J Bioenerg Biomembr (2013) 45:561–568
DOI 10.1007/s10863-013-9528-6

Early changes in membrane potential of *Saccharomyces cerevisiae* induced by varying extracellular K^+, Na^+ or H^+ concentrations

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Received: 28 May 2013 / Accepted: 9 September 2013 / Published online: 20 September 2013

Abstract Recently we introduced a fluorescent probe technique that makes possible to convert changes of equilibrium fluorescence spectra of 3,3'-dipropylthiadicarbocyanine, diS-C_3(3), measured in yeast cell suspensions under defined conditions into underlying membrane potential differences, scaled in millivolts (Plasek et al. in J Bioenerg Biomembr 44: 559–569, 2012). The results presented in this paper disclose measurements of real early changes of plasma membrane potential induced by the increase of extracellular K^+, Na^+ and H^+ concentration in *S. cerevisiae* with and without added glucose as energy source. Whereas the wild type and the Δ*tok1* mutant cells exhibited similar depolarization curves, mutant cells lacking the two Trk1,2 potassium transporters revealed a significantly decreased membrane depolarization by K^+, particularly at lower extracellular potassium concentration $[K^+]_{out}$. In the absence of external energy source plasma membrane depolarization by K^+ was almost linear. In the presence of glucose the depolarization curves exhibited an exponential character with increasing $[K^+]_{out}$. The plasma membrane depolarization by Na^+ was independent from the presence of Trk1,2 transporters. Contrary to K^+, Na^+ depolarized the plasma membrane stronger in the presence of glucose than in its absence. The pH induced depolarization exhibited a fairly linear relationship between the membrane potential and the pH_o of cell suspensions, both in the wild type and the *Δtrk1,2* mutant strains, when cells were energized by glucose. In the absence of glucose the depolarization curves showed a biphasic character with enhanced depolarization at lower pH_o values.

Keywords Plasma membrane potential · Fluorescent probe · Plasma membrane depolarization by extracellular cations · *Saccharomyces cerevisiae* · Potassium-transport deficient mutants

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Introduction

One of the crucial features of cellular metabolism is the maintenance of cellular ion homeostasis characterized by stable intracellular pH, high K^+ and low Na^+ concentrations even under rapidly changing external or environmental conditions. In plants and fungi, the driving force for ion fluxes across the plasma membrane is the electrochemical proton gradient $\Delta\mu_{H+}$ that is generated by the plasma membrane proton ATPase, Pma1. High intracellular concentrations of K^+ thus play a pivotal role in a wide range of essential cellular processes, such as osmotic regulation, protein synthesis, and enzyme activities (Evans and Wildes 1971; Leigh and Jones 1984). The transport of H^+ and K^+ ions is interlinked and electrically coupled.

In the yeast *Saccharomyces cerevisiae* two transport systems are responsible for K^+ uptake, Trk1 and Trk2. The efflux of surplus cations is mainly mediated by Ena1, a primary active Na^+ transporter, and Nha1, a proton/Na^+ or K^+ antiporter–for review see (Arino et al. 2010). The main role of the latter two systems appears to be the extrusion of toxic Na^+ indicative for *S. cerevisiae*'s salt tolerance. However, they are also able to transport K^+ at high intracellular potassium concentrations (Banuelos et al. 1998; Nakayama et al. 2004). Additionally *S. cerevisiae* employs an outward rectifying K^+-channel Tok1 that opens at $\Delta\Psi$ less negative than –40 mV. K^+ efflux through Tok1 contributes under physiological conditions to stabilize the

membrane potential (Bertl et al. 1998; Zahradka and Sychrova 2012).

Current knowledge on ion transporters and channels, comprising gene expression, transport activity, regulation and function, is based mainly on studies with isogenic mutant strains, derived from the parental *S. cerevisiae* strain BY4741, lacking the individual K^+-transporting systems or a combination of them (Petrezselyova et al. 2011). However, despite considerable experimental work, much less is understood concerning integration of individual transport systems into a more general network of transmembrane cation fluxes, which are energized by the electrochemical proton gradient generated by Pma1–for review see (Corratge-Faillie et al. 2010; Rodriguez-Navarro 2000).

