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Electron paramagnetic resonance studies and effects of vanadium in *Saccharomyces cerevisiae*

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Vanadium uptake by whole cells and isolated cell walls of the yeast *Saccharomyces cerevisiae* was studied. When orthovanadate was added to wild-type *S. cerevisiae* cells growing in rich medium, growth was inhibited as a function of the VO_4^{3-} concentration and the growth was completely arrested at a concentration of 20 mM of VO_4^{3-} in YEPD. Electron paramagnetic resonance (EPR) spectroscopy was used to obtain structural and dynamic information about the cell-associated paramagnetic vanadyl ion. The presence of EPR signals indicated that vanadate was reduced by whole cells to the vanadyl ion. On the contrary, no EPR signals were detected after interaction of vanadate with isolated cell walls. A 'mobile' and an 'immobile' species associated in cells with small chelates and with macromolecular sites, respectively, were identified. The value of rotational correlation time τ_r indicated the relative motional freedom at the macromolecular site. A strongly 'immobilized' vanadyl species bound to polar sites mainly through coulombic attractions was detected after interaction of VO^{2+} ions with isolated cell walls.

Keywords: EPR, *Saccharomyces cerevisiae*, uptake, vanadate, vanadyl

Introduction

Recently, increasing interest has been shown in the regulation of the gene expression of anion and cation transport systems in biological organisms. Living organisms have evolved several mechanisms to respond to the toxic effects of heavy metal ions; one of the most common mechanisms is the production of proteins such as metallothioneins in response to metal ion uptake (Thiele 1992). Metallothioneins are able to bind the metal ion, neutralizing the toxic effects. For example, resistance to the copper ion toxic effect in the yeast *Saccharomyces cerevisiae* is mediated by the induction of a cystein-rich protein specifically binding copper (Butt 1987).

Yeast cells share many features with higher eukaryotic cells and therefore can be used as model systems for studying several biological processes in eukaryotes. In addition, the use of microorganisms in biotechnological exploitation such as depuration and metal-regulated gene transcription has provided an incentive for further studies in this field.

Heavy-metal pollution of wastewater is a problem for which bioremediation by microorganisms may be a natural solution. In this context, the control of the transport of metal ions might make microorganisms more effective in extracting deleterious ions from the environment.

The yeast *S. cerevisiae* has been used to study retention and absorption mechanisms as well as processes involved in metal ion resistance to toxicity (Kihn 1987, Ross 1977, Ross and Walsh 1981, Gadd 1984).

Among the essential trace elements that are toxic at higher concentrations for eukaryotic cells, vanadium is of particular interest since it is widely distributed in rocks, soil and to a lesser extent in water (Schwartz & Milne 1971).

Vanadium exhibits a wide range of stable oxidation states, V(III), V(IV), V(V), all found in living systems. Most of the biochemistry of vanadium deals with its redox properties as well as its coordination chemistry (Rehder 1991).

The study of the essentiality and toxicity of vanadium is an area of great interest at present (Willsky 1990). For example, vanadate *in vitro* is a very potent inhibitor of protein phosphatases, while vanadyl *in vivo* appears to stimulate protein phosphorylation. Further investigation into the role of oxovanadium compounds in the regulation

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and alteration of metabolism could clarify how inorganic processes are involved in cellular regulation.

An extensive study of vanadate uptake and metabolism in *S. cerevisiae* has been reported by Willsky (Willsky *et al.* 1984, 1985, Willsky & Dosch 1986). They found that yeast cells absorbed vanadate and released vanadyl ions when VO_4^{3-} was present at a concentration of less than 1 mM, and that 5 mM VO_4^{3-} was the toxic concentration for cells grown in buffered synthetic medium containing yeast nitrogen base and tryptophan. Furthermore, some vanadate-resistant mutants of *S. cerevisiae* have been isolated and characterized (Kanik-Ennulat & Neff 1990). The results suggest that (1) vanadate is easily reduced by the cells under physiological conditions and (2) vanadate resistance is conferred by mutations in a number of genes and the products of these genes are important for the regulation of the phosphorylation of a number of proteins which may be involved in growth control.

While much work has been carried out on vanadium transport in biological systems from the point of view of the biochemical and genetic effects, little is known about the cellular sites responsible for metal uptake or the possible complexes and functional groups of potential ligands involved in vanadyl retention by cells.

