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Vanadium uptake by yeast cells

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During incubation with vanadyl, *Saccharomyces cerevisiae* yeast cells were able to accumulate millimolar concentrations of this divalent cation within an intracellular compartment. The intracellular vanadyl ions were bound to low molecular weight substances. This was indicated by the isotropic nature of the electron paramagnetic resonance (EPR) spectra of the respective samples. Accumulation of intracellular vanadyl was dependent on presence of glucose during incubation. It could be inhibited by various di- and trivalent metal cations. Of these cations lanthanum displayed the strongest inhibitory action. If yeast cells were exposed to more than 50 μM vanadyl sulfate at a pH higher than 4.0, a potassium loss into the medium was detected. The magnitude of this potassium loss suggests a damage of the plasma membrane caused by vanadyl. Upon addition of vanadate to yeast cells surface-bound vanadyl was detectable after several minutes by EPR. This could be the consequence of extracellular reduction of vanadate to vanadyl. The reduction was followed by a slow accumulation of intracellular vanadium, which could be inhibited by lanthanum or phosphate. Therefore, permeation of vanadyl into the cells can be assumed as one mechanism of vanadium accumulation by yeast during incubation with vanadate.

Introduction

There is experimental evidence that the transition metal vanadium is an essential trace element in mammalian nutrition. It can influence many biological functions and is used in biochemistry and cell biology as a tool to characterize various molecular processes [1].

The chemistry of this element is rather complex and will often complicate experimentation. Two vanadium species are considered to be predominant in living systems: The anion vanadate contains vanadium in oxidation state +5 and can be reduced to form vanadyl, a divalent cation, containing vanadium in oxidation state +4. Vanadate is monomeric only at low concentrations and higher pH and will polymerize on elevation of concentration and lowering of pH. One of such polymers, decavanadate, is very stable and decomposes only slowly [2]. Vanadyl ions are stable only at low pH and will be hydrolysed at a pH higher than 4. Hydrolysis is inhibited to a variable extent by chelators of the vanadyl ion. In the presence of oxygen, vanadyl is readily oxidized to vanadate, whereas within living cells vanadate will be reduced to vanadyl by intracellular reducing agents like glutathione [3].

Thus, it is often not possible to restrict experiments concerning biochemical or biological aspects of vanadium to one single vanadium compound, especially when whole cells or organs are involved. For example, in experiments with cultured cells vanadium usually is added as vanadate, because only this anion is thought to be able to cross the plasma membrane of such cells [2]. Uptake is followed by slow intracellular reduction of vanadate to vanadyl, so that both compounds will be present within the cells. Therefore, it will be difficult to ascribe an observed action to a certain vanadium species.

Yeast cells share many features with highly developed mammalian cells [4] and may be used as model systems for eukaryotic biology [5], if certain limitations are respected. On the other hand they are much more resistant to variations in extracellular conditions than higher animals cells. Because yeast can be exposed to pH 4 or less, incubation with vanadyl is possible at an acidic pH, at which hydrolysis of the vanadyl ion is avoided.

It was the aim of this study to examine whether yeast

Abbreviations: Mes, 2-*N*-morpholinoethanesulfonic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; AAS, atomic absorption spectrophotometry; EPR, electron paramagnetic resonance; NMR, nuclear magnetic resonance.

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cells can take up vanadyl ions under these conditions and therefore potentially are able to contribute to the exploration of vanadium functions, serving as a model for actions of vanadyl within eukaryotic cells.

For selective detection of vanadyl compounds electron paramagnetic resonance (EPR) spectroscopy was used: The vanadyl ion is rendered paramagnetic by its unpaired *d*-electron. In contrast to vanadium(IV), vanadium(V) compounds lack unpaired *d*-electrons and thus do not give an EPR signal. Moreover, information concerning the ligands in vanadyl complexes can be obtained from EPR spectra [6].

Materials and Methods

Preparation of yeast cells

Yeast cells, *Saccharomyces cerevisiae* H 1022 (ETH Zürich), were grown on YEPD medium, containing 1% yeast extract, 2% peptone and 2% glucose (w/v), and harvested during exponential growth. Harvested cells were washed three times with ice cold distilled water and used immediately after. Yeast suspensions of a defined cell content were prepared by the cytocrit method.

