Uptake of Selenite by Saccharomyces cerevisiae Involves the High and Low Affinity Orthophosphate Transporters

Myriam Lazard, Sylvain Blanquet, Paola Fisicaro, Guillaume Labarraque, and Pierre Plateau

From the Laboratoire de Biochimie and CNRS UMR7654, Laboratoire de Biochimie, Ecole Polytechnique, F-91128 Palaiseau Cedex, France and the Department of Biomedical and Inorganic Chemistry, Laboratoire National de Métrologie et d’Essais, 75015 Paris, France

Although the general cytotoxicity of selenite is well established, the mechanism by which this compound crosses cellular membranes is still unknown. Here, we show that in Saccharomyces cerevisiae, the transport system used opportunistically by selenite depends on the phosphate concentration in the growth medium. Both the high and low affinity phosphate transporters are involved in selenite uptake. When cells are grown at low Pi concentrations, the high affinity phosphate transporter Pho84p is the major contributor to selenite uptake. When phosphate is abundant, selenite is internalized through the low affinity Pi transporters (Pho87p, Pho90p, and Pho91p). Accordingly, inactivation of the high affinity phosphate transporter Pho84p results in increased resistance to selenite and reduced uptake in low Pi medium, whereas deletion of SPL2, a negative regulator of low affinity phosphate uptake, results in exacerbated sensitivity to selenite. Measurements of the kinetic parameters for selenite and phosphate uptake demonstrate that there is a competition between phosphate and selenite ions for both Pi transport systems. In addition, our results indicate that Pho84p is very selective for phosphate as compared with selenite, whereas the low affinity transporters discriminate less efficiently between the two ions. The properties of phosphate and selenite transport enable us to propose an explanation to the paradoxical increase of selenite toxicity when phosphate concentration in the growth medium is raised above 1 mM.

Although toxic at high concentrations, selenium is required in many cells, because it is translationally incorporated as selenocysteine into selenoproteins that perform specific and essential functions (1). Cells must ensure selenium uptake to sustain this metabolism. However, little is known about selenium transport. Because selenium and sulfur are chalcogen elements that have many chemical properties in common, selenium shares metabolic pathways with sulfur. Accordingly, selenium was shown to be taken up by the yeast Saccharomyces cerevisiae sulfate permeases (2). Similarly, in plants, selenate is taken up by roots via the high affinity sulfate transporters (3).

The high affinity phosphate transporter has been evidenced (8). In S. cerevisiae, which does not possess selenoproteins, an energy-dependent uptake of selenite, distinct from that of selenate, was reported. Characterization of the kinetics of selenite uptake suggested the existence of two transport systems: a high affinity system at low selenite concentration and a low affinity system at higher concentration (6). Recently, a study of selenite uptake by wheat (Triticum aestivum) roots showed it to be an active process competitively inhibited by phosphate, suggesting a role for the plant phosphate transporters in selenite uptake (7). Interestingly, in S. cerevisiae, a correlation between resistance to the toxicity of selenite and the expression of a high affinity phosphate transporter has been evidenced (8).

In this study, we asked whether the selenite and phosphate oxyanions, which have similar sizes and charges at pH 6, share the same pathways to enter S. cerevisiae cells. Phosphate is an essential nutrient required for numerous biological processes such as biosynthesis of nucleic acids and phospholipids. In S. cerevisiae, the inorganic phosphate (Pi) acquisition system is composed of five transporters (9). Three of them (Pho87p, Pho90p, and Pho91p) are constitutively transcribed and take up phosphate with low affinity (10). The high affinity transport system, composed of Pho84p and Pho89p, is transcriptionally up-regulated by the phosphate signal transduction (PHO)2 pathway in response to Pi starvation (11–13). This well-characterized regulatory pathway requires the transcription factor Pho4p, whose nuclear or cytoplasmic localization depends on the cyclin/cyclin-dependent kinase complex Pho80p-Pho85p and the cyclin-dependent kinase inhibitor Pho81p. When phosphate is limiting, the cyclin-dependent kinase inhibitor Pho81p inactivates the Pho80p-Pho85p complex, leading to accumulation of unphosphorylated Pho4p in the nucleus and subsequent activation of phosphate-responsive genes (14–17). When phosphate is abundant, Pho4p is phosphorylated by the Pho80p-Pho85p complex and exported to the cytoplasm by the receptor Msn5p, where it becomes unable to activate transcription. One of the genes up-regulated by the PHO pathway is SPL2, a negative regulator of the low affinity phosphate transporters (18). Inhibition of the low affinity phosphate transport is likely to occur through a physical interaction with Spl2 because both Pho87p and Pho90p have an SPX (SYG1, pho81, XPR1) domain that has been shown to bind the regulatory protein.