As part of an investigation program focused on interaction between yeast cells and metal ions (Zoroddu *et al.* 1989, 1991, Berardi *et al.* 1990), in this paper we report on vanadate interaction with *S. cerevisiae* cells growing in rich medium and with isolated cell walls of *S. cerevisiae*.

Electron paramagnetic resonance (EPR) spectroscopy was used to probe the interaction of vanadium with whole cells or isolated cell walls and thereby to identify the metal oxidation state and the chromophores involved in coordination to the metal. Since the $3d^1$ ground state of vanadyl complexes is orbitally non-degenerate with no electronic excited states nearby in energy, well resolved EPR spectra at room temperature can be obtained; furthermore, they are very sensitive to the rotational correlation time of the ion, allowing information to be drawn about the size of the complexes involved. EPR experiments can therefore give *in vivo* information about the environment involved in the metal ion uptake.

Material and methods

S. cerevisiae, strain S288c, was used in all experiments. Cells were grown in YEPD (1% yeast extract, 2% peptone, 2% glucose; pH = 5–6) medium.

Cells were grown at 30°C in a shaking incubator until cell concentration was 2×10^6 cells ml^{-1}. Cultures were then aliquoted into 250 ml flasks containing 50 ml of YEPD medium and VO_4^{3-} at different concentrations.

Cell growth was monitored by carrying out a cell count using a haemocytometer slide under a light microscope.

The method to obtain cell walls was derived from the literature (Campbell & Duffus 1988).

Sodium orthovanadate was purchased from Aldrich (99% pure grade), Vanadyl sulphate pentahydrate from Fluka (96% pure grade) and deuterium oxide from Janssen (99.8% pure grade).

A stock solution of orthovanadate was prepared in distilled water. When the pH was adjusted to 5–5.5, the colourless solution turned orange–yellow due to the conversion of vanadate to the polymeric decavanadate $(V_{10}O_{28})^{6-}$. Only at below 10^{-4} M concentration of vanadium and at pH above 12 did orthovanadate monomeric VO_4^{3-} occur.

Appropriate volumes of the filter-sterilized stock solution were added at the start of each experiment to obtain the desired 5, 10 and 20 mM concentrations of monovanadium units in the growth medium.

In addition, YEPD-VO^{2+} or aqueous-VO^{2+} (5, 10 and 20 mM) medium were used in the uptake experiment with isolated cell walls.

The pH of the supernatants did not change significantly after the growth experiments in YEPD-VO_4^{3-} media.

Pelleted cells, cell walls and supernatants were analysed by EPR spectroscopy.

NMR spectroscopy was used to identify the vanadate species in the growth medium YEPD. ^{51}V-NMR spectroscopy was carried out on a Varian VXR300 with $VOCl_3$ as standard. Free induction decays were collected using a 7 μs pulse. Sweep widths of 50 kHz were employed. A total of 1020 scans with 50 K data points were collected.

X-band EPR spectra were recorded at room temperature and at 115 K by using a Bruker 220 D SRC spectrometer. The microwave frequency was calibrated against powder DPPH samples ($g = 2.0036$). Presumed errors in the reported g_{iso} values were less than 0.010, while in A_{iso} values were about 2×10^{-4} cm^{-1}. Spin Hamiltonian parameters for vanadyl species were obtained by simulating the experimental spectra by means of a revised version of the MONOCLIN program (Troy *et al.* 1971).

The EPR spectra were obtained on whole cells, on supernatant and on cell walls using capillary tubes at room temperature or quartz tubes for frozen samples after adding a few drops of ethylene glycol; 5 10 db microwave power, 4–8 G modulation amplitude, 0.25 s time constants, 4 min scan time, 5×10^3–5×10^5 spectrometer gain were used.

The yeast cell samples were examined immediately after centrifugation at 3000 r.p.m. for 10 min, removal of the supernatant and washing with either distilled water (×4) or with EDTA (×6) and then triple washing with distilled water. Isolated cell wall samples were examined after the uptake experiment with vanadate or vanadyl in YEPD or water medium, immediately after removal of the supernatant and washing either with distilled water (×4) or with distilled water (×4) and then with D_2O (×3).

Results

As a first step to study uptake of vanadium in *S. cerevisiae*, we tested the toxic effect of vanadate on yeast cultures growing in rich medium.

