BioMetals 1997, **10**, 239–246

Reduction of vanadate to vanadyl by a strain of *Saccharomyces cerevisiae*

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Received 11 October 1996; accepted for publication 4 March 1997

Three strains of *Saccharomyces cerevisiae*, SC-1, DBVPG 6173 and DBVPG 6037, were studied for vanadate resistance in complex Sabouraud medium since they did not thrive in different minimal media (yeast nitrogen base with and without amino acids). The strain SC-1 was resistant up to 16 mM of vanadate, whereas the strains DBVPG 6173 and DBVPG 6037 were inhibited by 8 mM and 4 mM vanadate, respectively. The vanadate resistance in strain SC-1 was constitutive and due to the reduction of this oxyanion to vanadyl, which was detected by EPR spectroscopy and visible spectroscopy. The transformation of vanadate to vanadyl took place during the exponential growth phase; 10 mM of vanadate was reduced to vanadyl outside the cells since the oxyanion was not detected in the cell biomass and only a negligible concentration of vanadyl (25 nmoles mg^{-1} cells dry weight) was found in the biomass. The other two vanadate-sensitive yeast strains only accumulated vanadate and did not reduce the oxyanion to vanadyl.

Keywords: NMR and EPR spectroscopy, speciation, transformation, vanadate, vanadyl, yeast

Introduction

Vanadium is a transition metal of biological importance because of its role in metabolic regulation and in the inhibition and/or stimulation of several cell processes (Simons 1979). Both anionic and cationic forms of vanadium are involved in biological reactions. Under physiological conditions, the most common ones are the protonated vanadate anions ($H_2VO_4^-$ and HVO_4^{2-}) and the vanadyl cation (VO^{2+}) (Rehder 1992). Vanadate is easily reduced to V(IV) by reducing agents such as glutathione, ascorbate and NADH, whereas vanadyl undergoes autoxidation to vanadate in the presence of oxygen (Rehder 1992). This facile change between V(V) and V(IV) and its double anion and cation nature allows vanadium to play different roles in interactions with biological molecules. As HVO_4^{2-}, it competes with HPO_4^{2-}; on the other hand, the vanadyl cation competes with other transition metal ions for coordination to biological compounds (Rehder 1992). Vanadium is essential in several organisms; for example, some species of *Ascidians* accumulate V(IV) and V(III) in specialized blood cells. The physiological function of vanadium in these organisms is unclear, though it has been suggested to play a role in tunic tissue biosynthesis (Macara 1980, Michibata *et al*. 1990, Hawkins 1991, Rehder 1991). Vanadium has been determined in the fly-agaric toadstool, associated with a low molecular weight compound, amavadine, (Macara 1980, Armstrong *et al*. 1991, Rehder *et al*. 1991). In some species of seaweeds, such as *Ascophyllum nodosum* and *Laminaria saccharina*, it has been found in vanadate-dependent bromo-peroxidases which catalyze halogenation of organic substrates (De Boer *et al*. 1986a,b, Butler *et al*. 1991, Wever & Tromp 1991). Other important enzymes containing vanadium include special nitrogenases purified from the nitrogen-fixing bacteria *Azotabacter* and *Nostoc muscorum* (Hales *et al*. 1986, Robson *et al*. 1986, Fallik *et al*. 1993, Singh *et al*. 1993). Vanadate can be used by *Pseudomonas* strains as a terminal electron acceptor for anaerobic respiration in vanadium-rich environments (Lyalikova & Yurkova 1992).

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The toxic effects of vanadium are related to the structural similarity between vanadate and phosphate (Rehder 1991). As a phosphate-analog, vanadate inhibits enzymes which bind phosphates, such as glyceraldehyde-3-phosphate dehydrogenase (Gresser & Tracey 1990). Vanadate inhibition has mainly been studied with ATP-dependent enzymes, which are involved in several metabolic activities in the cell. It has been demonstrated that Na^+/K^+ pumps in mammals, and membrane ATPases in *Neurospora crassa* and *Saccharomyces cerevisiae*, are inhibited by vanadate because it interferes with a reaction mechanism involving a vanadate-sensitive phosphoprotein (Cantley *et al*. 1977, 1978a,b, Bowman & Slayman 1979, Cantley & Aisen 1979, Willsky 1979, Willsky *et al*. 1985). It has been suggested that vanadate toxicity in *S. cerevisiae* is due to NADH oxidation of the cell membrane. It has been shown that all vanadate-dependent NADH oxidation activities require oxygen, although aerobic microorganisms were not found to be more sensitive than anaerobic ones (Minasi *et al*. 1990). Thus, this vanadate-oxidizing activity is indirectly involved in the toxicity towards *S. cerevisiae* cells (Minasi *et al*. 1990, Minasi & Willsky 1991). Yeast growth is inhibited by 5 mM vanadate (Willsky *et al*. 1984), whereas only 35 μM vanadate is required to stimulate vanadate-dependent NADH oxidation (Minasi *et al*. 1990).