1 To whom correspondence should be addressed. Tel.: 33-1-69-33-49-00; Fax: 33-1-69-33-49-09; E-mail: myriam.lazard@polytechnique.edu.

2 The abbreviations used are: PHO, phosphate signal transduction; SD, synthetic dextrose; MES, 4-morpholineethanesulfonic acid; ICP/MS, inductively coupled plasma mass spectrometry.
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Spl2p (19). Thus, although the low affinity phosphate transporters are not transcriptionally regulated in response to external phosphate availability, their transport activity is inhibited post-transcriptionally following phosphate starvation. Overall, the above regulatory mechanism results in cells that use either the high affinity or the low affinity transport systems, depending on phosphate availability.

In this study, we show that selenite toxicity is dependent on phosphate concentrations in the growth medium. Selenite uptake measurements allow us to establish that both the high affinity phosphate transporter Pho84p as well as the low affinity carriers mediate selenite uptake in S. cerevisiae and that phosphate and selenite compete for uptake by both these systems. Primacy of one transport system on the other depends on the phosphate concentration conditions used to grow the cells.

EXPERIMENTAL PROCEDURES

Strains and Media—The S. cerevisiae strains used in this study are derived from strain BY4742 (MATa his3Δ1 leu2Δ lys2Δ0 ura3Δ0). The parental and all the single mutants were purchased from Euroscarf. The double and triple mutant strains were constructed by replacing the entire reading frame of the reference gene with a PCR-generated marker cassette containing either the URA3kl or the LEU2 genes (20). All of the disruptions were verified by PCR analysis. The constructed strains are as follows: Δpho87-Δpho90 (BY4742 pho87::KanMX4; pho90:: URA3kl), Δpho87-Δpho91 (BY4742 pho87::KanMX4; pho91:: URA3kl), Δpho90-Δpho91 (BY4742 pho90::KanMX4; pho91:: URA3kl), Δpho84-Δspl2 (BY4742 pho84::KanMX4; spl2:: URA3kl), and Δpho87-Δpho90-Δpho91 (BY4742 pho87:: KanMX4; pho90::URA3kl; pho91::LEU2). Plasmid pRS426 and its derivatives expressing PHO84 (pPHO84) or PHO87 (pPHO87) from the ADH1 promoter are described in Ref. 9.

YPD medium contained 1% yeast extract (Difco), 1% Bactotryptone (Difco), and 2% glucose. Standard synthetic dextrose (SD) minimal medium contained 0.67% yeast nitrogen base (Difco), 2% glucose, and 50 mg/liter of histidine, leucine, lysine, and uracil and was buffered at pH 6.0 by the addition of 50 mM potassium phosphate. This medium contained 7.3 mM phosphate. Phosphate-depleted SD medium was prepared as described (21). Then 2% glucose, 50 mg/liter of histidine, leucine, lysine, and uracil and was buffered at pH 6.0 by the addition of 50 mM MES-NaOH. This medium contained 7.3 mM phosphate. Phosphate-depleted SD medium was prepared as described (21). Then 2% glucose, 50 mg/liter of histidine, leucine, lysine, and uracil and was buffered at pH 6.0 by the addition of 50 mM MES-NaOH. This medium contained 7.3 mM phosphate. Phosphate-depleted SD medium was prepared as described (21). Then 2% glucose, 50 mg/liter of histidine, leucine, lysine, and uracil and was buffered at pH 6.0 by the addition of 50 mM MES-NaOH. This medium contained 7.3 mM phosphate. The cells were pregrown in phosphate-defined medium at an OD650 of 0.12 and left to grow at 30 °C for 1 h. Then 5 mM Na2SeO3 (Sigma) was added to half of the cultures, and growth was monitored by following the OD650 as a function of time. For viability assays, the cells were inoculated in phosphate-defined medium to obtain an OD650 of 0.25 and left to grow at 30 °C. When the OD650 reached 1.5, the cells were harvested by centrifugation and washed with SD medium without phosphate. The cells were resuspended in SD medium without phosphate at a density of 1 OD650/ml and incubated at 30 °C for 5 min prior to the addition of [32P]Pi, (PerkinElmer Life Science). Uptake measurements were initiated by the addition of 0.1 ml of [32P]Pi, (final concentrations from 5 to 7500 μM; specific activity between 104 and 106 dpm nmol−1) to 0.9 ml of cell suspension. The assays were terminated at 3 and 6 min by the addition of 1 ml of ice-cold 0.5 mM phosphate, pH 6.0. Cell suspensions were then filtered using 0.45-μm nitrocellulose filters (Schleicher & Schuell) and washed twice with 3 ml of 0.25 mM phosphate, pH 6.0. The radioactivity retained on the filter was measured by liquid scintillation counting. The rates of transport are given in pmol/min and per OD650. The Km and Vmax values were derived from iterative nonlinear fits of the theoretical Michaelis equation to the experimental values, using the Levenberg-Marquardt algorithm as described previously (22). When experimental values could not be fitted to a single hyperbola, a partition method was employed, as recommended (23). Km,1 and Vmax,1 values were derived from the experimental data obtained for phosphate concentrations ranging from 5 to 50 μM. The theoretical contribution of the first uptake system was subtracted from the subsequent experimental values (100–5000 μM). The resulting data were used to determine Km,2 and Vmax,2 values for the second uptake system.

