Amino acid uptake is strongly affected during exponential growth of *Saccharomyces cerevisiae* in 0.7 M NaCl medium

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Abstract

Labelling of *Saccharomyces cerevisiae* grown in 0.7 M NaCl with $^{35}$S-methionine revealed a 5–6 fold lowering of the methionine incorporation into protein, which could not be attributed solely to the approximately 50% longer generation time of cells grown in 0.7 M NaCl. Subsequent studies of the high affinity methionine uptake system showed a strongly reduced uptake of methionine during growth in 0.7 M NaCl medium. This reduced uptake was shown to be strain-independent and caused mainly by an approximately 20-fold lowered maximum velocity ($V_{\text{max}}$) of the transport system, while the substrate affinity ($K_m$) displayed only a minor change. A salt-instigated reduction of uptake was furthermore demonstrated for the leucine and histidine high affinity uptake systems and also for a mixture of 15 different amino acids. We therefore suggest that the reduced amino acid uptake is a general phenomenon observed in salt-grown cells.

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1. Introduction

To counter the adverse effects of osmotic stress by NaCl, *S. cerevisiae* induces two main responses: an increased exclusion of sodium ions and an osmotic potential compensatory accumulation of the three-carbon polyol glycerol [1]. The tolerance to osmotic shock, as well as the protein response during growth under osmotic stress, was found to be highly strain-dependent [2–4]. The expression of glycerol-3-phosphate dehydrogenase, encoded by the *GPD1* gene was, however, always found to be induced by a lowered water potential, while a more shock tolerant strain in addition displayed major changes in the expression of many proteins [4].

Processes involving membrane gradients are important during osmotic stress. It is vital for the cell to retain an excess of the produced glycerol intracellularly and this is seemingly accomplished through the osmotically instigated closing of Fps1p, a glycerol facilitator [5]. During NaCl stress the expression of the sodium pumping ATPase *ENA1* is increased [6] and the deletion of this gene accordingly renders cells highly sensitive to lithium and sodium ions. The effect of osmotic stress on the nutrient uptake systems of the plasma membrane is a field of research which has not received much attention, although it is likely that many nutrient uptake systems will be affected by changes in the water potential of the sur-
rounding medium. This study deals with the transport of amino acids into *S. cerevisiae* during salt stress which we have found to be highly impaired.

2. Methods

2.1. Strains and media

*Saccharomyces cerevisiae* strain Y41 (ATCC 38531) was used in most experiments except when strain-dependent methionine transport was investigated, where the diploid SKQ2n (ATCC 44827; Mata/MataIα; ade1+/+; ade2+/his1) and haploid X2180-1A (ATCC 26786; Mata; SUC2; mal0; gal2; CUP1) were included. For all growth and uptake experiments a defined minimal medium (YNB) with 5 g/l of D-glucose added was used under the conditions described previously [7]. When indicated the YNB medium was supplemented with 0.7 M NaCl.

2.2. Measurement of amino acid uptake

Cells were grown to an OD$_{610}$ of 0.5 (corresponding to $5 \times 10^6$ cells/ml) in YNB medium as described above. For studies on the methionine permease a methionine solution of 50 Ci/mol was added to final concentrations of 2, 5, 12, 25 and 50 µM and experiments were performed in duplicate. At 30, 60, 90, 120 and 150 s 750 µl of culture was withdrawn and mixed with 750 µl of ice-cold non-labelled 0.1 M methionine solution. The cells were pelleted (13 000 g, 3 min) and washed once with 1 ml of 0.1 M methionine solution. The cells were then resuspended with $2 \times 50$ µl milliQ H$_2$O and transferred to scintillation vials with 5 ml Aquassure (cat. no. NEF-965; DuPont Biotechnology Systems). Counting was performed in a Beckman LS 6000 LL scintillator. Rate of uptake per mg protein was calculated by use of the following relations: $1 \times 10^7$ cells equals 0.3 mg dry weight of cells and 50% of the cell dry weight corresponds to protein.

Studies on the uptake kinetics for the other amino acids were performed with $^{13}$C-leucine (11.8 GBq/mm; NEC 279E, DuPont), $^{14}$C-histidine (11.8 GBq/mm; CFB 140, Amersham Inc.) or a $^{14}$C-amino acid mixture (CFB 104; Amersham Inc.) containing 15 amino acids except Met, Trp, Cys, Asn and Gln in the range 5.6 GBq/mmol (Ala) to 11.7 GBq/mmol (His). The procedure was similar to the methionine case with the following modifications: values presented represent amino acids taken up after 60 s, and 0.1 M non-radioactive leucine or histidine solutions, or in the case of the amino acid mixture a non-radioactive 10% (w/v) casamino acid solution, were used to stop the uptake.