Incubations with vanadyl

Vanadyl was added from a stock solution of 200 mM vanadyl sulfate, which had been adjusted to pH 2.0. For incubations at pH 4.0, 100 mM Mes buffer was used, adjusted with NaOH. Mes buffer was considered advantageous in spite of its low buffering capacity at pH 4.0, because it shows only negligible binding of metals. In the presence of 2% yeast cells, 250 mM glucose and 1 mM vanadyl sulfate the pH dropped to 3.7 and 3.2 after 1 h and 4 h, respectively.

Nitrogen was bubbled through the suspensions to inhibit hydrolysis of vanadyl by reducing oxygen concentration. Incubation was performed at 25 °C, the same temperature was chosen for all other incubations of this study. If not indicated otherwise, vanadium was always added to suspensions already containing the yeast cells.

Incubation was performed slightly different for potassium loss experiments: 50 mM Mes buffer, adjusted with NaOH to the desired pH value, was used, glucose was omitted and 0.5% of yeast cells were added. In addition, the medium contained 150 mM NaCl to slow down re-uptake of potassium by monovalent cation transport, as such re-uptake can interfere with measurements of unspecific potassium leakage through permeabilized plasma membranes [7]. No nitrogen was used in these experiments.

Incubations with vanadate

Vanadate was added from a stock solution of 20 mM sodium metavanadate (pH 8.5), which had been boiled during preparation to dissolve all polymeric vanadate species. 50 mM Mes buffer (pH 6.0) or 50 mM Hepes buffer (pH 7.5) were used, both adjusted with NaOH. 250 mM glucose was added 10 min before addition of vanadium. The incubation mixtures contained 2% yeast cells.

Measurement of total vanadium content of the cells with atomic absorption spectrophotometry (AAS)

Washed cells were diluted and the vanadium content of these suspensions was determined directly, using a Perkin Elmer model 372 spectrophotometer, which was equipped with the graphite furnace HGA 76 B for electrothermal atomization. Calibration was performed by addition of standards to the samples. All values of vanadium content refer to wet weight of yeast cells, which was deduced from the volume of yeast present in the incubation mixtures (as measured by the cytocrit method).

To remove cell surface-bound vanadium, the cells were washed with 50 mM EDTA (pH 7.0) by suspending them in this buffer, centrifuging the cells and removing the supernatant. This was repeated four times, if not indicated otherwise.

Electron paramagnetic resonance (EPR) measurements of cell-associated vanadyl

Spectra were recorded at room temperature, using Varian E 9 and E 12 spectrometer, under the following recording conditions: Microwave frequency 9.51 GHz, microwave power 50 mW, modulation amplitude 5 gauss, time constant 0.25 s and scan time 4 min for 1000 gauss. Samples contained approx. 50% yeast cells and were injected into quartz tubes with 1 mm internal diameter for recordings. The exact yeast content of each sample was determined by the cytocrit method.

The cells were washed with a solution of 10 mM lanthanum chloride and 20 mM Mes buffer (pH 4.0) after incubation with vanadyl at pH 4.0 or 100 mM potassium phosphate buffer (pH 2.5) after incubation with vanadate at pH 6.0. These washing buffers were as effective in removing surface-bound vanadium as EDTA. To determine cell surface-bound vanadium(IV), which was the product of vanadate reduction by yeast at pH 6.0, the cells were not washed but suspended in pH 2.5 phosphate buffer to reverse potential hydrolysis and convert all vanadium(IV) into EPR-detectable vanadyl ions.

Measurement of potassium loss

Potassium loss of yeast cells was determined by measuring potassium activity in the medium with an ion-selective electrode. The electrode was constructed using a polyvinyl chloride membrane, which contained valinomycin to achieve selectivity for potassium. It was checked that no compounds were present during in-

cubation that interfered with the measurements. The changes in potassium content of the medium were transformed to give the amount of potassium lost by the cells (referring to kg wet weight).

Growth measurements of cells after loading with vanadyl

Yeast cells were loaded with vanadyl as described above, for 2 h at pH 4.0, in the presence of glucose. Control cells were submitted to the same procedure, except that vanadyl was omitted.

Both types of cells then separately were washed four times with EDTA and resuspended in fresh YEPD medium at a cell density of $5 \cdot 10^6$/ml. Growth was followed by measuring the optical density of the suspensions at 600 nm.

