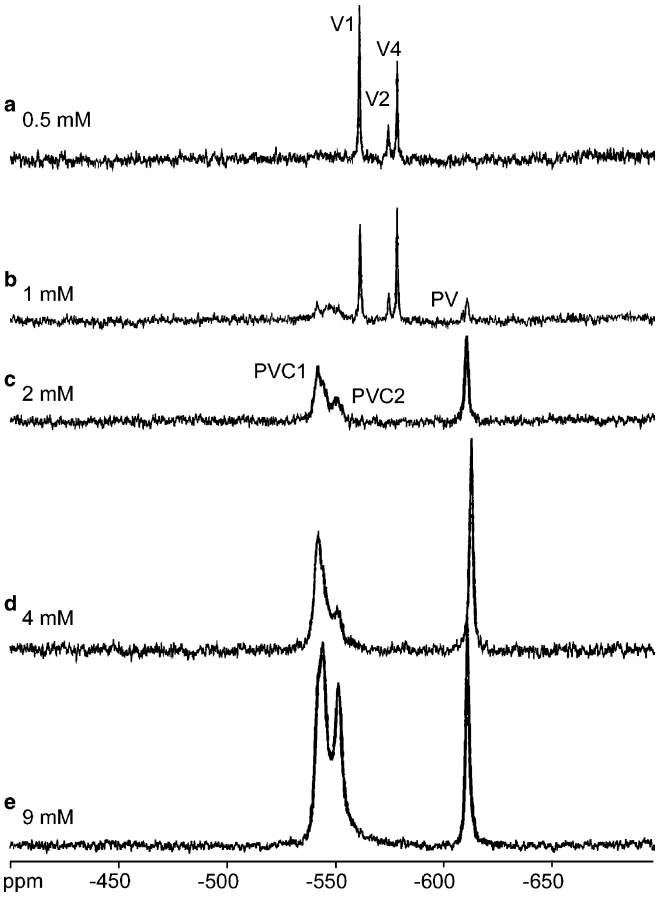

Fig. 4. 105.2 MHz ^{51}V NMR spectra, at 22 °C, of different concentrations of a peroxovanadium(V)–citrate (PVC) complex in a medium containing 25 mM Hepes, pH 7.0, and 0.1 M KCl: 0.5 mM (a); 1 mM (b); 2 mM (c); 4 mM (d) and 9 mM (e) total vanadium. V1 and V2 NMR signals correspond, respectively, to monomeric ($H_2VO_4^-$) and dimeric ($HV_2O_7^{3-}$ and $H_2V_2O_7^{2-}$) vanadate species irrespective of the protonation state, whereas V4 correspond to cyclic tetrameric ($V_4O_{12}^{4-}$) vanadate species. PVC1 and PVC2 stands for peroxovanadium(V)–citrate species, while PV stands for oxoperoxovanadium(V) species.

in order to investigate the interactions and effects of the complexes with the SR calcium pump, higher concentrations were used.

3.2. ^{51}V NMR study on the interaction of the complexes with SR calcium pump

Upon addition of sarcoplasmic reticulum vesicles (SRV, 2.5 mg/ml of total protein) to the VC complex solution (2 mM total vanadium) prepared in the reaction medium, the ^{51}V NMR signal at −548 ppm broadens (from 506 to 657 Hz) and its intensity decreases (Fig. 6(a)). This solution, however, contains vanadate oligomers, with the tetrameric vanadate signal resonance sustaining an enormous decrease in intensity upon SRV addition. In contrast, as it was observed by ^{51}V NMR, the PVC complex solution (2 mM total vanadium) prepared in the reaction medium does not contain vanadate oligomers and thus the interactions with the SR calcium pump can only be related to the