3. Results and discussion

3.1. Effects of NaCl growth on methionine transport

In a previous study cells of *S. cerevisiae* strain Y41 were labelled with $^{35}$S-methionine for 30 min in mid-exponential growth phase and the salt-instigated changes in global protein expression were subsequently studied by two-dimensional gel electrophoresis (2D-PAGE) [3]. The amount of methionine incorporated during the 30-min labelling period, measured as TCA precipitable material in the final protein extract, was 5–6 times greater in the control cells from non-saline medium than for 0.7 M NaCl grown cultures: $68 \pm 6 \pm 10$ dpm/g protein/30 min and $12 \pm 800 \pm 30$ dpm/g protein/30 min, respectively. If the slower growth rate of the NaCl-grown cells (a generation time of 3.2 h as compared to 2.2 h for the control cells) is taken into account, incorporation expressed as dpm/µg protein/generation, a roughly 4-fold discrepancy was still apparent between stressed and non-stressed cells. This indicated that specific mechanisms, and not only general growth rate effects, influenced the level of $^{35}$S-methionine labelling.

$^{35}$S-methionine is considered a good substrate for the labelling of proteins in *S. cerevisiae* because of the low intracellular concentration of this amino acid and thus rapid isotope saturation of the intracellular methionine pool, which is reported to occur within 30 s [8]. A small intracellular methionine pool also ascertains a high steady state specific activity of the label and thus a rapid labelling of proteins. This implies that a dramatically increased pool of intracellular methionine would lead to a slower rate of incorporation, a conceivable scenario since bacteria osmoregulate by accumulation of amino acids or amino acid derivatives [9]. However, measurements
of the impact of NaCl on the internal methionine pool in yeast, as well as the pool size of other amino acids, have revealed only minor fluctuations [10,11].

We therefore decided to investigate the possibility of an impaired methionine transport in response to NaCl stress as the cause of decreased methionine incorporation.

Uptake of methionine into *S. cerevisiae* was until recently thought to occur via two distinct transport systems, one of high affinity (\(K_m\) about 12 \(\mu\)M) and one with a low affinity (\(K_m\) about 770 \(\mu\)M) [12]. The high affinity system was largely confirmed in a recent study [13] and found to be mediated by the *MUP1* gene product, an amino acid transporter belonging to a new family distinct from other amino acid transporters in yeast. In contrast, the low affinity system was in the latter study found to be composed of two systems, one with a \(K_m\) of approximately 220 \(\mu\)M and one very low affinity system with a \(K_m\) of approximately 1 mM; the latter was mediated by the product of the *MUP3* gene [13]. Since the concentration of methionine added during our labelling regime was initially only 15 nM, a kinetic study comparing the high affinity system from cells grown at 0 or 0.7 M NaCl was conducted. Evidence of an impaired transport capacity of salinity grown cells of strain Y41 was observed at the reported \(K_m\) concentration of the high affinity transport system for methionine (12 \(\mu\)M), with the rate of methionine transport of the control cells being 13-fold higher than that of the salt grown cells (Fig. 1A): 600 pmol/10^6 cells/min for the control and 45 pmol/10^6 cells/min for salt grown cells. This high NaCl impact on transport capacity for methionine is contradictory to what was reported earlier [14]. Thus, to rule out strain specific variations and possible salinity induced transport defects of strain Y41, two lab strains, one haploid (X2180-1A) and one diploid (SKQ2n), were tested for NaCl impact on the high affinity uptake system for methionine (Figs. 1B and 1C). All three strains displayed roughly similar degrees of salinity impact on methionine transport at 12 \(\mu\)M methionine (X2180-1A: 460 to 36 pmol/10^6 cells/min and SKQ2n: 420 to 35 pmol/10^6 cells/min). Thus, the salinity instigated defect in high affinity methionine transport is a strain independent phenomenon. A similar effect was recently shown for the salt-tolerant fungus *Candida membranefaciens* in which the uptake of most amino acids was strongly reduced during growth in medium with 1.35 M NaCl [15].

### 3.2. Kinetic properties of the salt impaired methionine transporter

Kinetic studies in the 2 to 50 \(\mu\)M substrate range revealed that the major impact on this high affinity transport system was on its total transport capacity, and a roughly 20-fold lower \(V_{max}\) value for salinity grown cells was displayed. However, the \(K_m\) value was less affected and for either culture estimated to be in the range 10–30 \(\mu\)M (Fig. 2), in good agreement with what has been reported earlier for this
high affinity system [12]. In addition to the specific transport systems for methionine, a general amino acid transport system, encoded by the \textit{GAP1} gene, with broad substrate specificity has been reported for \textit{S. cerevisiae} [16]. In the present study cells were cultivated in ammonium rich media, conditions under which the GAP system is strongly repressed [17], and should thus not contribute to the uptake of methionine. However, to further substantiate that the specific high affinity transport system was under study in both control and NaCl grown cells and thus to exclude that the GAP-system was upregulated during saline growth even in the presence of ammonium, transport inhibition studies were performed. Ethionine is one of the few compounds reported to inhibit specifically the high affinity methionine transport at rather low concentrations [12]. We found that addition of ethionine to 200 \(\mu\text{M}\), at a concentration of methionine of 12 \(\mu\text{M}\), hampered the rate of methionine uptake to similar degrees for the two types of cells: 87\% inhibition for salt grown and 95\% inhibition for control cells (data not shown). The similar \(K_m\) values and inhibitor responses indicated that the same high affinity transport system was under study in both salt grown and control cells, the capacity, but not the affinity of this permease, being strongly affected during NaCl growth.