Chemicals

All chemicals used were obtained from commercial sources and were of analytical or reagent grade.

Results

Effect of vanadyl ions on yeast cell membrane permeability

Studies of divalent cation uptake in yeast in some cases have been complicated by an unspecific increase in plasma membrane permeability, which may be caused by certain divalent cations, for example copper and cadmium [8]. Such membrane damage will be accompanied by increased permeation of the respective divalent cation into the cells and leakage of potassium into the medium. So in this study possible vanadyl effects on plasma membrane permeability were examined first, by measuring potassium loss from yeast cells in the presence of vanadyl.

A considerable potassium loss was observed during incubation with vanadyl sulfate at pH 4.5 or higher pH (Fig. 1). Fig. 2 shows the dependence of potassium loss at pH 5.5 on the amount of vanadyl sulfate added.

Yeast cells are known to extrude potassium during uptake of various non-toxic divalent cations, two potassium ions being released for one divalent cation taken up [9]. However, the potassium loss observed here is too large to represent such stoichiometric potassium extrusion only. Its magnitude rather indicates increased permeability of the plasma membrane.

Potassium loss could be prevented either by pretreatment of the cells with iodoacetic acid or by addition of lanthanum chloride to the medium (Fig. 3). All experiments concerning this vanadyl sulfate-induced potassium loss were performed without addition of glucose to the incubation medium, because presence of glucose did not appear to have any influence on the generation of potassium loss. However, this is not necessarily in contradiction to the inhibitory action of iodoacetic acid on potassium loss, which did indicate involvement of metabolism in this process. Yeast cells for these experi-

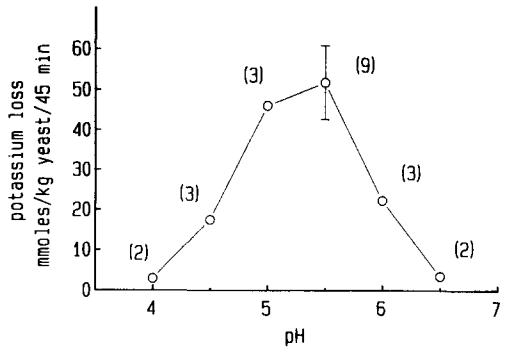

Fig. 1. Potassium loss of yeast cells during 45 min incubation with 500 μM vanadyl sulfate at varied pH. 0.5% yeast cells, 50 mM Mes buffer, 150 mM NaCl, 25 °C. Numbers of experiments are printed in parentheses next to the symbols. The bar denotes standard error of the mean.

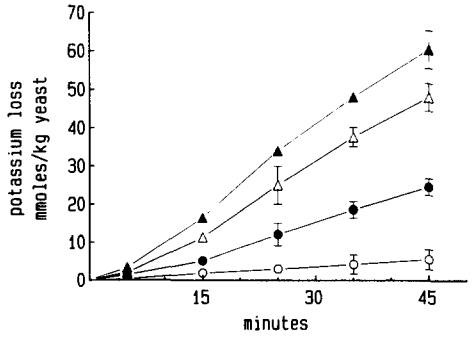

Fig. 2. Dependence of vanadyl-induced potassium loss of yeast cells on the amount of vanadyl sulfate added. 50 mM Mes buffer (pH 5.5), 0.5% yeast cells, 150 mM NaCl, 25 °C. Addition of 750 μM vanadyl sulfate (▲———▲), 500 μM (△———△), 250 μM (●———●) or 100 μM (○———○). Symbols refer to the mean of four experiments, bars denote standard deviation.

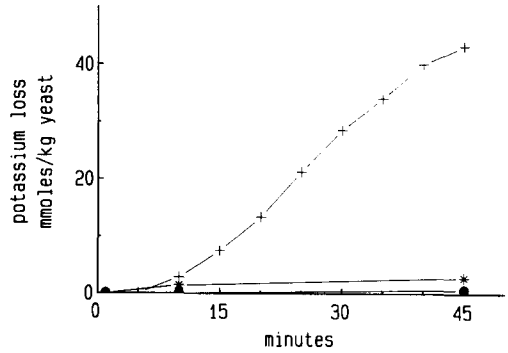

Fig. 3. Inhibition of vanadyl-induced potassium loss by lanthanum or iodoacetic acid. 50 mM Mes buffer (pH 5.5), 0.5% yeast cells, 150 mM NaCl, 25 °C, 500 μM vanadyl sulfate added. Inhibition of efflux by lanthanum (●———●): Addition of 0.5 mM lanthanum chloride to the medium 1 min before addition of vanadyl. Inhibition of potassium loss by iodoacetic acid (*———*): Pretreatment with 20 mM iodoacetic acid for 15 min at pH 4.0, then washing of the cells three times with 50 mM Mes buffer (pH 8), finally suspension in incubation buffer and addition of vanadyl. Control (+———+): No inhibitor present.