Selenite Uptake—The strains were pregrown overnight as indicated above. The cells were inoculated in phosphate-defined medium to obtain an OD650 of 0.4 and left to grow at 30 °C. When the OD650 reached 1.5, the cells were harvested by centrifugation and washed with SD medium without phosphate. The cells were resuspended in SD medium without phosphate at a density of 1 OD650/ml and incubated at 30 °C for 5 min prior to the addition of Na2SeO3. For determination of the kinetic parameters for selenite uptake, selenite concentrations were in the range 1–20 mM. At time intervals, 10 ml of cell suspension were removed from the incubation mixture, and the reaction was stopped by the addition of 1 ml of ice-cold 1 mM phosphate, pH 6.0. The samples were centrifuged (5000 × g for 5 min), washed twice with 10 ml of water, and lyophilized.

Selenium content of the cells was determined by inductively coupled plasma mass spectrometry (ICP/MS) on a Thermo Fisher PQ Excell quadrupole spectrometer in the collision cell mode. The mass of each yeast sample was estimated by the mass of each yeast sample was estimated by the mass of each yeast sample was estimated by the mass of each yeast sample was estimated by the mass of each yeast sample was estimated by
the difference of weight between the filled and empty microtubes (microbalance Sartorius ME 36S, range 30 g/1 μg). The yeast samples were digested with 3 ml of HNO₃ (67–69%; Plasmapur SGS) and 1 ml of H₂O₂ (30%; Suprapur Merck) in a closed vessel microwave oven (Ethos 900; Thermo Fisher). The residues were then diluted with 10 ml of pure water. The ICP/MS mass spectrometer was calibrated (range, 0–200 μg/liter) against a reagent blank solution, and several selenium standard solutions were obtained after dilutions from a concentrated certified standard (selenium, 999 ± 5 mg/liter; Certipur Merck). The data were recorded for the four selenium isotopes ⁷⁶Se, ⁷⁸Se, ⁸⁰Se, and ⁸²Se. Selenium concentrations calculated from each isotope were analogous. The results are the means of data obtained on each isotope and are expressed in μg of selenium g⁻¹ (dry weight). Conversion in pmol-OD₆₅₀⁻¹ was made using a mean atomic mass of 79 and by considering that 1 g of yeast (dry weight) corresponds to 6200 OD₆₅₀.

RESULTS

Effect of Phosphate on Selenite Toxicity—The toxicity of selenite in yeast is well documented, although the mechanisms of toxicity are less well understood (24, 25). Selenite uptake is the first step in selenium metabolism that ultimately leads to toxicity. To investigate the possibility that selenite enters yeast cells via the orthophosphate transport system, the influence of the concentration of phosphate in the growth medium on the toxicity of sodium selenite was analyzed. We compared the effect of the addition of 5 mM of sodium selenite on the growth of yeast cells (strain BY4742) cultivated in the presence of increasing concentrations of potassium phosphate (SD with 0.1, 0.2, 0.4, or 0.8 mM Pᵢ or standard high Pᵢ SD (7.3 mM Pᵢ)) (Fig. 1A). In the absence of selenite, whatever the concentration of phosphate, growth rates were identical (t₁/₂ = 135 min at 30 °C). We observed, however, that in the medium supplemented with 0.1 mM phosphate, upon reaching the late exponential phase, phosphate depletion became limiting for cell growth. In the presence of 5 mM selenite, growth rates and plateau values were clearly dependent on phosphate concentrations. Up to 0.8 mM Pᵢ, the cells were much more affected by selenite toxicity in the medium containing the lowest phosphate concentration. Surprisingly, at high phosphate concentration, selenite toxicity increased.

To determine whether this inhibition of growth was due to increased selenite lethality, cell viability was assayed after a short term exposure to selenite. The cells, grown exponentially in medium containing various concentrations of phosphate, were incubated for 1 h in the same medium with 5 or 10 mM selenite, diluted, and plated on rich medium plates (high phosphate). The survival rates were determined by counting colonies after 2 days growth at 30 °C (Fig. 1B). In a medium containing 0.1 mM Pᵢ, exposure to 5 mM selenite reduced cell survival by nearly 90%. In contrast, at 0.4 mM Pᵢ, more than 80% of the cells were resistant to 5 mM selenite. When the phosphate concentration was raised further, viability of the cells decreased to reach ~50% in high phosphate medium. These results show that selenite not only inhibits the growth of yeast cells but also induces mortality.