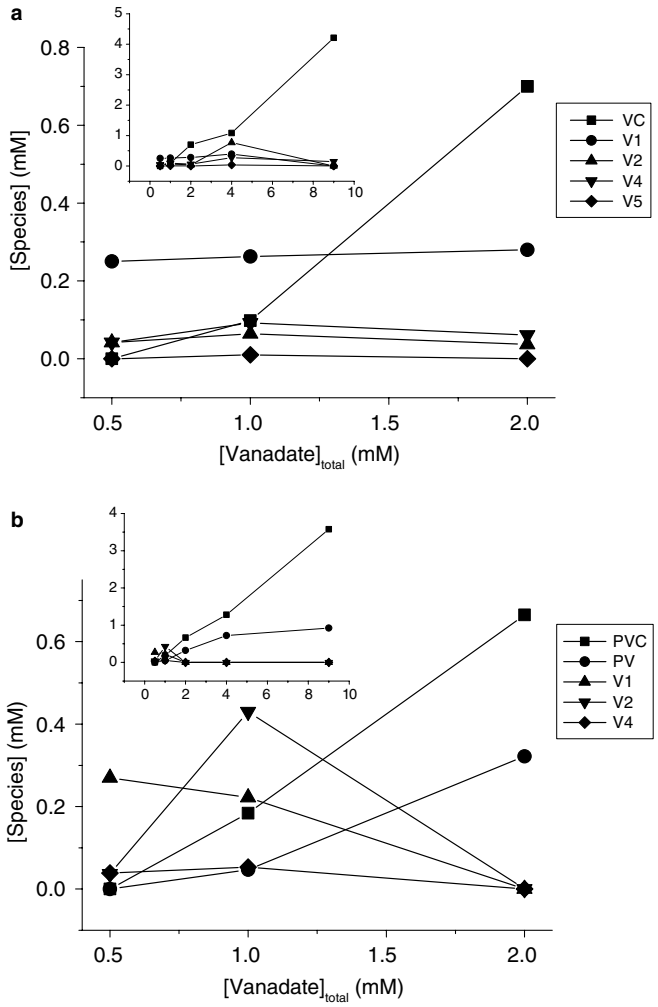

Fig. 5. Vanadium(V) species composition of vanadium(V)–citrate (a) and peroxovanadium(V)–citrate (b) solutions used in the assays. A series of ^{51}V NMR spectra of vanadium(V)–citrate and peroxovanadium(V)–citrate solutions were recorded at concentrations up to 9 mM, in a medium containing 25 mM Hepes, pH 7.0, 0.1 M KCl, at 22 °C. The concentrations of each vanadium(V) species were calculated from the fractions of the total integrated areas observed in the spectra.

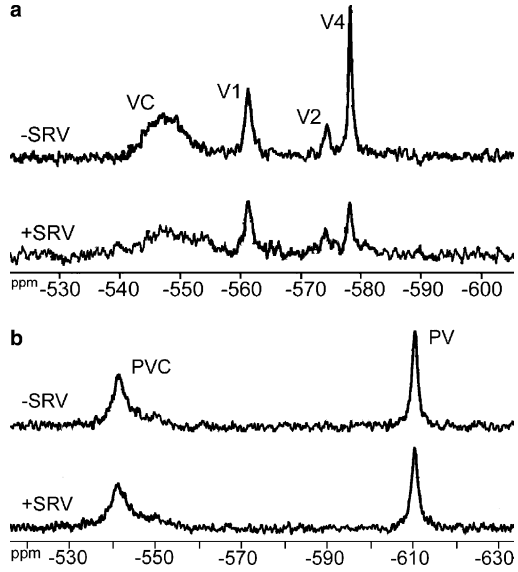

Fig. 6. 105.2 MHz ^{51}V NMR spectra of 2 mM vanadium(V)–citrate complex (a) or peroxovanadium(V)–citrate (b) in a medium containing 25 mM Hepes, pH 7.0, 0.1 M KCl, 5 mM $MgCl_2$, in the absence (−SRV) or in the presence (+SRV) of sarcoplasmic reticulum vesicles (2.5 mg/ml total protein).

peroxovanadium(V) complex affinity for the protein. Both signals detected at −542 and −609 ppm, ascribed to the PVC complex and to PV, broadened from 175 to 281 Hz and from 93 to 112 Hz, respectively, and decreased in intensity in the presence of the calcium pump (Fig. 6(b)). Putting all together, the relative order of the half width line broadening for the NMR signals, which reflect the interaction of the complexes with protein, was V4 > PVC > VC > PV > V2 = V1 = 1, with no effects observed for the V1 and V2 signals. Moreover, upon phosphorylation by Pi or ATP following the methodology described elsewhere [12], the different forms E2 and E1 of the enzyme did not affect the NMR signals of the complexes observed following SRV addition (not shown).

Early ^{51}V NMR studies on the binding of various ionic forms of vanadate to SRV have clearly shown that the NMR resonances for the free oligomeric species of vanadate, present at pH 7.4, decrease upon addition of the protein [21]. More recently, it was demonstrated that the changes in the NMR spectra are caused by alterations in the Ca^{2+}-ATPase induced by ATP [12]. These authors described that the signals for tetrameric vanadate increase in intensity and become narrower, whereas the signal for monomeric vanadate become broader, suggesting that ATP increases the affinity of monomeric species for the enzyme but decreases the affinity for the tetrameric species. In studies with myosin, similar findings were reported for tetrameric and monomeric species following ATP addition [22].