ments were harvested from exponentially growing cultures and used immediately. Such cells will possess considerable amounts of metabolic energy. Cells from stationary phase cultures showed significantly lower potassium loss on incubation with vanadyl sulfate and potassium loss was lowest using starved yeast cells (not shown).

As potassium loss occurred only at a pH higher than 4, it might have been caused by any of the various vanadium(IV) hydroxides, which include soluble species and are produced by hydrolysis of vanadyl ions in aqueous solutions above pH 4 [6].

However, no potassium loss at all could be detected, when vanadyl sulfate was added to the assay 10 min before the addition of yeast cells, allowing extensive hydrolysis of vanadyl to take place before the cells were exposed to the incubation mixture (not shown). It therefore is unlikely that the observed potassium loss was caused by vanadium(IV) hydroxides. The potassium loss rather seems to be associated with vanadyl ions, which probably are protected from hydrolysis by complexation with the negatively charged yeast cell surface.

The trivalent cation lanthanum should readily displace vanadyl ions from such binding sites. The inhibitory action of lanthanum on potassium loss might be related to such phenomena.

To check whether the vanadyl sulfate-induced potassium loss indeed was associated with plasma membrane damage, yeast cells that had been incubated with vanadyl were examined by electron microscopy, using the freeze-fracture technique. Plasma membranes from cells, which had been incubated with vanadyl sulfate at pH 4, displayed the same morphology as membranes of control cells, whereas cells after vanadyl incubation at pH 5 showed pronounced morphological alterations of their plasma membranes, mostly impressions and invaginations of varying size (Bode, Fuhrmann and Wehrli, unpublished results).

The injurious effect of vanadyl on yeast plasma membranes observed here may have been caused by lipid peroxidation, since vanadyl ions recently have been shown to induce such processes [10].

However, no further attempts to reveal the mechanisms of this effect were made, because it had been sufficiently established that plasma membrane damage could be avoided, if incubation was performed at pH 4 or lower. In addition, it should be mentioned that on incubation of yeast cells with vanadate or a polyvanadate solution, prepared as described by Csermely et al. [11], neither a potassium loss nor any effects on membrane morphology were seen.

Uptake of vanadium by yeast cells during incubation with vanadyl, as determined by AAS

To assay vanadyl uptake yeast cells were incubated with vanadyl at pH 4. Nitrogen was bubbled through the suspensions to prevent oxidation of vanadyl. EPR measurements confirmed that the vanadium(IV) content of the medium did not decrease significantly during these incubations.

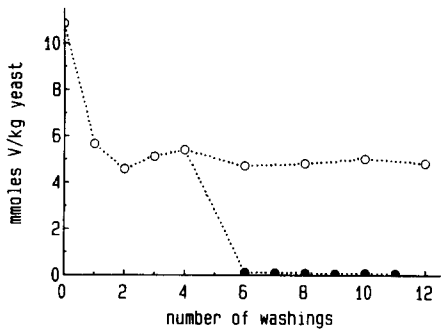

Fig. 4. Effect of repeated washings with EDTA on vanadium content of yeast cells after incubation with vanadyl at pH 4. After incubation with 1 mM vanadyl sulfate and glucose for 4 h (as described in Materials and Methods), cell samples were submitted to the indicated number of washings with 50 mM EDTA (pH 7.0) and vanadium content was determined by AAS (○·····○). Other samples were first washed four times in this manner, then frozen in liquid nitrogen and thawed twice and finally submitted to further washings (●·····●). Symbols referring to six or more washings represent mean of two experiments.