When orthovanadate was added to growing *S. cerevisiae* cells, growth was inhibited as a function of the VO_4^{3-}

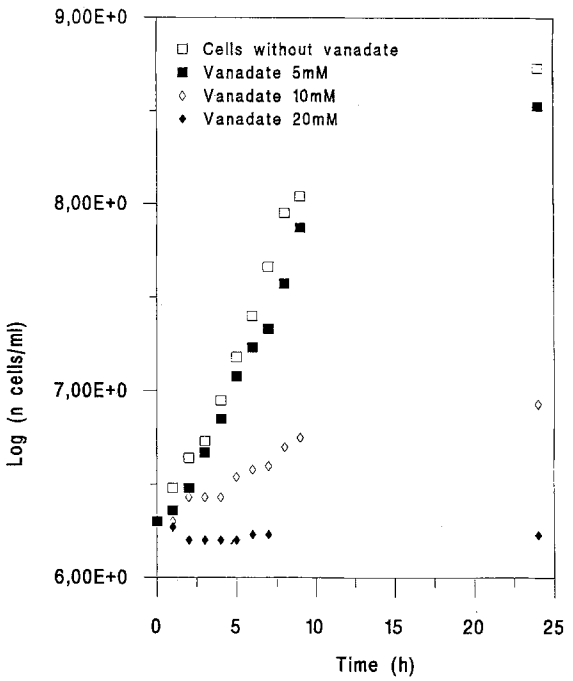

Figure 1. Growth curves for *S. cerevisiae* on YEPD medium at VO_4^{3-} concentrations of 5, 10 or 20 mM.

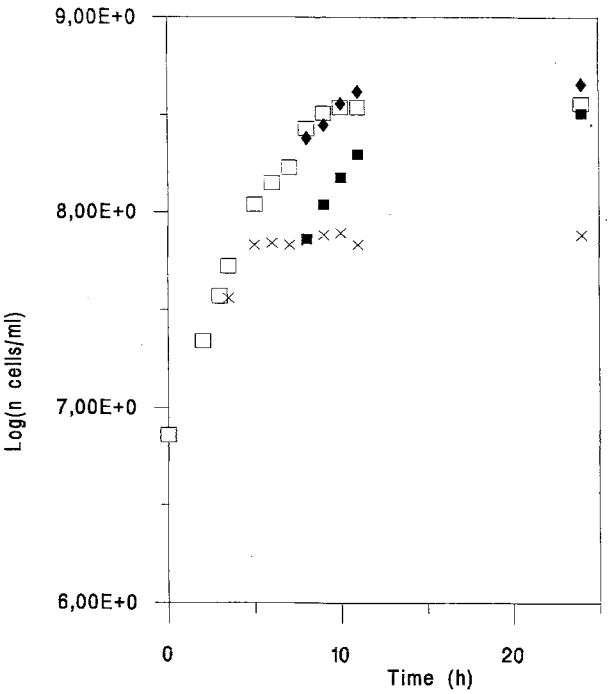

Figure 2. Growth response of *S. cerevisiae* to added vanadate. Cells grown as described; after 3 h the log-phase culture was divided into two portion: one part was 20 mM of vanadate (×), the other part without vanadate (□); after 4 h: one part without vanadate (■), the second portion as a control (◆).

concentration in the growth medium (Figure 1). The percentage of the total external vanadium taken up was about 4% at 24 h.

While the growth rate was not significantly affected in YEPD containing 5 mM VO_4^{3-}, it was severely affected by 10 mM VO_4^{3-} and completely inhibited by 20 mM VO_4^{3-}. These results suggest that the type of nutrient medium used in the growth experiment is important to determine the toxic concentration of the vanadate. In fact, similar experiments performed in synthetic YNB (yeast nitrogen base) medium showed complete growth arrest in the presence of 5 mM VO_4^{3-} (Willsky *et al.* 1984). The complete inhibition by 20 mM VO_4^{3-} did not involve cell death as demonstrated by the data reported in Figure 2. In this experiment, cells grown in YEPD as described in the experimental section were harvested after 3 h of stationary phase and resuspended at a concentration of 1×10^7 cells ml^{-1} in fresh YEPD medium containing either 20 mM VO_4^{3-} or no vanadate. After 4 h of growth, the vanadate-containing culture was divided into two portions: one part was harvested, washed with distilled water (×3) and resuspended in fresh medium without vanadate, while the second portion of the culture was left as a control.