The aim of this study was to investigate vanadate resistance in three strains of *S. cerevisiae*: the most vanadate-sensitive strain, namely the phenotype of the species; an intermediate vanadate-resistant strain; and the most resistant strain of *S. cerevisiae*. They were studied in relation to the biological reduction of vanadate to vanadyl (less toxic form) and total vanadium uptake during exposure to high concentrations of vanadate.

Materials and methods

Growth of S. cerevisiae *strains*

At the beginning of the experiments, six strains of *S. cerevisiae* were used. Five were obtained from the Industrial Yeast Collection of the Dipartimento di Biologia Vegetale, sezione di Microbiologia Applicata, Università Perugia, Perugia, Italy (DBVPG): *S. cerevisiae* DBVPG 6173; *S. cerevisiae* DBVPG 6174; *S. cerevisiae* DBVPG 6176; *S. cerevisiae* DBVPG 6037; *S. cerevisiae* DBVPG 6220. The sixth strain, *S. cerevisiae* SC-1, was of pharmaceutical origin. The strains were grown in Sabouraud complex medium (Difco) at pH 5.6. In some experiments *S. cerevisiae* DBVPG 6173, *S. cerevisiae* DBVPG 6037 and *S. cerevisiae* SC-1 strains were grown in the minimal salt medium yeast nitrogen base (YNB) with and without amino acids (Difco), using 20 g l^{-1} maltose as the carbon source.

MIC tests in Sabouraud medium

The six strains of *S. cerevisiae* were tested for vanadate resistance. Aliquots (1 ml) of overnight cultures were diluted in 99 ml of Sabouraud medium. 10 ml of this subsample were distributed in 20 ml test tubes with radial caps. Vanadate was added to the test tubes at different concentrations, (0, 4, 6, 8, 10, 12, 14, 16, 17 and 20 mM for SC-1; 0, 0.2, 0.5, 1, 2, 4, 6, 8, 10, 12 mM for the other five strains). The cultures were aerated in a rotary drum for 24 h and incubated at 28°C. Yeast growth was measured in terms of absorbance at 600 nm, using a UV–visible spectrophotometer (Shimadzu UV-160). The absorbances of the vanadyl species in Sabouraud medium were measured at 767 nm on the cell-free supernatant. On the other hand, the absorbance of the vanadyl species at 600 nm was negligible with respect to that of the cells and did not interfere in measurements. Other tests with lower vanadate concentrations (0.1–10 mM) were carried out with only three strains: *S. cerevisiae* SC-1, DBVPG 6037 and DBVPG 6173.

MIC tests in yeast nitrogen base medium

Vanadate resistance of *S. cerevisiae* DBVPG 6173, DBVPG 6037 and SC-1 was tested in minimum salt medium yeast nitrogen base with and without amino acids and with 20 g l^{-1} maltose, by adding increasing vanadate concentrations (0, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 8.0 and 10 mM). The same experimental protocol as in the previous tests was used.

Growth of SC-1 strain in different media

S. cerevisiae SC-1 was grown in two types of yeast nitrogen base media, with and without amino acids, both containing 20 g l^{-1} maltose, and in a third one, the complex Sabouraud medium. In the exponential growth phase, SC-1 strain was inoculated (1:100; v:v) into 50 ml of the three media and aerated in 250 ml conical flasks at 28°C. Aliquots of 3 ml were sampled to measure absorbance at 600 nm at regular time intervals.

Vanadate resistance induction tests

Tests for inducible growth in the presence of sodium metavanadate were performed with vanadate-resistant *S. cerevisiae* SC-1. This strain was grown in duplicate in separate 20 ml tubes with radial caps for five sequential transfers into Sabouraud medium, both in the presence and absence of 10 mM vanadate. Then, 250 ml of Sabouraud medium containing 10 mM vanadate were added to four 1000 ml conical flasks. Two conical flasks were inoculated with vanadate-exposed cells and the other two with cells not exposed to vanadate for five transfers. These cultures were incubated at 28°C in a rotary shaking bath (New Brunswick) at 200 rpm. Aliquots of 3 ml were drawn from each of the four conical flasks to measure growth (in terms of absorbance at 600 nm).