When glucose was present, yeast cells took up vanadium into a compartment, from which it could not be released by repeated washings with EDTA (Fig. 4). Twice freezing in liquid nitrogen and thawing again of the same cells, however, made it possible to remove all accumulated vanadium by EDTA washings. As cellular membranes are rendered leaky by freezing and thawing, this experiment demonstrated uptake of vanadium into an intracellular, membrane-bounded compartment.

Yeast cells accumulated vanadium continuously during 4-h incubation with vanadyl sulfate (Fig. 5).

In the absence of glucose, accumulation of intracellular vanadium was very low. After 30 min, it did not exceed 0.05 mmol per kg yeast (wet weight). Intra-

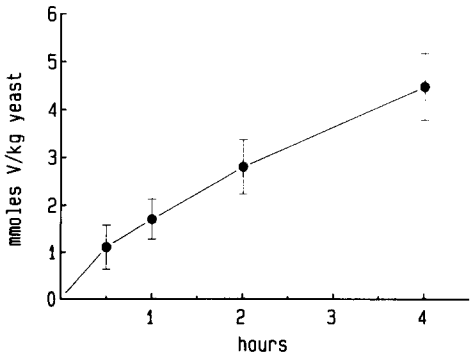

Fig. 5. Accumulation of vanadium by yeast cells during incubation with vanadyl at pH 4.0. Cells were incubated with 1 mM vanadyl sulfate and glucose at pH 4 (as described in Materials and Methods). Cell samples were washed four times with 50 mM EDTA (pH 7.0) and vanadium content was determined by AAS. Symbols represent mean of four experiments, bars denote standard deviation.

cellular vanadium after 1 h or longer incubation without glucose could not be determined, because even after eight washings with EDTA no plateau could be found.

This may have been due to undetected oxidation of vanadyl and formation of vanadate polyanions, which then were tightly bound to the yeast cell surface. A slight yellow-greenish colour of the respective cells indeed indicated presence of the decavanadate anion [1].

The obtained data sufficiently demonstrate that the vanadium accumulation described here is an energy-dependent process. It could be inhibited by addition of Ca^{2+}, Mn^{2+} or La^{3+} (added as chloride salts) to the incubation medium (not shown). Lanthanum, displaying the strongest inhibitory action, reduced vanadium accumulation to 35% of the control (1 mM of lanthanum chloride added 1 min before addition of 1 mM vanadyl sulfate, incubation with vanadyl as described above, 30 min). These metal cations are competitive inhibitors of divalent cation uptake by yeast, lanthanum not entering the yeast cells [8,9]. So apparently crossing of the plasma membrane by the divalent cation vanadyl is involved in vanadium accumulation during incubation with vanadyl.

Growth of yeast cells after incubation with vanadyl

Yeast cells, which had accumulated vanadium for 2 h as described, could continue growth in fresh medium nearly as well as control cells (not shown).

EPR measurements of yeast cells after incubation with vanadyl sulfate

The recordings of washed yeast cells after 4-h incubation with vanadyl sulfate, at pH 4 and in the presence of glucose, showed the typical eight lines of an isotropic vanadyl spectrum [1].

One representative spectrum is shown in Fig. 6b, in comparison with the isotropic spectrum of a vanadyl aquo complex (Fig. 6a). At least the second line of the yeast spectrum is divided into two peaks and other lines show broad peaks, so that probably two very similar complexes were present. The isotropic nature of this spectrum suggests that the intracellular vanadyl was bound to substances of low molecular weight [1]. An isotropic hyperfine coupling constant in the order of 102–103 gauss was deduced from the spectrum by measuring average horizontal peak-to-peak distance. Coupling constants of this order characteristically will be displayed, when the vanadyl ion is coordinated to oxygen donor atoms of carboxylic groups, as in vanadyl complexes with organic acids [12]. For example, the same hyperfine splitting as observed here has been obtained with malonate or oxalate as ligands, but also with glutathione at pH 5 [13].

During incubation of yeast with vanadyl sulfate and glucose the intensity of the vanadyl signal of washed cells increased continuously, whereas washed cells did not show any signal when glucose had been omitted.