Although cell growth was stopped in the culture containing 20 mM VO_4^{3-}, after the cells were harvested from this culture, washed in distilled water (×3) and resuspended in fresh medium without vanadate, the growth was resumed within one generation time.

^{51}V-NMR spectroscopy was used to identify the vanadate species in the growth medium. A very broad resonance at chemical shift in the range −497 to −500 from the standard $VOCl_3$ was obtained by ^{51}V-NMR spectrum of YEPD-VO_4^{3-} medium. The signal can be attributed to the presence of the decavanadate ion $(V_{10}O_{28})^{6-}$ and of some vanadate complexes formed with diols or monosaccharides (Rehder 1991).

No EPR signals were observed in YEPD-vanadate medium or in cells alone.

The EPR spectrum obtained on cells in the logarithmic phase of growth (4 h) and harvested from YEPD-VO_4^{3-} (5 mM) showed the presence of signals due to the paramagnetic vanadyl ions (Figure 3). This demonstrates that vanadate V(V) was reduced to vanadyl V(IV) ions after interaction with the cells.

The EPR spectrum exhibits an eight-line isotropic feature due to the coupling of the single d_{xy} electron with the nuclear spin of the vanadium atom ($I=7/2$, $^{51}V=99.8\%$ natural abundance) and to the averaging effect of molecular tumbling of a VO^{2+} complex, suggesting that low molecular weight ligands are, in this case, involved in the coordination to the VO^{2+} ions in the cells.

Although the EPR parameters can be sensitive to a number of factors, such as pH and viscosity of media (Frank 1988, Brand 1987), from the examination of the spectral pattern and evaluation of the EPR parameters, the presence of the aquo complex $[VO(H_2O)_5]^{2+}$ could be excluded.

By increasing the concentration of vanadate in the growth medium, the intensity of the vanadyl signals increased in the EPR spectra.

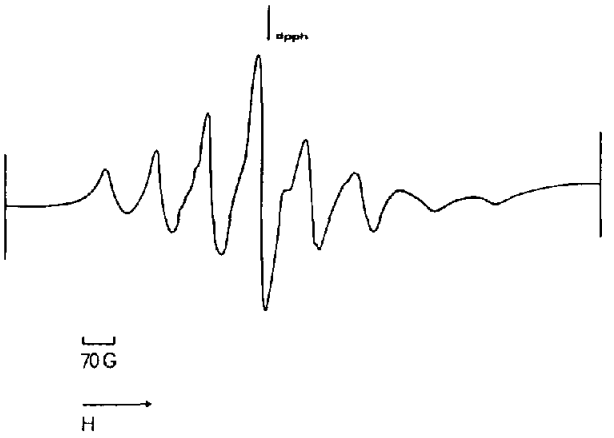

Figure 3. Room temperature EPR spectrum for exponential cells grown on YEPD-VO_4^{3-} (5 mM) at 4 h.

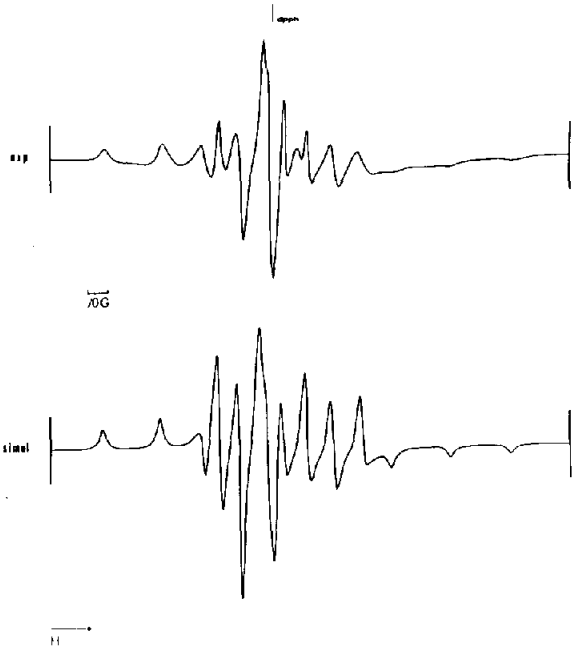

Figure 4. Room temperature EPR spectrum for stationary cells grown on YEPD-VO_4^{3-} (5 mM) at 24 h.