Total vanadium uptake

Vanadium uptake was determined in the V(V)-resistant strain, *S. cerevisiae* SC-1, and in the V(V)-sensitive strains, *S. cerevisiae* DBVPG 6173 and DBVPG 6037. Cells of the three strains at the exponential phase in Sabouraud medium were harvested by centrifuging at $1000 \times g$ for 20 min and washed twice with 10 mM PIPES (piperazine-*N*,*N*′-bis(2-ethanesulfonic-acid) buffered at pH 7.4. The pellets were transferred in 250 ml conical flasks containing 140 ml PIPES and 1% maltose as the sole carbon source. An aliquot of 20 ml of suspension was distributed into 100 ml conical flasks and different concentrations of V(V), ranging from 0.01 mM to 2.0 mM, were spiked into the medium. The inoculated conical flasks were incubated at 28°C in an orbital shaker at 200 rpm. After 18 h, 10 ml of sample were removed and centrifuged at $1800 \times g$ for 20 min. The pellet was washed twice with PIPES buffer and mineralized with 2 ml concentrated HNO_3 at 60°C for 1 h. The sample was cooled and made up to 10 ml with double distilled water (DDW). The samples were analyzed for total vanadium by inductively coupled plasma atomic emission spectrophotometry (ICP–AES) (Perkin Elmer 400). Standard additions from a stock solution of 1 g l^{-1} sodium vanadate were made for calibration curves and standard additions to the cell pellet. The coefficient of variation of the five replicates was 3.4%.

^{51}V NMR spectroscopy

NMR spectra were obtained from samples on a Varian XL 200 spectrometer equipped with a multinuclear broad band probe. The spectrometer was operated at 4.7 tesla (frequency for ^{51}V, 52.7 MHz) and 298 K in 10 mm diameter tubes. Free induction decays were obtained using a 10 μs with a 50 μs deadtime. Sweep widths of 50 KHz were employed. 3000–2000 scans were recorded at a 10 ms repetition rate for each spectrum. The technique was used to qualitatively detect V(V) in the samples, without any attempt to assign the observed resonances. The samples used for ^{51}V NMR spectroscopy were: (1) the uninoculated Sabouraud culture medium containing 10 mM, 4 mM and 2 mM sodium metavanadate (samples a, b, c); (2) washed cells of *S. cerevisiae* SC-1, grown in the presence of 10 mM sodium metavanadate in Sabouraud medium with incubation at 28°C (301 K) for 24 h, harvested by centrifuging at $1800 \times g$ for 20 min (sample d); (3) the supernatant obtained by filtering the cell cultures with 0.2 μm nitro-cellulose filters (sample e). All samples were at 253 K for spectroscopic analysis.

EPR spectroscopy

EPR spectra were obtained on a Bruker ER 200 spectrophotometer operating in the X band at 9.86 GHz with a modulation amplitude of 10 G, time constant of 10 ms and spectrophotometer gain of 5×10^4. All spectra were obtained at room temperature. The arrows in Figures 3 and 6 indicate the *g* value of diphenylpicrylhydrazyl, namely 2.0365. EPR spectroscopy was used as a qualitative tool to detect the presence of the paramagnetic vanadyl species in the samples. The samples used for EPR measurements were: (a) aliquots of the supernatant of cell cultures of *S. cerevisiae* SC-1 grown in Sabouraud medium in the presence of 10 mM sodium metavanadate (see above) taken at 2 h intervals during the growth experiment; (b) aliquots of the supernatant of cell cultures of *S. cerevisiae* DBVPG 6173 grown in Sabouraud medium in the presence of 2 mM sodium metavanadate (see above) sampled at 2 h intervals during the growth experiment; (c) cells of *S. cerevisiae* SC-1 and DBVPG 6173 washed twice with PIPES buffer after 24 h of incubation (as described above).

Results

The six strains of *S. cerevisiae* showed a variable vanadate resistance; among these, *S. cerevisiae* SC-1 was the most resistant strain with a minimum inhibitory concentration of 16 mM. It was four times more resistant than the 'typical' strain of the species, DBVPG 6173. The other strains had intermediate vanadate resistance, between 4 mM and 8 mM (Table 1). Three strains: the most resistant (SC-1), the less resistant (DBVPG 6037) and the most sensitive (DBVPG 6173) were also tested for Mo(VI), As(V) and As(III) resistance, because all these oxyanions compete with phosphate transport systems (Silver & Walderhaug 1992).