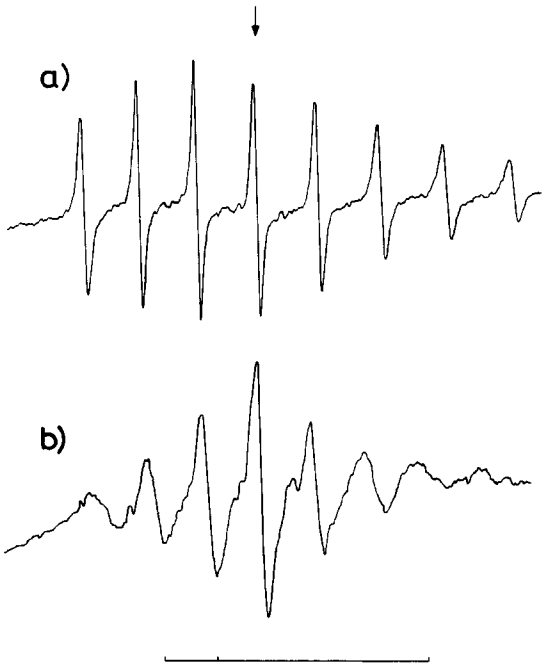

Fig. 6. EPR spectrum of yeast cells after incubation with vanadyl at pH 4. (a) Spectrum of a vanadyl aquo complex in comparison. Concentration of vanadyl 1 mM. (b) Spectrum of yeast cells after 4 h incubation with 1 mM vanadyl sulfate and glucose and washing of the cells four times with lanthanum chloride/Mes (pH 4) (as described in Materials and Methods). The sample contained 51% cells. Recording conditions: Microwave frequency 9.51 Ghz, microwave power 50 mW, modulation amplitude 5 gauss, time constant 0.25 s, scan time 4 min for 1000 gauss, receiver gain 2000. The bar in total represents a span of 500 gauss, its first segment covering 100 G. The arrow is positioned at 3300 G.

Exact determination of the intracellular vanadyl content could not be performed, because the spectrometer used was not equipped for this purpose. However, comparison of the cell-associated signal with signals of standard solutions gave similar values as determinations of total vanadium content by AAS.

EPR measurements of cell-associated vanadyl after incubation with vanadate

A cell-associated vanadyl signal appeared also, when yeast cells had been incubated with vanadate, at pH 6. However, when the cells were washed after incubation, intracellular vanadyl was found only after prolonged incubation. Cell-associated vanadyl already was present after 10 min incubation (Fig. 7a), but it could be removed from cell samples taken after 10 or 30 min by washing (Fig. 7b). This would be in accordance with rapid extracellular reduction of vanadate to vanadyl, followed by slower uptake of vanadyl into the cells.

When the incubation was performed at pH 7.5 instead of pH 6.0, even after 3 h no cell-associated vanadyl could be detected.