In a previous paper, we showed that extracellular reduction of selenite into hydrogen selenide (HSe⁻) led to increased cellular accumulation and toxicity of selenium (26). To determine whether sodium selenide toxicity was dependent on phosphate concentration, we measured cell survival after a 5-min exposure to 40 μM Na₂Se in medium containing increasing concentrations of Pᵢ. This resulted in ~50% mortality of the cells, independently of phosphate concentration, suggesting that these two forms of inorganic selenium (selenite and selenide) are internalized by different pathways, as suggested previously (27). Because our sample of sodium selenite might contain traces of selenite, sodium selenate toxicity was also assayed. Exposure to 5 mM selenate resulted in low mortality (<20%), irrespective of phosphate concentration. Thus, the toxicity of selenite cannot originate from trace amounts of selenite contaminating the selenite solution.
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Selenite Toxicity in Mutants of the PHO Pathway—In a first set of experiments, the toxicity of selenite was analyzed in \( \text{P}_i \)-limited medium. Viability assays were performed in strains disrupted for one of the phosphate transporter genes (\( \text{PHO84}, \text{PHO87}, \text{PHO89}, \text{PHO90}, \) and \( \text{PHO91} \)). The cells were pregrown with increasing \( \text{P}_i \) concentrations, exposed to 5 mM selenite, plated, and scored to assess cell survival (Fig. 2A). Whatever the concentration of phosphate in the range 0.1–0.8 mM, each of the single mutants in the low affinity carriers (\( \text{\Delta pho87}, \text{\Delta pho90}, \) and \( \text{\Delta pho91} \)) displayed curves similar to that of the parental strain, with high selenite toxicity at low \( \text{P}_i \) concentrations. However, a triple mutant \( \text{\Delta pho87-\Delta pho90-\Delta pho91} \) was slightly more sensitive to selenite than the wild-type strain. At 0.1 mM \( \text{P}_i \), survival was 1.4% for the triple mutant as compared with 15% for wild type. In contrast, the \( \text{\Delta pho84} \) strain was very resistant to selenite toxicity at low \( \text{P}_i \) concentrations. The \( \text{\Delta pho4} \) strain, in which \( \text{PHO84} \) is not expressed, was also very resistant to selenite toxicity at low \( \text{P}_i \) concentrations. As shown in Fig. 2B, expression, in the \( \text{\Delta pho84} \) strain, of \( \text{PHO84} \) from the constitutive \( \text{ADH1} \) promoter restored selenite toxicity values close to those observed in the wild type. These results suggest that \( \text{Pho84p} \) plays a major role in the toxicity of selenite, at least up to 0.4–0.5 mM phosphate.

In the case of a \( \text{\Delta pho91} \) strain, the survival curve was similar to that of the wild type. This indicates that, under our experimental conditions, this transporter is not involved in selenite toxicity. However, both the transcription and the activity of \( \text{Pho89p} \) are strongly pH-dependent, with an optimum pH of >8.0 (13). Although no effect of the inactivation of its gene was observed, a potential role for \( \text{Pho89p} \) in selenite toxicity cannot be excluded but must be studied in different conditions. For this reason, this high affinity transporter was not considered in the remainder of our study.

![FIGURE 2. Selenite toxicity in mutants of the phosphate transport pathway.](image)

A, various strains, as indicated in the figure, were grown at 30 °C in SD medium supplemented with the indicated phosphate concentration. When the OD\(_{650}\) reached 0.1, 5 mM Na\(_2\)SeO\(_3\) was added to the cultures. After 1 h of incubation at 30 °C, the samples were diluted and plated onto YPD-agar. Cell viability was determined after 2 days growth at 30 °C. The results are expressed as percentages of survival compared with control samples incubated in the absence of selenite. The values are the means of at least three independent experiments. Standard deviations between these experiments were lower than 15%. B, cells expressing either the control plasmid pRS426 or pPHO84 expressing \( \text{PHO84} \) from the \( \text{ADH1} \) promoter were grown and treated as in A. The error bars represent the means and ranges of two independent experiments.
reduced selenite uptake. These results show that, in Pi-limited conditions, transport of selenite is ensured mostly by Pho84p. In addition, the kinetics of selenite uptake were measured in the presence of 0.5 mM Pi in the uptake medium (Fig. 3 and Table 1). Whatever the selenite concentration, accumulation of selenium was reduced at least 10 times, showing that selenite uptake by Pho84p is inhibited by phosphate.