SC-1 was also resistant to levels of Mo(VI) four times higher than the other two strains. On the other hand, all three yeast strains showed the same minimum inhibitory concentrations of As(V) and As(III) (Table 1). These tests were performed in complex Sabouraud medium, in which resistance may increase due to the reduced availability of metal ions which are chelated by organic ligands. MIC tests in complex medium (Figure 1A) showed that *S. cere-*

Table 1. Minimum inhibitory concentrations (MICs) of several strains of *Saccharomyces cerevisiae*, exposed to various concentrations (mM) of sodium metavanadate ($NaVO_3$), sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$), sodium arsenate dibasic heptahydrate ($Na_3AsO_4 \cdot 7H_2O$) and sodium meta-arsenite ($NaAsO_2$).

Strains	V(V)	Mo(VI)	As(V)	As(III)
SC-1	16	4	0.3	0.6
DBVPG 6173	4	1	0.3	0.6
DBVPG 6174	6	n.d.	n.d.	n.d.
DBVPG 6176	6	n.d.	n.d.	n.d.
DBVPG 6037	8	1	0.3	0.3
DBVPG 6220	8	n.d.	n.d.	n.d.

n.d. = not determined

visiae SC-1 resisted vanadate toxicity at concentrations up to 12 mM, whereas strain DBVPG 6037 showed an intermediate vanadate-resistance between the vanadate-sensitive DBVPG 6173 and the vanadate-resistant SC-1 (Figure 1A). This is why the MIC test is commonly performed in the minimal medium, yeast nitrogen base, which has a low metal binding capacity. In this medium strain SC-1 grew with a significantly lower cell density ($A_{600} = 0.45$) with respect to the others ($A_{600} = 1.55$) (Figure 1B). Strain SC-1 showed a constant cell density up to the maximum additions (10 mM) and was still more resistant than the other two strains (Figure 1B). However, all three strains grew poorly in this minimal medium (Figure 1B) with respect to the complex medium (Figure 1A).

To check for deficiency of growth factors in the minimal medium, SC-1 was grown in three different media: Sabouraud medium, YNB with amino acids and YNB without amino acids. The growth of SC-1 in both minimal media leveled off at $A_{600} = 0.45$, whereas in the complex medium it reached $A_{600} = 4.5$ after dilution of the dense yeast culture (Figure 2). Minimal salt media were therefore deficient in some important, still unidentified, growth factor for strain SC-1.

Vanadate resistance in the SC-1 yeast strain was constitutive as demonstrated by a control experiment. Two cultures were prepared with (10 mM) and without vanadate by transferring SC-1 cells in Sabouraud medium for five consecutive times.

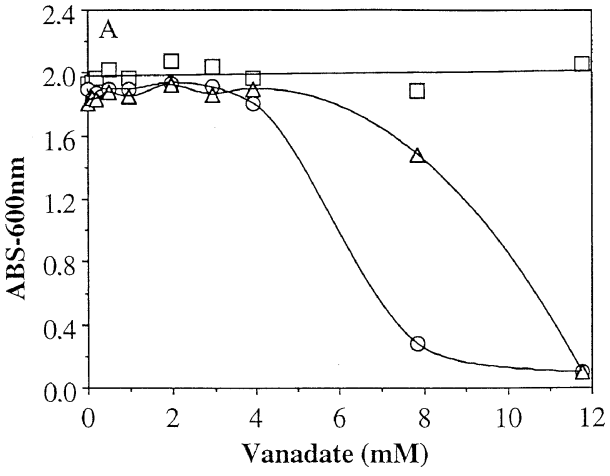

Figure 1A. Minimum inhibitory concentration test (MIC) of three *S. cerevisiae* strains grown in complex Sabouraud medium amended with different concentrations of vanadate (mM) as sodium metavanadate ($NaVO_3$). Vanadate-resistant strain SC-1 (□) and two vanadate-sensitive strains, DBVPG 6037 (△) and DBVPG 6173 (○).