Different SR ATPase conformational forms arisen from the presence of specific ligands can be ascribed to vanadium(V) affinities for the protein. Our data indicate that the affinity of both vanadium(V) complexes is not apparently affected when the different forms E2 and E1 of the enzyme are induced. Moreover, the affinity for both forms of the ATPase does not change upon phosphorylation by Pi or ATP. It has been suggested that ''monovanadate'' species may bind to the ATPase in the vicinity of the aspartyl phosphate acceptor, resulting in the blockage of the active site by avoiding phosphoenzyme intermediate formation, whereas the decavanadate binding site to the enzyme has been reported to be very close to the ATP binding site [13,15]. In the present work, it is suggested that the vanadium(V) complexes may modulate the calcium pump through a different interaction than the one observed for vanadate oligomers. This indicates that the complexity of vanadium effects is not only due to the presence of different oligomers in solution, which were shown to promote different in vivo effects [23], but also to the influence of potential ligand binders of vanadium in biological systems.

3.3. Inhibition of ATPase hydrolysis and Ca^{2+} accumulation by VC and PVC complexes

The inhibition promoted on the SR calcium pump ATP hydrolysis process was similar for both VC and PVC complex solutions, with a 50% inhibition at 0.5 mM (Fig. 7). However, with respect to Ca^{2+} accumulation by the SR calcium pump, the PVC complex solution exerted a stronger inhibition on this process, with an IC_{50} value of 1.1 mM (Fig. 7(b)), compared to a 2.0 mM IC_{50}value for the VC complex solution promoting the same effect (Fig. 7(a)). Under the same experimental conditions, it was observed that metavanadate does not affect Ca^{2+} accumulation by the calcium pump. However, as it can be observed in Fig. 7, 1 mM solution of the PVC complex inhibits the Ca^{2+} accumulation by 75 %, whereas a solution of the VC complex of the same vanadium concentration inhibits the calcium pump activity by 33 %, meaning that the IC_{50} value obtained for the latter complex is eventually lower than the obtained through the function that fits the graph. Even so, if we considered the obtained IC_{50} values, the Ca^{2+}/ATP stoichiometry was affected differently by both complex solutions: the VC complex induced an increase in pump efficiency (thereby increasing Ca^{2+}/ATP ratio), whereas the PVC complex did not affect Ca^{2+}/ATP stoichiometry. For the former complex the effects may not be exclusively attributed to it, without define the influence of the PV species on the inhibition of ATPase hydrolysis and Ca^{2+} accumulation.