The electrochemical proton gradient consists of the proton concentration gradient ΔpH and the electrical potential gradient $\Delta\Psi$, the so called membrane potential. Whereas the intracellular pH_{in} and thus, ΔpH can be reliably determined by different methods such as measurement of pHluorin fluorescence, (Brett et al. 2005; Miesenbock et al. 1998), quantitative measurements of $\Delta\Psi$ have been so far hampered by several pitfalls. Lipophilic cationic fluorochromes have been repeatedly shown to be a suitable tool for examination of membrane potential changes in yeast cells (Gášková et al. 1998; Madrid et al. 1998; Maresova et al. 2006; Pena et al. 1984). By analyzing equilibrium diS-C_3(3) fluorescence spectra measured under defined experimental conditions in yeast suspension it is possible to convert their changes into underlying membrane potential differences in the scale of millivolts (Plášek et al. 2012). Briefly, the assessment of membrane potential changes of yeast cells incubated with different extracellular ion concentrations is based on the assumption that the ratio of respective diS-C_3(3) fluorescence intensities of the dye accumulated in the cells and that in their aqueous sorrounding is directly proportional to the ratio of intra- to extracellular dye concentrations and that the distribution of the dye follows the Nernst equation. By means of the spectral analysis of synchronously scanned diS-C_3(3) fluorescence spectra it is possible to assess the amount of dye accumulated in cells without otherwise necessary sample taking and following separation of cells from the medium. For the above assumption it is essential to use dye concentrations low enough to ensure direct proportionality between the amount of intracellular bound dye and the intracellular free dye concentration resulting from the Nernstian accumulation of diS-C_3(3) in the cells. For detailed description of the principles underlying the methodology see (Plášek et al. 2012).

Unlike previous applications of fluorescent probes for measuring membrane potential of yeast allowing only a qualitative monitoring of membrane potential changes, see e.g. (Madrid et al. 1998; Maresova et al. 2006, 2009; Pena et al. 2010; Petrezselyova et al. 2010), the method used in this work leads to quantitative assessment of membrane potential changes in mV. In the current study this method has been used to assess the extent of cell depolarization caused by changing external concentrations of K^+, Na^+ and H^+ in *S. cerevisiae* suspensions and thus, to characterize yeast plasma membrane permeability for these cations. By comparison of the membrane depolarizing curves obtained in suspensions of *S. cerevisiae* wild type and various potassium-transport-deficient mutant cells conclusions were drawn as to the role of Trk1, Trk2 and Tok1 in plasma membrane permeability for the three cations. The presented results provide first reliable quantitative information about the response of the membrane potential to defined changes of concentration gradients of K^+, Na^+ and H^+ across *S. cerevisiae* plasma membrane. Using this information it will be possible to manipulate selectively the components of the electrochemical proton gradient (ΔpH and $\Delta\Psi$) by varying the extracellular pH_o or the extracellular cation concentration.

Materials and methods

Chemicals

Fluorescent probe diS-C_3(3), 3-propyl-2-[3-[3-propyl-2(3H)benzothiazolylidene]-1-propenyl]benzothiazolium iodide (often abbreviated as 3,3′-dipropylthiadicarbocyanine iodide), CAS no. 53336-12-2, was purchased from Fluka, and added to cell suspensions from a 20 μM stock solution in ethylalcohol for UV spectroscopy (Lach-Ner, Czech Republic). D-glucose was from Penta (Czech Republic); yeast extract and potassium chloride from Fluka; choline chloride, peptone, MES hydrate and triethanolamine (TEA) from Sigma-Aldrich. All these compounds were of p.a. quality, except of peptone (Cell Culture Tested), MES hydrate (Biotech. Performance Certified) and TEA (Ultra, >99.5 %).