Inhibition of Phosphate Uptake by Selenite in Cells Grown in Low Pi Conditions—Because the addition of phosphate in the assay reduced selenium accumulation, phosphate uptake was expected to be inhibited by selenite. Therefore, \[^{32}P\]Pi transport kinetic parameters were measured in the presence of increasing concentrations of selenite (Table 2).

FIGURE 3. Uptake of selenium by BY4742 cells grown in SD medium supplemented with 0.3 mM Pi. A, cells were harvested by centrifugation, washed, and resuspended in SD medium without phosphate. Uptake of 1 mM ( □ ), 2.5 mM ( ▲ ), 5 mM ( ▼ ), and 10 mM ( ◇ ) Na\(_2\)SeO\(_3\) was measured as described under “Experimental Procedures,” in the absence (filled symbols) or the presence (open symbols) of 0.5 mM potassium phosphate. The samples were analyzed for their total selenium content. B, rates of selenite uptake in the absence of phosphate ( ▲ ), determined from A, were fitted to the Michaelis-Menten equation as described under “Experimental Procedures.” In the presence of 0.5 mM phosphate ( ▼ ), the rate of selenite uptake increased roughly linearly with the selenite concentration.

First, we analyzed phosphate uptake in conditions identical to those previously used to measure selenite uptake (strain BY4742, 0.3 mM Pi). The rate of phosphate accumulation at concentrations ranging from 5 to 500 \(\mu\)M could be fitted to a single Michaelis-Menten equation, giving a \(V_{\text{max}}\) of 860 pmol of Pi incorporated per min/OD\(_{650}\) and a \(K_m\) of 20 \(\mu\)M. The \(K_m\) for Pi uptake is in the micromolar range, suggesting that only high affinity phosphate transport is operative at this phosphate concentration. To support this idea, we compared the rate of phosphate uptake in the wild-type strain and the \(\Delta\)pho84 mutant grown in 0.3 mM Pi, using a phosphate concentration of 0.3 mM in the assay. Rates of 840 and 43 pmol of Pi/OD\(_{650}\) min\(^{-1}\) were determined, respectively. These results indicate that in the growth conditions used above, Pho84p is the major contributor to phosphate uptake. Kinetic studies were also performed in the presence of 1, 2.5, 5, and 10 mM selenite. The results show that selenite competitively inhibits phosphate uptake by BY4742 cells with a \(K_i\) of 4.6 mM (Table 2). It is noteworthy that this value is close to the \(K_m\) determined for selenite uptake. This is in agreement with the conclusion that the same transporter, Pho84p, is responsible for the major part of the uptake of both phosphate and selenite. In a previous paper (8), the authors found that selenite did not compete with phosphate transport. However, they did not go beyond a 10-fold molar excess of selenite, which may not have been enough to observe an effect, because of the large difference of \(K_m\) for phosphate or selenite transport.

We also measured the \(K_i\) of selenite toward phosphate uptake in the \(\Delta\)pho87-\(\Delta\)pho90-\(\Delta\)pho91 strain. Experimental conditions were identical to those used above with the wild-type strain. As expected, the \(K_i\) for phosphate (25 \(\mu\)M) and the \(K_i\) for selenite (5.5 mM) were similar to those determined with the BY4742 strain, which confirms that the selenite transport kinetic values determined above correspond to Pho84p-mediated uptake.

Selenite Toxicity in High Phosphate Medium—Transport of selenite by Pho84p cannot explain the increase in selenite toxicity observed with cells grown in high Pi conditions, because Pho84p is not significantly expressed in these conditions (11,
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In agreement with this idea, when assayed for cell survival after exposure to 5 or 10 mM selenite, the single mutants in the high affinity transporter genes (PHO4 and PHO89), as well as the \( \Delta pho4 \) and \( \Delta spl2 \) strains, grown in high phosphate medium, displayed selenite survival values comparable with those of the wild-type strain (Fig. 4). In contrast, single mutant strains in the low affinity Pi transporters, as well as the double and triple mutants, were more resistant to selenite. An increased resistance of the \( \Delta pho87 \), \( \Delta pho90 \), and \( \Delta pho91 \) mutants has been previously reported by Pinson et al. (8). Several studies have shown that mutations in the low affinity phosphate transporters resulted in derepression of the PHO-regulated genes (8, 10, 28), leading to increased transcription of PHO4 and to inactivation of the low affinity transporters through derepression of SPL2. Thus, in these mutants, Pho84p is responsible for most of the phosphate uptake, whatever the phosphate conditions.

To confirm the induction of the PHO pathway upon the deletion of a single low affinity transporter gene, we compared whatever the phosphate conditions.