3.3. General NaCl impact on amino acid transport

A possibly general impact on transportation of amino acids during growth in 0.7 M NaCl was investigated by uptake studies with other amino acids. The kinetic characteristics of some other amino acid transport systems are known, and the \(K_m\) values of the high affinity systems for leucine and histidine are reported to be 84 and 20 \(\mu\text{M}\), respectively [16]. The uptake of histidine or leucine at concentrations below their respective high affinity \(K_m\) values was drastically decreased in NaCl grown cells (Fig. 3). Furthermore, if a mixture of 15 different amino acids was used the same pattern was observed and the overall rate of transport for this amino acid mixture was only 8\% of the rate found for cells grown in basal medium (Fig. 3). Clearly, the NaCl impact on the transportation of amino acids was not restricted to the uptake of methionine. Furthermore, our kinetic studies revealed that the major salinity impact on the transport system for methionine was observed for the maximum transport capacity \(V_{\text{max}}\) while \(K_m\) displayed no significant changes. Even though our kinetic studies have been restricted to the uptake of methionine, we hypothesize similar salinity induced behaviour of \(V_{\text{max}}\) and \(K_m\) for the other amino acid transport systems because of the high degree of functional and structural conservation [18].

The mechanism responsible for the lowered capacity might be a lower expression of the high affinity uptake systems. We therefore made attempts to examine the expression of \textit{MUP1}, encoding the high affinity uptake system for methionine [13], by Northern blotting. Despite the fact that a strong \textit{ACT1} signal was seen on the filters, no band potentially corresponding to \textit{MUP1} could be detected even after prolonged exposure.

An alternative mechanism explaining the reduced methionine uptake is that the driving force of the transport is reduced in a salt-dependent manner. The active uptake of amino acids is believed to be driven by the simultaneous inward transport of protons [16,19], dissipating the proton gradient over the membrane generated by the activity of the plasma membrane bound \(\text{H}^+\text{-ATPase}\). Thus, anything that affects the membrane potential will obviously influence amino acid transport. Speaking against the plasma membrane ATPase as a crucial determinant

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**Fig. 3.** Impact on amino acid high affinity uptake in \textit{S. cerevisiae} by growth medium salinity. Exponentially growing cultures in 0 M NaCl (open bars) or 0.7 M NaCl (filled bars) were tested for amino acid transport defects by the addition of radioactive amino acids at the following concentrations; 0.3 \(\mu\text{M}\) leucine (Leu), 0.16 \(\mu\text{M}\) histidine (His), 2-30 nM mixture of 15 amino acids (Mix), and 12 \(\mu\text{M}\) methionine (Met). Rate of uptake of a particular amino acid at 0 M NaCl was set to 100\%, which for the different amino acids corresponds to an absolute value of \(\text{Leu} = 2\), \(\text{His} = 0.6\) and \(\text{Met} = 20\) nmol/mg protein/min. Values are the mean of two independent experiments.

of amino acid transport is the fact that salt affected the PMA activity differentially in \textit{C. membranefaciens} (increased) and \textit{S. cerevisiae} (decreased) [15] even though both species displayed the uptake deficiency phenotype in saline medium.

Manipulation of the ATPase content by promotor deletions resulted in impaired transport capacities for amino acids [20]. Despite these mutant cells being auxotrophic for histidine and adenine (which accordingly was supplied into the media), no salinity-induced impact on growth was observed even at 1.15 M NaCl. This indicates that the amount of ATPase will not be of apparent significance for the cells to cope with NaCl-stressed conditions, at least when synthesised to certain threshold levels. Apparently, under these growth conditions neither ATPase content nor transportation of amino acids is the growth rate limiting factor.

In the context of NaCl growth it is of interest to note that amino acid transport is believed to be coupled to the outward flow of potassium for electroneutrality reasons [16]. Transport of this ion will influence the generation of the membrane potential. Sodium efflux is mediated by a P-type ATPase encoded by the \textit{ENA1} gene, which is transcriptionally induced by NaCl [6]. Conceivably, the increased amount of this sodium specific ATPase will at least partly be involved in the enhanced utilisation of ATP in maintenance during saline growth [21], and could thus influence the generation of the membrane potential. There is furthermore experimental support that the Na\textsuperscript{+} efflux is linked to the influx of protons [22], which might cause an effect on the membrane potential during NaCl stress. In summary, there are a number of candidates for molecular mechanisms relating salt stress to impaired amino acid transport.

References