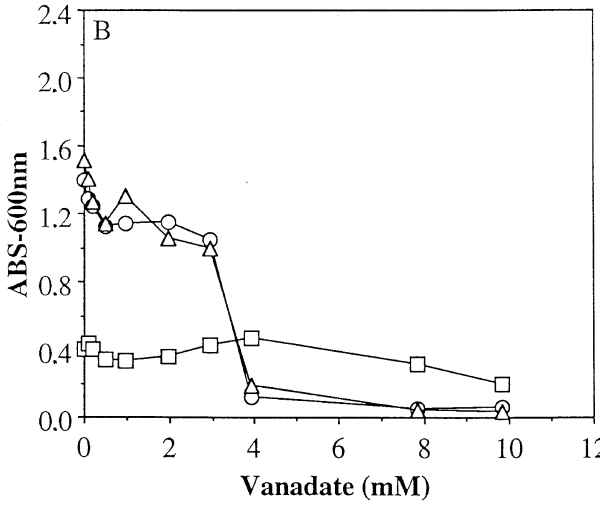

Figure 1B. Minimum inhibitory concentration test (MIC) of *S. cerevisiae* vanadate-resistant SC-1 strain (□) and two vanadate-sensitive strains, DBVPG 6037 (△) and DBVPG 6173 (○), grown in minimal salts medium 'yeast nitrogen base' without amino acids with 2% maltose, and different concentrations of vanadate (mM) as sodium metavanadate ($NaVO_3$).

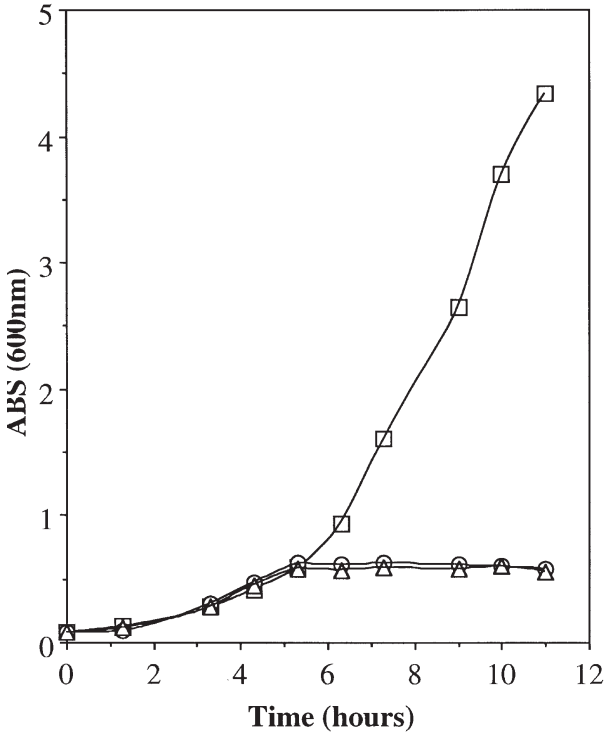

Figure 2. Growth of *S. cerevisiae* vanadate-resistant strain SC-1, in Sabouraud complex medium (□) and in two types of minimal salts medium 'yeast nitrogen base' with (○) and without (△) amino acids.

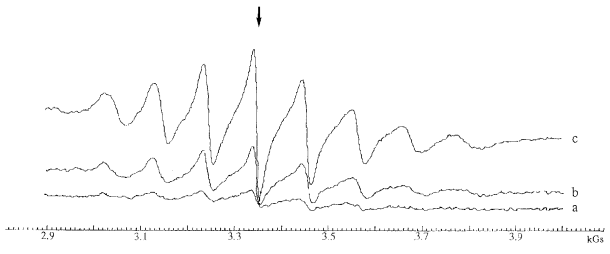

Figure 3. Electron paramagnetic resonance (EPR) spectra of vanadyl in the supernatant of *S. cerevisiae* vanadate-resistant SC-1 culture at different times after inoculation: (a) 6 h; (b) 16 h; and (c) 20 h.

Samples from both cultures were then exposed separately to 10 mM vanadate. Significant differences between the two lag phases of induced and uninduced yeast cultures were not observed.

During the growth of SC-1 with 10 mM vanadate, the complex medium turned dark green in color. Without vanadate, the color of the yeast suspension remained pale yellow, the original color of Sabouraud medium. The solution color did not change by adding vanadate to the uninoculated Sabouraud medium and its EPR spectrum was featureless. The dark green color displayed by the inoculated medium spiked with vanadate was similar to the color of uninoculated medium (control) spiked with 10 mM vanadyl ion.