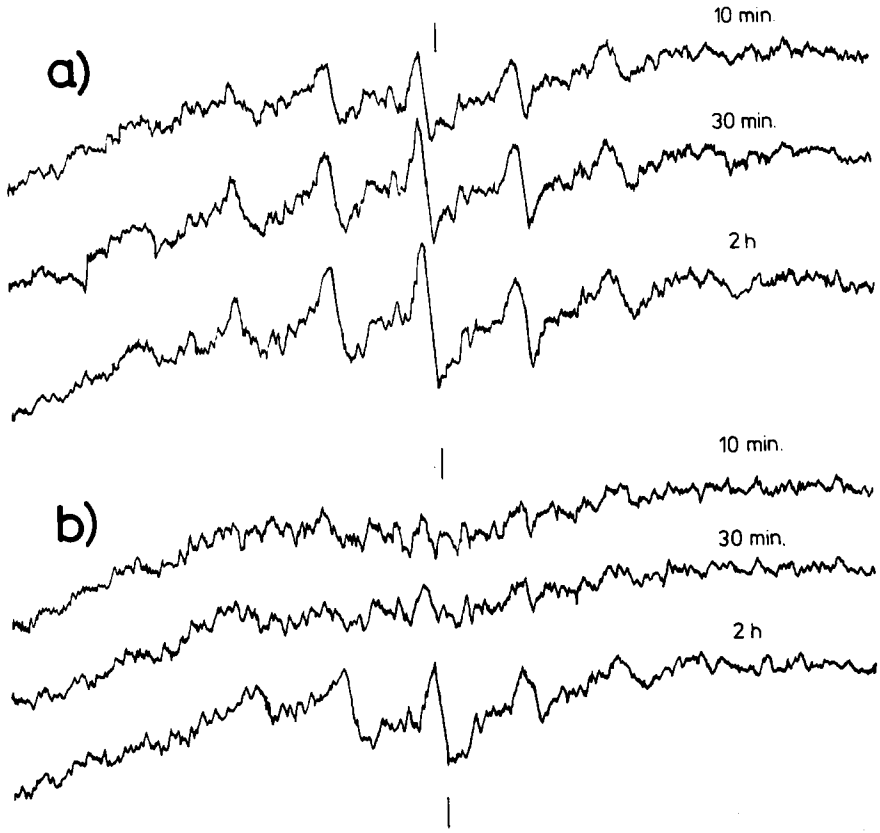

Fig. 7. EPR spectra of cell-associated vanadyl after incubation of yeast cells with vanadate at pH 6.0. 50 mM Mes buffer (pH 6.0), 2% yeast cells, 250 mM glucose, 25 °C, 1 mM vanadate. (a) Cell samples not washed, but suspended in 50 mM phosphate buffer (pH 2.5). Samples were taken after 10-min incubation for the top spectrum, after 30 min for the intermediate and after 2 h for the bottom one. (b) Cell samples washed four times with 50 mM phosphate buffer (pH 2.5) and suspended in this buffer for recordings. Same times of sampling as in (a). Conditions of recordings as described in Fig. 7, except that receiver gain was 4000. The vertical bars are positioned at 3300 gauss.

Determination of total cell-associated vanadium after incubation with vanadate

Results of AAS measurements of vanadate-treated yeast cells corresponded to the results obtained by EPR.

Intracellular vanadium was found after prolonged incubation with vanadate at pH 6. No intracellular vanadium could be detected after prolonged exposure of cells to vanadate at pH 7.5.

For further examination of vanadium accumulation after 2 h incubation with vanadate at pH 6, lanthanum as an inhibitor of vanadyl uptake and phosphate as a putative inhibitor of vanadate entry into cells were used. Both substances inhibited vanadium accumulation. Lanthanum was effective at rather low concentrations (Table I).

The inhibitory effect of phosphate could as well have

TABLE I

Inhibition of vanadium uptake by yeast cells during incubation with vanadate by lanthanum and the anions phosphate and succinate

Cells were incubated with 1 mM vanadate and glucose for 2 h (as described in Materials and Methods) in the presence of the indicated substances and washed with EDTA afterwards. Vanadium content was then determined by AAS. Results are given as percent of vanadium uptake of control cells, which had been incubated in the same way, except that no inhibitors were present.

Lanthanum chloride					
concn. added (mM)	0.01	0.05	0.1	0.2	0.5
Vanadium uptake (% of control)	121	61	55	31	12
Phosphate					
concn. added (mM)	1	10	40		
Vanadium uptake (% of control)	48	22	9		
Succinate					
conc. added (mM)	1	10	40		
Vanadium uptake (% of control)	111	109	87		

been due to its ability to bind vanadyl ions [14], thereby inhibiting their entry into the cells.

In addition, the effect of succinate was examined, which may complex metals through its carboxylic groups. However, the effect of succinate on vanadium accumulation was negligible (Table I).

Discussion

The results presented here show that yeast cells under certain conditions are able to accumulate millimolar concentrations of vanadium without significant loss of viability. If yeast cells are incubated in the presence of vanadyl, hydrolysis and oxidation being prevented by low pH and reduction of oxygen concentration, vanadyl ions can cross the yeast plasma membrane. They exist within the cells as low molecular weight complexes. This is an energy-dependent process.

No definite conclusions about the localization of the intracellular vanadyl complexes can be drawn yet, but, in parallel with the fate of several other divalent cations [8], vanadyl ions are probably taken up into the yeast cell vacuole. The vacuole serves as a storage organelle that protects the cells from substances with potentially harmful effect on cytoplasmic functions. This would explain the low toxicity of such large concentrations of intracellular vanadium.

The vanadyl complexes described here have the same isotropic hyperfine coupling constant as one of two complexes which appear after addition of vanadate to rat adipocytes [13]. It was suggested that this latter complex could have been formed by coordination of vanadyl to glutathione, because a similar spectrum had been obtained in vitro by adding vanadate to a glutathione solution of pH 5. However, the authors of the respective article conceded that their spectroscopic data did not allow definite identification of this adipocyte-associated low molecular weight vanadyl complex. It will be a matter of future research to identify the ligands of vanadyl ions in such complexes as well as their association with intracellular compartments and potential role in the cellular metabolism of metals.