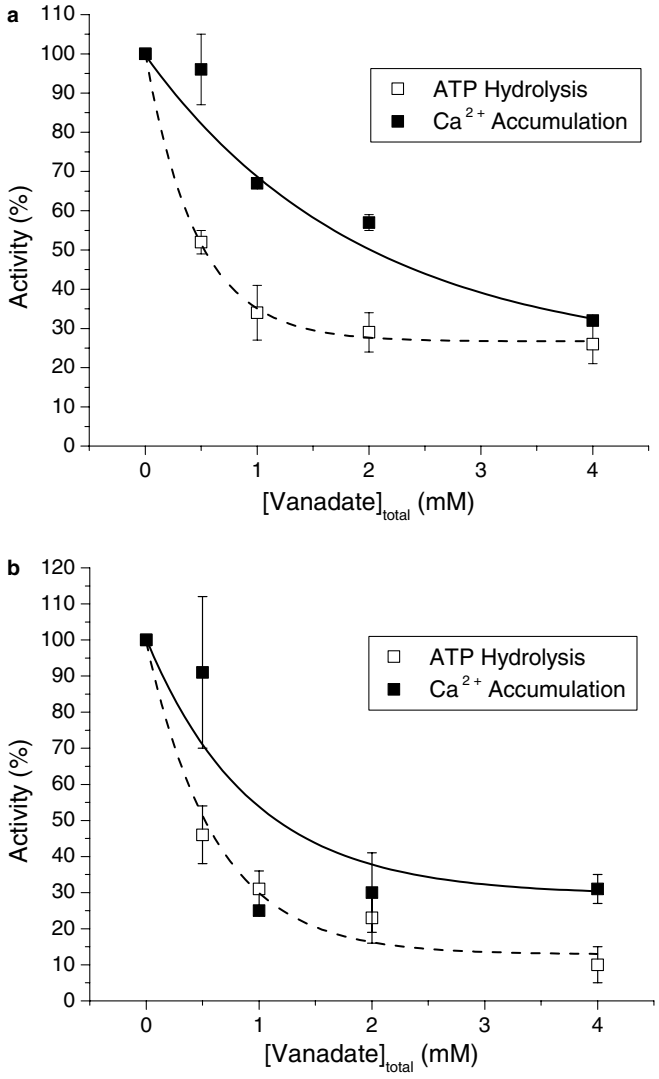

Fig. 7. Effects of the vanadium(V)–citrate complex (a) and peroxo vanadium(V)–citrate complex (b) solutions, on phosphate production during ATP splitting (dashed line, open squares) and Ca^{2+} accumulation (solid line, solid squares) by the SR calcium pump. The reactions were monitored at 25 °C, in an assay medium containing 0.1 M KCl, 5 mM $MgCl_2$, 50 μM $CaCl_2$, 285 μg/ml protein or 100 μg/ml (according to Section 2) and 25 mM Hepes, pH 7.0. The reactions were started upon addition of 500 μM MgATP and were monitored for 10 min. The provided rates reflect the means of at least six independent experiments.

In order to evaluate the effect of citrate alone, a structural component of these vanadium(V)–citrate complex solutions, on the inhibition registered by VC and PVC complexes, the ATP hydrolysis and Ca^{2+} accumulation processes were measured in the presence of several citrate concentrations. It was observed that citrate induced an increase in sarcoplasmic reticulum Ca^{2+} accumulation, with no discernible effect on the hydrolytic activity (not shown). In fact, Ca^{2+} accumulation increased by 23% and 70 % in the presence of 2 and 4 mM citrate, respectively. This effect may explain the increase in Ca^{2+}/ATP ratio observed for the VC complex (Fig. 7(a)). In contrast, citrate did not affect the inhibition by the PVC complex, even though it was verified that the complex dissociates, giving rise to oligomers and also to oxoperoxovanadium(V) and therefore inducing, to a lower extent than the VC complex, the appearance of citrate.

4. Concluding remarks

^{51}V NMR spectroscopy was used to determine the composition of the VC and PVC complex solutions in the reaction medium and then to calculate the concentrations of the various species present in solution relative to the total vanadium concentration. In the concentration range used, it was observed that the vanadium(V) complex solutions contain, besides vanadium(V) complexes and oxoperoxovanadium(V) species, different amounts of mono (V1); di (V2); tetra (V4) and pentameric (V5) species (Fig. 6). NMR spectra of a 1 mM VC complex solutions in the reaction medium indicated that the solutions contain 98 μM of complex, 263 μM monomeric (V1), 64 μM dimeric (V2) and 92 μM tetrameric (V4) species, whereas a 1 mM PVC complex solutions contain 184 μM of complex, 94 μM oxoperoxovanadium(V) species, 222 μM monomeric (V1), 43 μM dimeric (V2) and 53 μM tetrameric (V4) species. It should be noted that a 1 mM of metavanadate solution contains, under the same experimental conditions, about 460 μM monomeric, 90 μM dimeric and 80 μM tetrameric vanadate species. The unique nature of the title complexes investigated and their emerging patterns of degradation in aqueous media dictate a commensurately unique biological activity exerted in the presence of the biological target (the

SR system). These solutions, with equivalent vanadium(V) concentrations, inhibit Ca^{2+} accumulation to variable degrees, namely 75%, 33% and 2% for PVC, VC and metavanadate solutions, respectively. These results point out an interesting behaviour of the vanadate complexes. Specifically, based on total vanadium concentration of vanadium present in the ternary PVC and binary VC complex solutions of the same 1 mM concentration, the PVC complex is more potent (2.3 times higher) than the VC complex. Thus, the peroxovanadate complex appears to be a more potent inhibitor of the calcium pump.

It is considered to be of primary importance to precisely characterize the vanadium species that could interact with an enzyme system prior to attempting to investigate any effects promoted by vanadium(V)-containing solutions. To that end, it was not possible to clearly define the effects promoted by each individual vanadium(V)–citrate complex. Concomitantly, due to the complexity of the system, which contains several vanadium(V) species interacting with multiple sites on the Ca^{2+}-ATPase, that can adopt several conformations, it was not possible to completely define the effects of the vanadium(V):citrate species on sarcoplasmic reticulum Ca^{2+}-ATPase activity. It was clear, however, that enzyme activity inhibition increases in the presence of the title complexes, suggesting an interaction of vanadium with the sarcoplasmic reticulum Ca^{2+}-ATPase different from that exhibited by other vanadate oligomers.

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