For the highest concentration of VO_4^{3-} (20 mM) used in the experiments, VO^{2+} isotropic and anisotropic EPR signals could be seen at room temperature from cells after 4 h of growth. These signals can be attributed to the contemporary presence of freely-tumbling and slow-tumbling complexes, respectively associated with low and high molecular weight compounds.

The presence of anisotropic EPR signals due to the vanadyl associated with high molecular weight compounds became more evident with elapsing time for samples obtained from nutrient media with the same concentration of VO_4^{3-}.

Figure 4 shows the EPR, paired with the corresponding

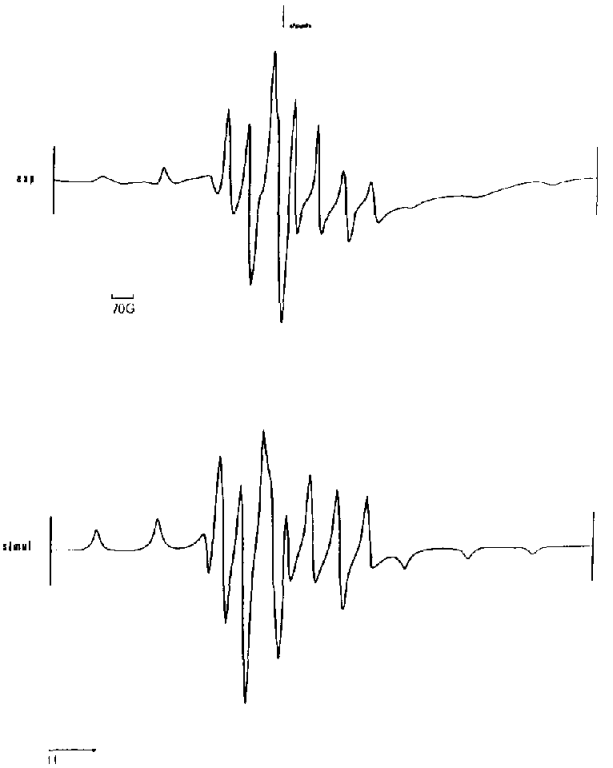

Figure 5. Frozen EPR spectrum for stationary cells grown on YEPD-VO_4^{3} (5 mM) at 24 h.

simulated spectrum, obtained at room temperature for cells in the stationary phase (concentration $> 10^8$ cells ml^{-1}), after 24 h of growth in YEPD-VO_4^{3-} (5 mM). The typical axial EPR spectrum with parallel and perpendicular overlapping lines reflects the presence of a VO^{2+}-slow tumbling species in the cells.

In Figure 5 the spectrum obtained on the same sample of cells at 115 K is reported. It is only slightly different from that obtained at room temperature, confirming that vanadyl ions are bound to an immobilized region in the cells, although a partial tumbling average is suggested.

Motional information can be extracted from the EPR spectrum: it is sensitive through the rotational correlation time τ_r to the size of the compound since the τ_r of the metal ion (VO^{2+}) is mainly governed by the tumbling rate of the complex.

As the rate of rotational diffusion slows ($\tau_r \geq 10^{-9}$ s rad^{-1}) the EPR spectrum approaches the 'rigid' limit type and parallel and perpendicular features become evident, as was seen in the EPR spectra of cells from YEPD-VO_4^{3-} 5 or 10 mM after 24 h of growth, or 20 mM starting at 4 h of growth, although, in this case, clearly superimposed by the isotropic signals.

Under the assumption of Brownian rotational diffusion, τ_r was tentatively estimated for the 'immobilized' species involved in the vanadyl retention in *S. cerevisiae* cells, by using the empirical formula valid for solution EPR spectra

with 'parallel' features (Bruno *et al.* 1977): $\tau_r = a(1-S)^b$, where $S = \Delta/\Delta'$, Δ is the separation of the high and low field extrema of the slow tumbling species obtained at room temperature, Δ' is the extrema separation measured from the frozen 'rigid' limit spectrum, a and b are the constants which depend on the residual line width. S increases with molecular weight of the slow tumbling complexes. Although limitations of the above equation have been reported (Bruno *et al.* 1977), the value of τ_r found for a residual line width from 1 to 7.5 Gauss, is in the range 6.1×10^{-9} to 5.4×10^{-9} s rad^{-1}.