### TABLE 1

Rates of selenite uptake and inhibition by phosphate of wild-type and Pi transporter-defective strains in the presence of 5 mM selenite

| Pi concentration in the growth medium | Strain          | V max (pmol of SeO\(_3^2\)−/min/OD\(_{650}\)−1) |
|--------------------------------------|-----------------|-----------------------------------------------|
|                                      |                 | 0.0 mM Pi\(\text{a}\) | 0.5 mM Pi\(\text{a}\) |
| 0.3 mM                               | BY4742          | 76 ± 10                                      | 6.4 ± 1                      |
| 7.3 mM                               | BY4742          | 27 ± 4                                       | 24 ± 4                      |
| 0.3 mM                               | \(\Delta pho84\) | 7 ± 2                                        | ND                          |
| 7.3 mM                               | \(\Delta pho84\) | 7 ± 2                                        | ND                          |
| 0.3 mM                               | \(\Delta pho84-\Delta spl2\) | 7 ± 2                                      | ND                          |
| 7.3 mM                               | \(\Delta pho84-\Delta spl2\) | 7 ± 2                                      | ND                          |
| 7.3 mM                               | \(\Delta pho87\) | 7 ± 2                                        | 14 ± 4                      |
| 7.3 mM                               | \(\Delta pho87\) (PHO487) | 7 ± 2                                      | 14 ± 4                      |
| 7.3 mM                               | \(\Delta pho85\) | 7 ± 2                                        | 11 ± 2                      |

\(\text{a}\) Concentration of phosphate in the uptake medium.

### TABLE 2

Phosphate uptake parameters and selenite inhibition constants of wild-type and Pi transporter-defective strains

| Pi concentration | Strain          | V max (pmol of Pi/min/OD\(_{650}\)) | \(K_m\) (μM) | \(K_i\) (μM) |
|------------------|-----------------|-----------------------------------|-------------|-------------|
| 0.3 mM           | BY4742          | 860 ± 80                           | 20 ± 4      | 4.6 ± 1.7   |
| 0.3 mM           | \(\Delta pho87\)-\(\Delta pho90-\Delta pho91\) | 1400 ± 300                      | 25 ± 3      | 5.5 ± 1.8   |
| 0.3 mM           | \(\Delta spl2\)  | 1600 ± 400                        | 430 ± 1600  | 9.0 ± 2.0   |
| 0.3 mM           | \(\Delta pho84\) | 1400 ± 300                        | 5000 ± 1600 | 9.5 ± 2.0   |
| 7.3 mM           | BY4742          | 1600 ± 400                        | 5000 ± 1600 | 9.5 ± 2.0   |
| 7.3 mM           | \(\Delta pho87\) | 1400 ± 300                        | 5000 ± 1600 | 9.5 ± 2.0   |
| 7.3 mM           | \(\Delta pho84\) | 1600 ± 400                        | 5000 ± 1600 | 9.5 ± 2.0   |

The uptake experiments were performed as described under “Experimental Procedures” on two independently grown cultures, apart from BY4742 grown in 0.3 mM Pi (four independent experiments), BY4742 grown in 7.3 mM Pi, and \(\Delta spl2\) grown in 0.3 mM Pi (three independent experiments). Seven different concentrations of phosphate were used to determine the kinetic parameters. The Pi concentrations were in the range 5–500 μM for high affinity uptake and 0.1–7.5 mM for low affinity uptake measurements.

To determine the phosphate uptake parameters in the \(\Delta spl2\) strain, 10 concentrations of phosphate were used (5–5000 μM). Selenite inhibition constants were determined from the measurements of the apparent \(K_m\) for phosphate in the presence of 2.5, 5, and 10 μM selenite. The \(K_i\) was deduced from the slope of the plot of \(K_m\)\(\text{app}\) as a function of selenite concentration. The errors are standard deviations.
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Then we assayed selenite toxicity in the Δspl2 strain. As shown in Fig. 5, inactivation of SPL2 resulted in increased selenite sensitivity, as compared with the wild-type cells. At 0.1 mM $P_i$, survival of the Δspl2 cells exposed to 5 mM selenite was less than 1%. These results show that deletion of SPL2 leads to the activation of the low affinity transport system with a concomitant increase in selenite toxicity.

Selenite Uptake by the Low Affinity Transport System—To demonstrate that the low affinity phosphate transporters can import selenite, we compared the initial rates of selenite uptake at a fixed selenite concentration of 5 mM in Δpho84 and Δpho84-Δspl2 cells grown in low $P_i$ medium (Table 1). Phosphate uptake was also determined in the same conditions (Table 2). In the strain that expresses Spl2p, activation of the PHO pathway led to down-regulation of the low affinity transporters. As a consequence, the $V_{max}$ of $P_i$ uptake was twice higher in the Δpho84-Δspl2 strain than in the Δpho84 strain. This higher activity of the low affinity transporters was paralleled by a 2-fold increase of selenite uptake, establishing that the low affinity transporters are able to carry selenite inside cells.