Yeast strains

Saccharomyces cerevisiae BY4741 (*MAT*a *his*3Δ1 *leu*2Δ *met*15Δ *ura*3Δ; EUROSCARF) strain (Brachmann et al. 1998) and its isogenic derivatives lacking one or various combinations of the *TRK1*, *TRK2* and *TOK1* genes were used in this study. For details see Table 1.

Yeast growth and sample preparation

Yeast strains were pre-cultured in YEPG medium (1 % yeast extract, 1 % peptone, 2 % glucose) at 30 °C for 24 h. Inocula were added to 10 ml of fresh YEPG medium (to OD = 0.1) and the main cultures were grown to early exponential phase (from 5 to 7 h) at 30 °C. Cells were harvested and washed twice by centrifugation, first with distilled water and then with MES-TEA buffer (25 mM MES, pH 6.2). The washed cells

Table 1 Yeast strains used in this study

Name	Genotype	Reference
BY471 *(wt)*	*MAT*a *his*3Δ1 *leu*2Δ *met*15Δ *ura*3Δ	(Brachmann et al. 1998)
Δ *tok1*	*BY4741, tok1::loxP*	(Zahradka and Sychrova 2012)
Δtrk1,2	*BY4741, trk1::loxP, trk2::loxP*	(Petrezselyova et al. 2011)
Δtok1,trk1,2	*BY4741, tok1::loxP, trk1::loxP, trk2::loxP*	(Zahradka and Sychrova 2012)

were resuspended in MES-TEA buffer to OD ≈ 0.2 (at 580 nm). Series of samples in 25 mM MES-TEA buffers containing required concentrations of K^+, Na^+ and H^+ were prepared. Within 30 min after the last washing equal volumes of the washed cell suspensions were added to individual samples of a particular titration series (typically about 50 μl of cell suspension to 3 ml of buffer). When required, glucose was also added to the samples at final concentration of 100 mM. After adding cells to the prepared buffer each sample was hand-shaken and left in this medium for 4 min before staining with diS-C_3(3). Actual pH values of all cell suspensions were directly measured with pH-meter (inoLab pH 720 with SenTix 81 electrode, WTW, Germany). In both the K^+ and Na^+ titration experiments, choline chloride was added to the cell suspensions together with KCl or NaCl to maintain constant both the ionic strength and extracellular Cl^- concentration.

Fluorescence measurements

Samples of cell suspensions were prepared and their fluorescence measured in disposable 1×1 cm UV-grade fluorometric cuvettes (Kartell, Italy). Autofluorescence spectrum of each sample was measured before adding diS-C_3(3). Upon adding the dye (to the final concentration of 40 nM), the samples were left for 20 min under occasional hand-shaking to allow dye equilibration across the plasma membrane. Then the synchronously-scanned fluorescence (SSF) spectrum of the stained cell suspensions was measured, as described in (Plášek et al. 2012). The SSF spectra were recorded using Fluoromax-3 spectrofluorometer (Jobin-Yvon Horiba), at 13 nm offset between λ_{exc} and λ_{em} with slit widths of 2.3 nm. With 5 nm large steps and λ_{exc} spectral range of 520–590 nm measured spectra comprised only 15 spectral data points. The low number of points was well sufficient for the spectral unmixing of these spectra using Nonlinear Regression module of SigmaPlot 11 software (Systat Software Inc., USA) and for the determination of the ratio of bound-to-free fluorescence intensities, B/A. With such a small number of spectral points and the integration time set to 0.5 s, a single SSF spectrum was measured in less than 10 s, and the average of seven spectra in 75 s, which is fast enough for monitoring membrane potentials in multiple samples in extensive titration experiments. The advantage of averaging the repeatedly measured spectra instead of more common use of longer integration times consists in the reduction of statistical weight of sudden fluctuations of fluorescence intensity caused by tiny bubbles of dissolved air released from cell suspensions. In this way we were able to reduce significantly the incidence of flawed points in the titration curves based on the determination of the B/A ratio from the measured SSF spectra.