Chasteen & Francavilla (1976) defined an 'order parameter' S, to describe molecular tumbling in vanadyl-labeled proteins, where the metal ion tumbling is mainly governed by the τ_r of the protein: $S = A'_p A_0 / A_p A'_0$, where the dipolar contribution to the hyperfine splitting $A_p = (A_\parallel - A_0)/2$, A_0 and A_p are in frozen solution, and A'_0 and A'_p are at room temperature.

For the 'immobilized' VO^{2+}-species in *S. cerevisiae* cells, we found $S = 0.935$. As reported in the literature, S increases with increasing molecular weight and τ_r. For comparison, the τ_r and S values obtained are typical for VO^{2+}-labeled proteins with molecular weight in the range 18 000–35 000 (Chasteen 1981).

The low intensity of the EPR signals obtained for the supernatants after 24 h of growth on 5 or 10 mM VO_4^{3-} media, shows that the quantity of V(IV) was not significant. However, despite the weak EPR signals, it was possible to calculate EPR parameters from the simulated spectra and identify an isotropic VO^{2+}-species in the supernatants.

No EPR signals were detected on isolated cell walls harvested from YEPD-VO_4^{3-} or from aqueous-VO_4^{3-} (5, 10 and 20 mM) medium. This demonstrates that isolated cell walls were not able to reduce vanadate to vanadyl paramagnetic species under our conditions. EPR signals were detected when isolated cell walls were harvested from YEPD-VO^{2+} or from aqueous-VO^{2+} (5, 10 and 20 mM) medium. The anisotropic EPR spectrum reflects the presence of a strongly immobilized species ($\tau_r > 10^{-9}$ s rad^{-1}; $S = 0.952$). Even if τ_r and S have an experimental error of roughly 10% and ± 0.01, respectively, an increase in the S value corresponds to an increase in the τ_r value and then in the molecular weight of the species involved.

In addition, dipolar spin–spin broadening overlapping anisotropic features of VO^{2+} ions were clearly visible in the spectrum of the cell walls (Figure 6).

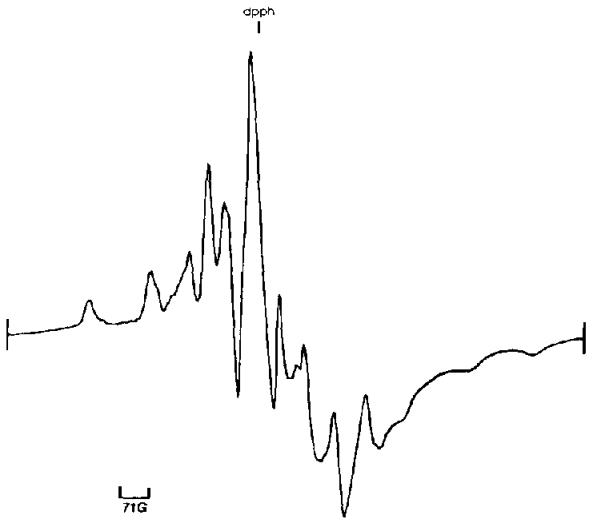

Figure 6. Room temperature EPR spectrum of isolated cell walls after uptake experiment with aqueous VO^{2+} (20 mM).

Discussion

To sum up our results, from an examination of the EPR spectra, two species of VO^{2+}-complexes can be identified in the *S. cerevisiae* cells grown under our conditions on YEPD medium at 5, 10 or 20 mM concentration of VO_4^{3-} ions: a 'mobile' species and an 'immobilized' species. The relative amounts of each are a function of the concentration of vanadate in the growth medium, as well as of the growth phase of the cells.

g_{iso} and A_{iso} parameters calculated directly from the spectrum for the mobile species gave the best fit for $g_{iso} = 1.97$ and $A_{iso} = 93.9 \times 10^{-4}$ cm^{-1}.