Some evidence reported here suggests that upon addition of vanadate to yeast cells they can reduce vanadate to vanadyl extracellularly, followed by uptake of vanadyl into the cells.

During incubation with vanadate the cell-associated vanadyl appeared rapidly. However, initially it could be completely removed by washing the cells with EDTA, suggesting binding of vanadyl to the cell surface. The subsequent uptake into the cells was strongly inhibited by the non-permeant cation lanthanum. So apparently vanadyl ions had crossed the plasma membrane after the reduction of vanadate to vanadyl.

Similar mechanisms involving extracellular reduction of metals have been described for iron uptake by yeast [15] and are discussed also in relation to iron acquisition in both higher plants and mammalian cells [16,17]. A plasma membrane oxidoreductase seems to take part in such processes. Further studies will have to examine the relation of vanadate reduction observed here to such oxidoreductase activities.

The fact that not only lanthanum but also phosphate inhibited vanadium accumulation after addition of vanadate seems to imply direct permeation of the vanadate anion into the cells, in addition to entry of the cation vanadyl. But other mechanisms also are conceivable to explain the inhibitory action of phosphate. As vanadyl readily binds to phosphate [14], formation of such complexes could inhibit permeation of vanadyl across the plasma membrane. This would justify the assumption that vanadium accumulation during incubation with vanadate was entirely or partly due to permeation of vanadyl into the cells. However, this question could not be answered definitively. It probably will not be solved easily until specific inhibitors of vanadate and vanadyl uptake are available.

As another study about vanadium metabolism by yeast has already been published, the conclusions presented by the respective authors have to be discussed at this point. Willsky and co-workers [18] argued that yeast cells take up vanadium by the same mechanism as erythrocytes [19] and several other types of eukaryotic cells, by permeation of vanadate through an anion transport protein, followed by intracellular reduction to vanadyl. These authors additionally used nuclear magnetic resonance (NMR) measurements to detect and distinguish the various mono- and oligomeric vanadate species. By this method some cell-associated vanadate was found after incubation of yeast cells with vanadate, being mostly di- and decameric vanadate.

However, as those cells had not been washed at all after incubation, the cell-associated vanadium compounds were not necessarily located within the cell, but may have been bound to the cell surface or remained in the medium entrapped in the sample.

Formation of oligomeric vanadate species will occur in the cell wall space rather than intracellularly, because it is supported by an acidic environment [1]. Yeast cells possibly can acidify the cell wall space even in buffered medium, due to the activity of their plasma membrane proton pump. Moreover, Willsky et al. found the inhibitory action of vanadate on yeast cell growth to be small under their conditions. 5 mM vanadate was necessary to inhibit growth, which was soon resumed after re-suspension of the cells in vanadium-free medium.

It has been shown for the yeast *Neurospora crassa* that as little as 10 μM vanadate may inhibit yeast growth significantly, provided that vanadate is taken up into the cells [20].

The results of Willsky and co-workers therefore rather

suggest accumulation of oligomeric vanadate species at the cell surface.

No sufficient experimental support for the assumption that vanadate directly is taken up into the cells is being provided by these authors.

In *Neurospora crassa* vanadate is toxic only when a high-affinity phosphate transport system has been induced by phosphate starvation prior to addition of vanadate [21]. Vanadate-resistant *Neurospora* mutants were deficient in this transport system.

Willsky et al. isolated several mutants of *Saccharomyces cerevisiae* that were even more resistant to mM concentrations of vanadate than the wild type [22]. However, these mutants neither showed altered phosphate transport nor profoundly different accumulation of mono- or oligomeric vanadate compounds, as measured by NMR. Again, this does not suggest permeation of vanadate into *Saccharomyces cerevisiae* cells.

In conclusion, our study has shown that yeast cells can be loaded selectively with vanadyl, the predominant intracellular vanadium species in living organisms [23].

Considering the suitability of yeast as a model for eukaryotic biology, experiments with yeast cells may be useful for the exploration of intracellular transport and effects of vanadyl ions in eukaryotic cells.

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