Accumulation and intracellular compartmentation of lithium ions in \textit{Saccharomyces cerevisiae}

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Abstract: Accumulation of Li$^+$ in \textit{Saccharomyces cerevisiae} X2180-1B occurred via an apparent stoichiometric relationship of 1:1 (K$^+$/Li$^+$) when \textit{S. cerevisiae} was incubated in the presence of 5 and 10 mM LiCl for 3 h. Other cellular cations (Mg$^{2+}$, Ca$^{2+}$ and Na$^+$) did not vary on Li$^+$ accumulation, although lithium chemistry dictates a degree of similarity to Group I and II metal cations. Compartmentation of Li$^+$ was mainly in the vacuole which accounted for 85% of the Li$^+$ accumulated after a 6-h incubation period. The remainder was located in the cytosol with negligible amounts being bound to cell fragments including the cell wall. Transmission electron microscopy of Li$^+$-loaded cells revealed enlarged vacuoles compared with control cells. This asymmetric cellular distribution may therefore enhance tolerance of \textit{S. cerevisiae} to Li$^+$ and ensure that essential metabolic processes in the cytosol are not disrupted.

Key words: Lithium; Potassium; Vacuole; Intracellular compartmentation; \textit{Saccharomyces cerevisiae}

Introduction

Interactions of Li$^+$ with \textit{Saccharomyces cerevisiae} and other yeasts have been documented in the areas of transport kinetics and metabolic effects. The former has accounted for accumulation occurring via K$^+$ transport system(s) \cite{1,2}. An efflux mechanism for Li$^+$ has also been demonstrated, dependent on the proton-motive force, and occurring via H$^+$/Li$^+$ antiport \cite{3,4}. Metabolic effects of Li$^+$ include the inhibition of yeast enolase by both Li$^+$ and Na$^+$ \cite{5} and a general inhibition of protein and RNA synthesis, not affecting respiration, has also been observed \cite{6}. Internal compartmentation data have not, however, been presented to date. In this paper we describe internal compartmentation of Li$^+$ in \textit{S. cerevisiae} as well as the effect of Li$^+$ accumulation on other important cellular cationic species (K$^+$, Na$^+$, Mg$^{2+}$ and Ca$^{2+}$).

Materials and Methods

Organisms, media and cultural conditions

\textit{Saccharomyces cerevisiae} X2180-2B was maintained at 25°C on MYGP agar of composition (g l$^{-1}$): d-glucose, 10; yeast extract (Lab M), 3; malt extract (Lab M), 3; neutralized bacteriological peptone (Oxoid), 5; agar (Lab M) No. 2, 15. The liquid basal medium (AP2) had the following composition (g l$^{-1}$): d-glucose, 20.0; KH$_2$PO$_4$, 0.4; MgSO$_4$·7H$_2$O, 0.1; d-bromocresol purple, 0.005. The \textit{Saccharomyces cerevisiae} was transferred from MYGP agar to AP2 liquid medium for 24 h at 25°C before use.
2.72; K₂HPO₄·3H₂O, 5.22; (NH₄)₂SO₄, 2.0; MgSO₄·7H₂O, 0.12; FeSO₄·7H₂O, 0.0022; ZnSO₄·7H₂O, 0.004; MnSO₄·4H₂O, 0.004; CuSO₄·5H₂O, 0.0004; yeast extract (Difco), 1.0. Experimental cultures (initial OD at 550 nm approx. 0.1) were prepared from 48 h old starter cultures grown from loop inoculation from MYGP agar. All cultures were incubated at 25°C on a rotary shaker (100 rev. min⁻¹). Cell numbers were determined using a modified Fuchs-Rosenthal haemocytometer after appropriate dilution with distilled water.