For the 'immobilized' species, the simulation which gives the best fit allows a precise evaluation of the magnetic parameters $g_\parallel = g_{zz} = 1.937$, $g_\perp = g_{xx} = g_{yy} = 1.975$, $A_\parallel = A_{zz} = 172 \times 10^{-4}$ cm^{-1}, $A_\perp = A_{xx} = A_{yy} = 64 \times 10^{-4}$ cm^{-1}. The simulation is based on a Lorentzian line-shape with line width $\sigma_\parallel = \sigma_{zz} = 25$ G and $\sigma_\perp = \sigma_{xx} = \sigma_{yy} = 28$ G. However, the EPR parameters obtained are 'apparent', in that the room temperature spectra reflect some motional averaging. This can be observed in both the intense central peak ($M(I)_{\parallel,\perp} = -1/2$) and the corresponding EPR parameters obtained from the frozen sample: $A_\parallel = 177 \times 10^{-4}$ cm^{-1}, $A_\perp = 62.5 \times 10^{-4}$ cm^{-1}, $g_\parallel = 1.935$, $g_\perp = 1.980$; the increase in $A_\parallel$ and the reduction in $A_\perp$ on passing from the room temperature to the frozen EPR spectrum confirms the presence of a partially averaged system.

However, since the 'apparent' anisotropic parameters from the room temperature sample are only slightly different from those obtained from the frozen sample, it is reasonable to use the equations $g_0 = 1/3\ (g_\parallel + 2g_\perp)$, $A_0 = 1/3\ (A_\parallel + 2A_\perp)$, which are valid when $A_\parallel$, $A_\perp$, $g_\parallel$ and $g_\perp$ are obtained from a completely anisotropic spectrum. From the room temperature spectrum, values of $g_0 = 1.965 = 1/3\ (1.937 + 2\ (1.98))$, $A_0 = 100 \times 10^{-4}$ cm^{-1} $= 1/3\ (172 + 2\ (64))$ were obtained compared to $g_0 = 1.965$ and $A_0 = 100.6 \times 10^{-4}$ cm^{-1} from the corresponding frozen sample. These slight differences suggest that no changes in the metal coordination occurred by changing the temperature.

The predominant 'immobilized' VO^{2+} species identified in the cell walls at room temperature gave EPR parameters $g_\perp = 1.932$, $g_\perp = 1.981$, $A_\parallel = 180 \times 10^{-4}$ cm^{-1}, $A_\perp = 72.8 \times 10^{-4}$ cm^{-1}, $g_0 = 1.964$, $A_0 = 108 \times 10^{-4}$ cm^{-1}, although broadening and doubling of the peaks indicated the presence of two slightly different binding centres for VO^{2+} ions; $g_\parallel = 1.93$; $g_\perp = 1.982$; $A_\parallel = 183 \times 10^{-4}$ cm^{-1} and

$A_{\perp} = 70 \times 10^{-4}\,cm^{-1}$ were obtained from frozen EPR spectrum. These values are very similar to those reported in the literature for $VO(H_2O)_5^{2+}$ (van Willigen & Chandrashekar 1983, Brand *et al.* 1987). These results suggest that the 'immobilized' species on the cell walls was mainly a VO^{2+} hydrated species. The interactions of VO^{2+} with cell walls were thus especially of ionic type and the metal ions were mainly absorbed by electrostatic forces between adjacent chains of cell wall polymers, the cation binding sites in the cell walls being phosphodiesters in the phosphomannans and carboxyl and amines in the peptides. In addition, the presence of a broad signal overlapping the anisotropic VO^{2+} EPR features can be due to a strong dipolar spin–spin interaction between neighbouring paramagnetic VO^{2+} ions bound to adjacent sites on the cell walls. The dramatic reduction of the linewidth in the $M(I) = +1/2$ perpendicular resonance observed after washing the VO^{2+}-cell walls with D_2O is a further demonstration that the VO^{2+} ions are mainly hydrated. The line width decreased approximately by 6 G in the frozen spectrum; it may be caused by the substitution of some equatorially coordinated water molecules with D_2O (Albanese & Chasteen 1978).

The nature of the binding sites of the VO^{2+} ion in the cells can be deduced by comparison of the anisotropic and isotropic g and nuclear hyperfine A parameters known for a large number of models of vanadyl compounds and for vanadyl found in biological systems. Correlation diagrams of $g_{\perp}$ versus $A_{\parallel}$ and of A_0 versus g_0 can be used to help identify the ligands involved in the coordination environment around the VO^{2+} ions, although caution must be exercised (Holyk 1979, Johnson & Shepherd 1978). The spin Hamiltonian parameters obtained for VO^{2+} species in *S. cerevisiae* cells are in agreement with a moderate in-plane ligand field strength, involving oxygen and/or nitrogen donor atoms (White & Chasteen 1979, Dickson *et al.* 1972).