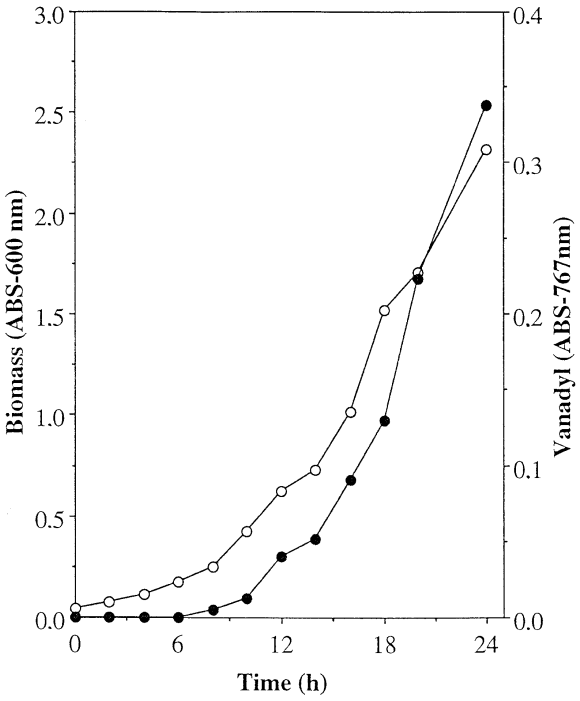

Figure 4. Growth curve of SC-1 strain (○) and the corresponding formation of vanadyl (VO^{2+}) (●) in cell-free supernatant.

The vanadyl content of the supernatant was analyzed by electron paramagnetic resonance spectroscopy (EPR). The dark green color of the SC-1 culture was found to be caused by the reduction of vanadate to vanadyl after different periods of growth (6 h, 16 h and 20 h) (Figure 3). The EPR spectrum was characteristic of the free aquated or weakly bound vanadyl ion, displaying eight absorption maxima due to coupling of the vanadyl unpaired electron with the vanadium nuclear spin moment.

The kinetics of vanadyl production due to vanadate reduction to vanadyl (dark green color) were also analyzed by UV–visible spectroscopy at 767 nm. The reduction of vanadate to vanadyl was only observed with SC-1, and did not occur in the other two yeast strain cultures. The results of the vanadyl production experiment demonstrated that vanadate reduction occurred during exponential growth of SC-1 in Sabouraud medium (Figure 4).

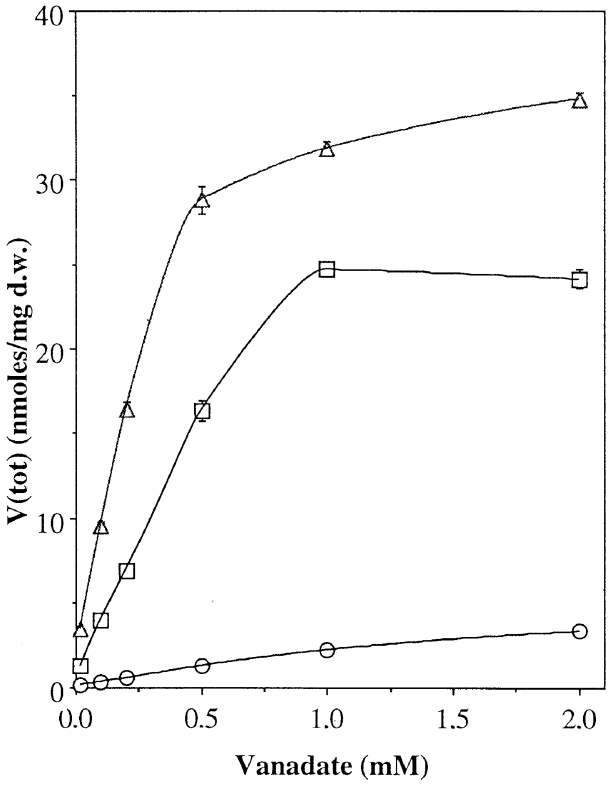

Figure 5. Uptake of total vanadium (nmoles mg^{-1} dry weight) by *S. cerevisiae* vanadate-resistant strain SC-1 (□) and two vanadate-sensitive strains, DBVPG 6037 (△) and DBVPG 6173 (○). Vanadium was determined by inductively coupled plasma atomic emission spectrophotometry (ICP–AES). Bars represent standard deviations of duplicate measurements of vanadium concentrations.