**Preparation of cells for lithium accumulation**

AP2-grown cells in the mid-exponential phase were centrifuged (1200 × g, 10 min), washed twice with and finally suspended in 5 mM piperazine-N,N'-bis[2-ethanesulphonic acid] (PIPES) buffer, adjusted to pH 6.5 using solid tetramethylammonium hydroxide pentahydrate. Loading of yeast cells with lithium was carried out using methods modified from Borst-Pauwels [2] and Conway and Gaffney [7]. Equilibration of cell suspensions (OD₅₅₀ approx. 5.0) on a rotary shaker (100 rev. min⁻¹) at 25°C was for 1 h prior to the addition of LiCl to a final concentration of 200 mM. Glucose was added to a final concentration of 50 mM 30 min before Li⁺ addition. The yeast cells were centrifuged (1200 × g, 10 min) washed twice with 5 mM PIPES buffer (pH 6.5) and resuspended in fresh Li⁺-containing buffer every 2 h. Accumulation of Li⁺ (from external LiCl concentrations of 1, 5 and 10 mM) was carried out using methods modified from Huber-Wälchi and Wiemken [8] and White and Gadd [9]. Li⁺-loaded yeasts were prepared as described previously. Using 1.5-ml samples, exchangeable ions at the cell surface were removed by washing with 3 × 1 ml 5 mM PIPES buffer (pH 6.5), followed by one washing with 10 mM Tris/MES buffer (pH 6.0) at 4°C. Cells were incubated in the washing medium for 1 min and separated by microcentrifugation (8000 × g, 30 s) at each step. Cytosolic contents were extracted by resuspending the pellet in 1 ml 10 mM Tris/MES buffer (pH 6.0) with 0.7 M sorbitol at 25°C. DEAE dextran was added (40 μl; 10 mg ml⁻¹), mixed and incubated for 30 s at 25°C. Cells were separated by microcentrifugation (8000 × g, 30 s) and the supernatants removed and retained. The permeabilized cells were washed with 3 × 1 ml 0.7 M sorbitol in 10 mM Tris/MES buffer (pH 6.0) at 4°C incubating for 1 min at each wash and separating by centrifugation as described. Supernatants were retained and combined with those from the permeabilization step. Vacuolar contents were extracted by suspending pellets in 60% (v/v) methanol at 4°C for 30 s, centrifuging (8000 × g, 30 s) and removing the supernatant. This was repeated three times and followed by three further washes in 10 mM Tris/MES buffer (pH 6.0) at 4°C, incubating for 1 min and centrifuging as above. The remaining pellet was digested and analysed for Li⁺ and K⁺ as described previously. All glassware was washed with 1 M HCl and rinsed thoroughly with distilled deionized water prior to use. Solutions were analysed for K⁺ contamination by atomic absorption spectrophotometry and values taken into account in final calculations.

**Transmission electron microscopy**

Fixation of cell pellets, obtained by centrifuging (8000 × g, 30 s) and washing twice with 5 mM PIPES (pH 6.5) occurred in 5% (v/v) glutaraldehyde in 5 mM PIPES (pH 6.5) overnight (17 h) by vacuum infiltration. After washing with buffer, post-fixation employed 0.2% (w/v) osmium tetroxide (17 h); cells were finally washed twice with 5 mM PIPES (pH 6.5). Dehydration was through a 25–100% (v/v) methanol (EtOH) series in distilled water, samples being left for 15 min at each stage. Three transfers were made in
Table 1

<table>
<thead>
<tr>
<th>LiCl concentration (mM)</th>
<th>Intracellular cations (nmol (10^8 cells)^{-1})</th>
<th>K^+</th>
<th>Na^+</th>
<th>Li^+</th>
<th>Mg^{2+}</th>
<th>Ca^{2+}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>1057.9 ± 6.6</td>
<td>6.2 ± 2.9</td>
<td>0</td>
<td>211.5 ± 2.7</td>
<td>8.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1027.6 ± 23.5</td>
<td>2.1 ± 0.5</td>
<td>54.2 ± 0.9</td>
<td>206.0 ± 5.0</td>
<td>6.7 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>948.5 ± 13.2</td>
<td>1.2 ± 5.6</td>
<td>128.9 ± 1.2</td>
<td>204.3 ± 2.1</td>
<td>6.7 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>921.5 ± 5.5</td>
<td>10.4 ± 3.9</td>
<td>195.2 ± 9.3</td>
<td>204.0 ± 3.4</td>
<td>7.7 ± 1.0</td>
<td></td>
</tr>
</tbody>
</table>

Values are shown ± S.E.M. (three replicates).

Results

Cation composition of S. cerevisiae on Li^+ accumulation

Intracellular cations (K^+, Na^+, Li^+, Mg^{2+} and Ca^{2+}) present in S. cerevisiae X2180-1B after incubation for 3 h in various concentrations of LiCl are shown in Table 1. The results indicate that only K^+ and Mg^{2+} form a large proportion of the intracellular cation pool in this organism. Ca^{2+} and Na^+ are present but in low amounts,
averaging approximately 7.4 and 5.0 nmol \((10^8 \text{ cells})^{-1}\), respectively. Accumulation of Li\(^+\) occurred at all concentrations of LiCl and increased with increasing external LiCl concentration (Table 1). The percentages of cell K\(^+\) replaced by Li\(^+\) were 5, 12 and 17\% at 1, 5 and 10 mM LiCl, respectively. Changes in the cellular levels of Mg\(^{2+}\), Ca\(^{2+}\) and Na\(^+\) were not observed on Li\(^+\) accumulation into the cells. A small exchange of Li\(^+\) for K\(^+\) occurred at 1 mM LiCl but at both 5 and 10 mM LiCl there appeared to be a stoichiometric Li\(^+\)/K\(^+\) exchange relationship of approximately 1:1 (calculated from Table 1).

**Cellular compartmentation of Li\(^+\) and K\(^+\)**

The cellular distribution of Li\(^+\) and K\(^+\) was measured, using a differential extraction procedure, in the vacuole, cytosol or bound to cellular compartments.