In addition, the isotropic nuclear hyperfine coupling constant A_0 can be used as an empirical measure of the ligands involved, with the average equatorial ligand environment around VO^{2+} defined by the additivity relationship:

$$A_{0,calc} = \Sigma_i n_i A_{0,i}/4$$

where $A_{0,i}$ is the isotropic coupling constant obtained experimentally from a complex with four identical donor groups.

In this way, the A_{iso} value obtained for the 'mobile' species can be ascribed to a $[VOO_4]^{2-}$ chromophore as in the $VO(oxalate)_2^{2-}$ complex in which the four oxygen atoms can derive from deprotonated carboxylic groups ($A_{0,exp} = 94.1$); but A_{iso} also agrees well with the value reported for a complex of vanadyl with a small reducing peptide such as glutathione (Degani *et al.* 1981) bound through two carboxylate groups, the thiol group and a water molecule. It turns out that glutathione can also be a possible reducing agent for vanadate (Degani *et al.* 1981).

For the 'immobilized' species, the calculated A_0 value can be ascribed to a $[VOO_4]^{2-}$ chromophore in which the four oxygen atoms are from carboxylate groups such as in the $VO(phthalate)_2^{2-}$ complex ($A_{0,exp} = 100.1 \times 10^{-4}\,cm^{-1}$).

The 'mobile' species identified in the supernatants gave EPR parameters $g_{iso} = 1.98$ and $A_{iso} = 91.9 \times 10^{-4}\,cm^{-1}$ which are consistent with a stronger ligand field compared with the 'mobile' VO^{2+}-species identified in the cells. By assigning the equatorially coordinating ligands of the VO^{2+} complex using the 'rule of average environment', we can predict a coordination environment with two aromatic nitrogens as donor atoms (such as from imidazole ligands) and two oxygens from carboxylate groups such as in the $VO(glycinate)_2$ complex ($A_{0,exp} = 91.6$). It is important to consider that the isotropic vanadyl signals in the supernatants became more evident with elapsing time and increasing concentration of VO_4^{3-} in the nutrient medium, except for the toxic 20 mM concentration of VO_4^{3-} which only gave very weak signals. It is thus possible to deduce that the viability of cells is essential in promoting an efflux of VO^{2+} species which can be correlated to the cellular metabolism of the metal ion in response to the ion stress; the efflux can be regarded as a detoxification process.

It is noteworthy that the intracellular 'mobile' species was not removed after washing the cells with EDTA (10 mM) (×6) and then with water (×3). The EDTA washing partially removed only the 'immobile' species, suggesting that this species is either mainly surface-bound or more accessible from the EDTA ligand. The EPR spectra on the EDTA wash media showed only the presence of the VO(EDTA) complex, easily identified by the spin Hamiltonian parameters ($g_0 = 1.972$, $A_0 = 97 \times 10^{-4}\,cm^{-1}$) (Smith *et al.* 1974).

Although no definite conclusion about the localization of the intracellular vanadyl complexes can be drawn, the vanadate interaction with the *S. cerevisiae* cells can be described as follows:

VO_4^{3-} (5 or 10 mM)-YEPD$\Rightarrow VO^{2+}$-cells ('mobile'—inside)
$\Rightarrow VO^{2+}$-cells ('immobile'—macromolecular components)

In the presence of an extensive ion uptake which is a function of the VO_4^{3-} concentration in the growth medium, as well as of the growth phase of the cells, macromolecular components are involved in metal binding and in immobilizing the VO^{2+}.

The isolated cell walls were not able to reduce vanadate to vanadyl suggesting that, once within the cells, vanadate can be reduced by metabolically active *S. cerevisiae*. The biopolymer systems forming cell walls were able to immobilize a hydrated VO^{2+} species on polar sites through mainly electrostatic attractions, when VO^{2+} ions were supplied externally.

No conclusions about the correlation between the type of compounds identified in the cells and the toxicity of vanadium can be drawn.

The τ_r and the order parameter S values yield information concerning the 'rigidity' of the local environment of the vanadyl oxocation in the cells. In fact, the slow tumbling EPR spectrum obtained at room temperature occurs when the VO^{2+} ion is bound to a macromolecule or when a vanadyl complex is dissolved in a viscous medium.

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