Selenite uptake by the low affinity transporters was also measured in cells grown in high $P_i$ medium. In these conditions, the wild-type, Δpho84, and Δpho84-Δspl2 strains exhibited phosphate uptake rate values very similar to that measured with the Δpho84-Δspl2 strain grown in low $P_i$ medium (Table 2). The rates of selenite uptake by the three strains grown in high $P_i$ medium were also very similar to that measured with the Δpho84-Δspl2 strain grown in low $P_i$ medium (Table 1).

Finally, we determined the kinetic parameters for selenite uptake in the wild-type strain grown in high $P_i$ conditions. A $K_m$ of 7.7 ± 3 mM and a $V_{max}$ of 40 ± 8 μg of selenium·g$^{-1}$·min$^{-1}$ (or 81 pmol of selenium·OD$^{-1}$·min$^{-1}$) were determined. Measurement of phosphate uptake in the presence of various concentrations of phosphate and selenite indicated that selenite competitively inhibited phosphate uptake, with a $K_i$ value close to 9 mM (Table 2). This $K_i$ value is comparable with the $K_m$ for selenite transport by low affinity carriers, as measured in selenite uptake experiments. This result confirms that the low affinity $P_i$ transport system is competent for selenite transport.

Phosphate Inhibition of Selenite Uptake—The experimental kinetic values determined above allow us to calculate a ratio between the $K_m$ values for selenite and phosphate of ~250 for Pho84p and ~2 for the low affinity transporters. Therefore, we anticipated that, in high $P_i$ medium, phosphate would inhibit selenite uptake much more efficiently in strains expressing Pho84p than in strains expressing the low affinity system. To verify this prediction, we compared the rates of sel-

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mutant. As expected from previous studies (18), deletion of SPL2 resulted in activation of the low affinity transporters (Table 2). The observed values of $[^{32}P]P_i$ incorporated could not be fitted with a single hyperbola but were correctly fitted with a double hyperbola curve. The results show that the high affinity system ($K_m = 19$ μM) uptakes phosphate with a $V_{max}$ of 600 pmol of P$_i$·OD$^{-1}$·min$^{-1}$. Activation of the low affinity transporters allowed us to determine a $K_m$ of 4 mM and a $V_{max}$ of 1700 pmol of P$_i$·OD$^{-1}$·min$^{-1}$ for low affinity $P_i$ transport.

FIGURE 4. Selenite toxicity in high $P_i$ medium. The cells were incubated in SD medium. When the OD$_{650}$ reached 0.1, Na$_2$SeO$_3$ was added to the cultures (0 mM, black bars; 5 mM, gray bars; 10 mM, white bars). After 1 h of incubation at 30 °C, the samples were diluted and plated onto YPD-agar. Cell viability was determined after 2 days of growth at 30 °C. The results are expressed as percentages of survival compared with control samples incubated in the absence of selenite. The error bars represent the means and ranges of two independent experiments. W.T, wild type.
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TABLE 3

| P_i transporter expressed | Strain and growth conditions | \( V_{\text{max}}/K_m \) for P_i | \( V_{\text{max}}/K_m \) for selenite | Selectivity* |
|---------------------------|------------------------------|---------------------------------|---------------------------------|-------------|
| Pho84p                    | BY4742 and 0.3 mM P_i        | 43000                           | 34                              | 1260        |
| Low affinity              | BY4742 and 7.3 mM P_i        | 370                             | 10.5                            | 35          |

* Selectivity is defined as the ratio of the \( V_{\text{max}}/K_m \) for P_i uptake and that for selenium uptake.

... enite uptake in the presence of 0.5 mM P_i in the assay of \( \Delta \text{pho}87 \) and \( \Delta \text{pho}85 \) cells to those of strains expressing low affinity transporters (wild type and \( \Delta \text{pho}87 \) (pPHO87)). The data reported in Table 1 show that, indeed, selenite uptake was severely inhibited by phosphate in Pho84p-expressing strains, whereas little inhibition was observed in strains expressing low affinity P_i transporters. These results indicate that, when extracellular P_i is abundant, selenite is taken up more efficiently by the latter transporters than by Pho84p.

**DISCUSSION**

Active selenite transport by *S. cerevisiae* has already been reported (6), but no transporter had been identified so far. In this study, we demonstrate that the high affinity phosphate transporter Pho84p, as well as the low affinity carriers, are able to uptake sodium selenite in *S. cerevisiae*. The transport system used opportunistically by selenite depends on the phosphate concentration in the medium. At low P_i concentrations (up to 0.4 mM), Pho84p is the major contributor to selenite uptake. When phosphate is abundant, the role of Pho84p becomes negligible, and selenite is internalized through one or all of the low affinity P_i transporters. Other toxic metalloids are taken up adventitiously by the existing transport system. For instance, the phosphate transport system is also known to take up arsenate in both prokaryotes and eukaryotes (30, 31). Another example is that of the sulfate transporters of yeast and plants that have low selectivity for sulfate versus analogous selenate or chromate (2, 3, 32).