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**Fig. 2.** Transmission electron micrographs of *S. cerevisiae* X2180-1B after a 6-h loading procedure in the presence (a, b, c) or absence (d) of 200 mM LiCl. Some irregularities in the vacuolar membranes may be artifacts resulting from preparative procedures; some of the dark spots present in the vacuole may result from accumulation and precipitation of osmium during the fixation process [25]. Bar marker represents 500 nm.
constituents including the cell wall. Figure 1b shows the ionic compartmentation of Li⁺ and K⁺ in *S. cerevisiae* X2180-1B after a 6-h loading procedure. Initially, cells possessed a high vacuolar concentration of K⁺ (approx. 890 nmol (10⁸ cells)⁻¹) with a smaller concentration in the cytosol (approx. 170 nmol (10⁸ cells)⁻¹); a negligible amount was bound to the wall or other cell components. During repeated incubation with 200 mM LiCl there was marked replacement of vacuolar K⁺ for Li⁺. After 6 h incubation, 85% of the total Li⁺ accumulated was located in the vacuole, the remainder being present in the cytosol with a negligible amount being bound. The results revealed that approx. 60% of initial cell K⁺ was replaced by Li⁺ after 6 h. Control cells initially showed a similar distribution of K⁺ which was present in higher concentrations in the vacuole than in the cytosol (approx. 970 and 160 nmol (10⁸ cells)⁻¹, respectively), negligible amounts being bound to cell wall fragments. During repeated incubation in 50 mM glucose, there was a re-distribution of K⁺ from the vacuole to the cytosol, to a final distribution after 6 h of about 570 and 410 nmol (10⁸ cells)⁻¹, respectively (Fig. 1a).

**Transmission electron microscopy of Li⁺-loaded cells**

The morphological consequences of Li⁺ accumulation on yeast intracellular structure is shown in Fig. 2a–c. Enlarged vacuoles are present in Li⁺-loaded cells compared with control cells (Fig. 2d). The presence of other small vacuoles and/or lipid droplets is also apparent. Assuming a yeast cell volume of 47 fl [10], the volume of the enlarged vacuole accounted for about 65% of the cell volume. Using these dimensions, a calculated vacuolar Li⁺ concentration of about 240 nM exists which is 4 times the concentration of Li⁺ in the cytosol after a 6-h Li⁺-loading period (Table 2).

**Discussion**

Analysis of the metal cations belonging to Group I and II of the Periodic Table reveals that lithium has the smallest ionic radius, compared with Na⁺, K⁺, Ca²⁺ and Mg²⁺, and is the lightest metal with the largest field density at its surface. Consequently, Li is the least reactive of the alkali metals and general chemical properties resemble those of magnesium and calcium in cell physiology [11,12]. This work has shown that in *S. cerevisiae*, uptake of Li⁺ occurs via K⁺ replacement. A reduction in Na⁺, Ca²⁺ and Mg²⁺ was not observed despite all these cations being classified as fast exchange elements [13]. However, calcium- and magnesium-dependent processes such as ribosome stability and enzyme activation [14] could still be affected by Li⁺ accumulation and result in toxic symptoms in *S. cerevisiae* [6].

In yeasts, the important role of vacuoles as storage organelles and their involvement in pH homeostasis and cytosolic ion regulation is established [15]. This work has indicated that the majority (approx. 85%) of Li⁺ accumulated in *S. cerevisiae* X2180-1B was located in the vacuoles. Uptake of Li⁺ resulted in K⁺ efflux from the vacuoles, yeast vacuoles already being identified as the major storage compartment for K⁺ as well as Mg²⁺ and orthophosphate [16,17]. A similar phenomenon has been observed in Na⁺-grown yeast [18]. This asymmetric ionic distribution may explain the tolerance of yeasts to high concentrations of Group I cations [19]. The presence of vacuolar ion channels in *S. cerevisiae* which conduct K⁺ but also have a broad selectivity for other monovalent cations including Li⁺ has been described [20,21]. Control cells of *S. cerevisiae* exhibited a redistribution of K⁺ from the vacuole to the cytosol. Reorganisation of ions during cel-
lular metabolism may reflect metabolic activation by K⁺ in the cytosol. It is known that K⁺ is involved in high-molecular mass polyphosphate synthesis in the yeast cell envelope [22] while a K⁺ gradient at the plasma membrane may also aid transport processes [23]. The absence of monovalent ions bound to the cell wall or other components is consistent with fungal biomass not absorbing alkali metal ions [24]. Finally, the total intracellular monovalent cation concentrations, as calculated for the total cellular monovalent cation pool (K⁺, Li⁺) is high (approx. 280 mM) but is in agreement with other studies for K⁺ (> 250 mM) [2].

The enlarged vacuoles observed during Li+-loading may be associated with an intracellular toxic response absent in the control cells. Previous observations of yeast vacuoles have shown wide variations in shape according to cultural and cellular conditions. Such differences range from several small, cytoplasmic vesicles to a fusion of the latter to form single large spherical bodies [25]. Cultural manipulation during the Li+-loading procedure might have provided the fundamental requirements of excess carbon (glucose) and lack of other nutrients such as Fe and Mg [26] leading to possible formation of lipid droplets.

To conclude, S. cerevisiae is able to accumulate Li⁺ via energy-dependent transport system(s) at the plasma membrane [27]. Once accumulated, Li⁺ is predominantly sequestered in the vacuole via vacuolar membrane transport which is also energy-dependent [20]. Such ionic compartmentation may reduce or prevent any toxic effects exerted by Li⁺.

Acknowledgements

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References

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