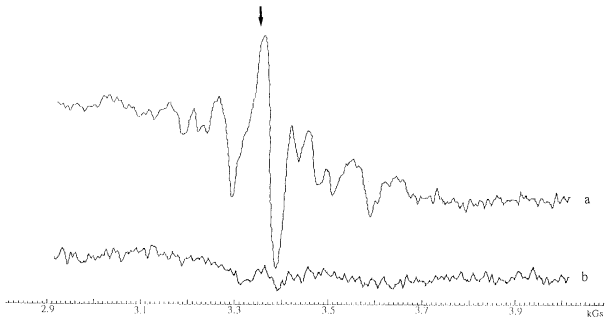

Figure 6. Electron paramagnetic resonance (EPR) spectra of vanadyl found in: (a) cells of vanadate-resistant strain SC-1; and (b) cells of vanadate-sensitive strain DBVPG 6173. Cells were grown for 24 h and washed twice in PIPES buffer solution. The amplification gain of these spectra is 10^3 times that of those reported in Figure 3, as can be observed from the different noise levels.

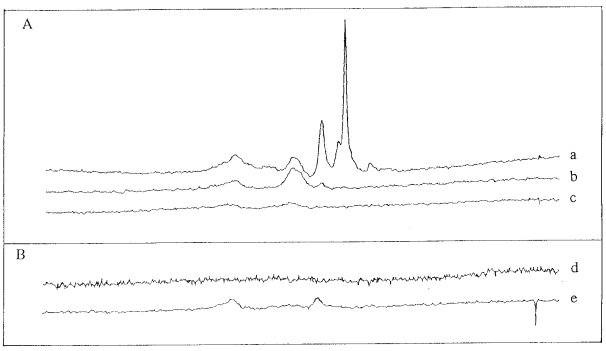

Figure 7. Nuclear magnetic resonance (NMR) spectra of sodium metavanadate added to uninoculated Sabouraud complex medium in the following concentrations: (a) 10 mM; (b) 4 mM; and (c) 2 mM; and 10 mM in cultures of: (d) strain SC-1 cells; and (e) their supernatants.

In order to associate the V(V) reduction with the vanadate resistance, total vanadium uptake was monitored by cultivating SC-1, DBVPG 6173, and DBVPG 6037 for 24 h with increasing concentrations of vanadate. This experiment unexpectedly complicated the interpretation of the results, since total vanadium uptake by vanadate-resistant strain SC-1 plotted between the accumulation curves of the other two strains (Figure 5). The vanadate sensitive strain DBVPG 6173 accumulated the lowest concentrations of total vanadium, but probably enough levels to inhibit its growth. On the other hand, the less vanadate-resistant strain, DBVPG 6037, accumulated ten times more vanadium, despite the fact that its MIC was only double that of the vanadate-sensitive DBVPG 6173.

EPR analysis of the vanadyl ion content of washed cell pellets of the three strains was carried out in an attempt to obtain an explanation for this atypical metal accumulation. A weak signal from vanadyl ions was only found in washed cells of SC-1 (Figure 6a). In the other two strains, no vanadyl was detected at all (Figure 6b).

NMR analysis of cells pellets of SC-1 confirmed that vanadium was accumulated in the cells of this vanadate-resistant strain only as vanadyl, since NMR spectroscopic analysis failed to detect the vanadate oxyanion (Figure 7d).

^{51}V NMR spectra of 10, 4, and 2 mM vanadate [V(V)] in uninoculated Sabouraud medium are shown in Figure 7a–c. We did not use external standards on purpose, because we only wanted to use this spectroscopy approach to check the presence of detectable V(V) species in the samples. In complex media like Sabouraud, which contains mixtures of organic compounds such as peptides, cations and other compounds that could interact with V(V) species, the identification of the different V(V) chemical forms would have been a study on its own, beyond the scope of the present research. However, it was interesting that at the lower concentrations of vanadate (4 and 2 mM), only broad resonances were detected (Figure 7b and 7c), suggesting that the medium favored the formation of large molecular weight vanadium polymeric species. When all these vanadate ligands were probably saturated at higher vanadate concentrations (10 mM), vanadate species of lower molecular weight become detectable (Figure 7a). Broad resonances, obtained from large molecular weight of vanadate species, were detected in the supernatant (Figure 7e) but not in washed cells of SC-1 (Figure 7d).