Selenite uptake measurements in cells expressing either the high affinity P_i transporter Pho84p or the low affinity Pho87p, Pho90p, and Pho91p indicate that both systems are able to transport selenite inside the cells. Comparison of the \( V_{\text{max}}/K_m \) of the transporters for P_i or for selenium (Table 3) shows that Pho84p is slightly more efficient than the low affinity carriers for selenite transport. However, Pho84p has a much higher affinity for P_i than for selenium. Thus, the high affinity transporter is very selective for P_i, whereas the low affinity system is much less discriminating.

Selenite toxicity results correlate well with selenium uptake measurements, indicating that mortality of *S. cerevisiae* cells is directly dependent on the amount of internalized selenium by the phosphate transport system. In the wild-type strain grown in very low P_i conditions (0.1 mM), selenite toxicity is high. When the phosphate concentration in the medium is increased up to 0.4 mM P_i, selenite toxicity is reduced. This effect is easily accounted for by phosphate inhibition of the Pho84p-mediated selenite uptake. When phosphate concentration in the culture medium is further increased, the transport of phosphate (and of selenite) is progressively taken over by the low affinity carriers. Because these carriers are less specific, the advantage of phosphate over selenite (in term of \( V_{\text{max}}/K_m \)) is reduced, and selenite uptake/toxicity increases. This mechanism implies that, at very high phosphate concentrations, selenite resistance should improve again. This effect was, indeed, observed previously (8). In the \( \Delta \text{pho}84 \) strain grown in low P_i medium, resistance to selenite toxicity can be attributed to the concomitant inactivation of PHO84 and down-regulation of low affinity transport that affects both phosphate and selenite transport (Tables 1 and 2) and thus results in low selenite (and phosphate) uptake.

The triple mutant \( \Delta \text{pho}87\Delta \text{pho}90\Delta \text{pho}91 \) was more sensitive to selenite than the wild-type strain in P_i-limited medium. In agreement with previous studies (10), we observed that in P_i-limited medium the triple-disruptant strain exhibited a 2-fold higher \( V_{\text{max}} \) of P_i uptake than the wild-type strain (Table 2). It was shown that the higher activity in this strain was accompanied by enhanced transcription of the PHO84 gene (10). Increased activity of Pho84p, in phosphate and selenite transport, explains the higher sensitivity of this strain to selenite as compared with the wild-type cells. Another strain that is very sensitive to selenite at low P_i concentrations is the \( \Delta \text{spl}2 \) strain. In this case, loss of the regulation by Spl2p results in higher selenite uptake and toxicity.

On the contrary, in high P_i medium, each of the single, as well as the double and the triple low affinity P_i transporter mutants, were more resistant to selenite than the wild-type strain or mutants of the high affinity transport system. Increased selenite resistance of the low affinity phosphate transporter mutants has been observed previously (8). The higher resistance to selenite of such mutants was ascribed to an overexpression of Pho84p. However, the mechanism by which expression of Pho84p led to selenite resistance remained unknown. The results obtained in this study provide a good explanation for this behavior. Phosphate uptake measurements in the single low affinity P_i transporter mutant \( \Delta \text{pho}87 \) confirm that, in high P_i conditions, Pho84p is overexpressed, and the low affinity transport system is concomitantly down-regulated. Additionally, we show that inhibition by phosphate of Pho84p-mediated selenite transport is much more effective than that of the low affinity transport system. As an example, with a simple calculation using the selenite and phosphate kinetic constants determined here, we find that in the presence of 5 mM selenite, the addition of 7.3 mM P_i (as in the high P_i medium) inhibits by more than 150-fold the uptake of selenite by Pho84p. In the same conditions, the uptake of selenite by the low affinity transporters is reduced by only 50%. Therefore, reduced uptake of selenite by cells expressing Pho84p, as compared with cells...
using the low affinity \( P_\text{i} \) transporters, is responsible for the paradoxical resistance of these strains to selenite, in high \( P_\text{i} \) conditions.

Competitive inhibition of \( \text{SeO}_3^{2-} \) uptake by phosphate has long been documented in various plant species \((7, 33)\), in the green alga \textit{Chlamydomonas reinhardtii} \((34)\), and in the yeast \textit{Candida albicans} \((35)\). Thus, selenite uptake by the phosphate transport pathway could be a general mechanism, at least in plants, algae, and fungi.

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