Discussion

The *S. cerevisiae* SC-1 strain was found to have a higher vanadate resistance (MIC > 16 mM) than other vanadate-resistant strains (MIC = 5 mM) reported so far (Willsky *et al.* 1984). The differences found in the phosphate analog (vanadate, molybdate and arsenate) resistances suggest that different toxicity mechanisms occur in *S. cerevisiae* (Table 1).

The fact that SC-1 only grew in complex medium increased the MIC value because of vanadate ion complexation by organic compounds. However, inhibition of cell growth was evident in the sensitive strains DBVPG 6037 and DBVPG 6173 spiked with 8 and 4 mM vanadate, respectively, in the medium.

EPR and visible spectroscopy revealed that the vanadyl species was formed in the supernatant from vanadate during the yeast growth (Figures 3 and 4, respectively). The dark green color developed by the SC-1 culture was due to vanadyl species in the supernatant. EPR spectroscopy also detected vanadyl cations in SC-1 yeast cells, but not in the vanadate-sensitive strain, DBVPG 6173. However, the signal was two to three orders of magnitude smaller in washed SC-1 cells than in the supernatant. Willsky *et al.* (1984) detected the vanadyl EPR signals of similar intensities (i.e. similar concentrations) in the supernatant and in cells of vanadate-sensitive *Saccharomyces* strains. Our observations show that SC-1 reduces vanadate to vanadyl, whereas the vanadate-sensitive strains do not. These results provide a likely explanation for vanadate uptake obtained by inductively coupled plasma atomic emission spectrometry (ICP–AES). Total intracellular vanadium was measured in the three tested strains. There was evidence that SC-1 accumulated vanadate only as V(IV), whereas the two sensitive strains only accumulated total vanadium as vanadate. The SC-1 strain therefore grows with high concentrations of the vanadate oxyanion because it reduces and accumulates the less toxic vanadyl cation (Willsky *et al.* 1984).

The SC-1 strain converts vanadate to vanadyl, over hours, during the exponential growth phase. Conversely, in bacteria which use vanadate as the final acceptor in anaerobic respiration, the oxyanion reduction occurs over days and the growth rate is consequently slower (Lyalikova & Yurkova 1992).

The reduction of vanadate to vanadyl has also been observed in other *S. cerevisiae* yeasts (Willsky *et al.* 1984, Minasi *et al.* 1990, Minasi & Willsky, 1991, Zoroddu *et al.* 1996), but the kinetics of conversion have not been reported. Our data do not provide evidence for vanadate uptake by SC-1 cells and for its reduction to vanadyl inside the cells. Vanadate (10 mM) was totally transformed to vanadyl before reaching the stationary phase of *S. cerevisiae* SC-1 and a negligible concentration (25 nM mg^{-1} cells dry weight of total vanadium, probably as vanadyl, was found in the biomass. Vanadate was not detected inside SC-1 yeast cells.

In another report (Willsky *et al.* 1984) it has been suggested that vanadate reduction occurs totally inside cells of certain *S. cerevisiae* strains. According to this hypothesis, the vanadate oxyanion would enter the cells of our vanadate-resistant *S. cerevisiae* SC-1 by the phosphate transport system (Bowman 1983), would be reduced by high concentrations of intracellular reducing agents and then pumped out as vanadyl. This presumes an inducible energy efflux system as described in other microorganisms (Silver & Walderhaug 1992). In our case there is no evidence of this, because vanadate reduction is constitutive and vanadium accumulates in the SC-1 strain as the vanadate is added up to 1 mM concentration (Figure 5). The present results suggest a different hypothesis: vanadate reduction occurs at the level of the cell envelope and not intracellularly. Previous studies on vanadate reduction by *S. cerevisiae* strains (Willsky *et al.* 1984, Zoroddu *et al.* 1996) suggested that the efflux of vanadyl from cells occurs only following its intracellular reduction. However, the occurrence of a vanadyl efflux system has yet been not demonstrated. From our accumulation curves (Figure 5), we can exclude any active export of vanadium species from the cell cytoplasm, because a total vanadium accumulation was observed. Conversely, in the presence of an efflux system a non-uptake (impermeabilization) of metals is expected (Silver & Walderhaug 1992). In addition, a conversion by a vanadate-reductase system embedded in the cell membrane would be more efficient in terms of energy consumption than an efflux system, as in the case of energy consuming metal-ATPases (Silver and Walderhaug 1992). Further investigations are needed to study the vanadate-reducing system in cell membranes of *S. cerevisiae* strain SC-1.

Acknowledgement

The authors thank A. Vivi and F. Briganti for their assistance with NMR and EPR experiments.

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