Results

K^+ induced depolarization of yeast cells harvested in the exponential phase

Series of titration experiments were carried out with *S. cerevisiae* wild type and the K^+-transport mutant strains deficient in either single potassium transport system (Δ*trk1,* Δ*trk2* and Δ*tok1*) or two potassium transport systems (Δ*trk1,tok1;* Δ*trk2,tok1* and Δ*trk1,trk2*) or all three potassium transport systems (Δ*trk1,trk2,tok1*)–detailed data not shown. All titration experiments resulted in a consistent set of data demonstrating that all Δ*trk1* strains behaved as strains in which both *TRK1* and *TRK2* genes were deleted whereas all Δ*trk2* strains behaved as the wild type strain. Hence, experiments presented in this paper focused on four *S. cerevisiae* strains only, the wild type and the mutants Δ*tok1,* Δ*trk1,trk2* and Δ*trk1,trk2,tok1*. Each series of titration experiments was carried out 2–4 times whereby any group of results in the repeated series did not differ by more than 20 %. Average values of the repeated titrations are presented in the figures (data in Fig. 1, four times repeated series, are provided with error bars, S.E.)

The titration experiments using the four yeast strains suspended in buffers containing seven increasing KCl concentrations revealed that the characteristics of K^+-induced depolarization of the examined strains split into two distinct patterns–that of the wild type and the *Δtok1* mutant, on one side, and that of the *Δtrk1,2* mutants (both the double mutant *Δtrk1,2* and the triple mutant *Δtok1,trk1,2*), on the other side, Fig. 1. Whether the functional Tok1 is present or not, the absence of Trk1 and Trk2 transporters renders the cell membrane potential to be less sensitive to the external potassium concentration compared to cells containing the two Trk transporters. Thus, the presence or absence of Tok1 itself plays

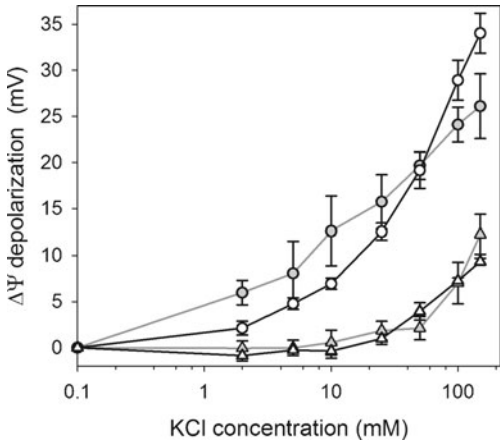

Fig. 1 K^+ induced depolarization of yeast cells (early exponential phase) suspended in MES-TEA buffer (pH 6.2) with 100 mM glucose. Wild type–*black line/empty circles*; *Δtok1* mutant–*grey line/grey circles*; *Δtrk1,2* mutant–*black line/empty triangles*; *Δtok1,trk1,2* mutant–*grey line/grey triangles*. Series of measurements repeated with four different cell suspensions on different days. Data represent arithmetic means ± S.E

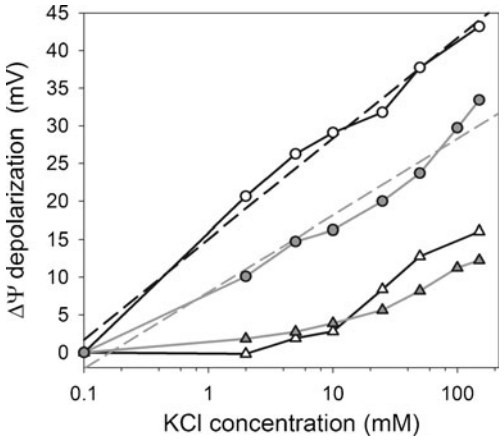

Fig. 2 K^+ induced depolarization of yeast cells (early exponential phase) suspended in MES-TEA buffer (pH 6.2) without glucose. Wild type–*black line/empty circles*; *Δtok1* mutant–*grey line/grey circles*; *Δtrk1,2* mutant–*black line/empty triangles*; *Δtok1,trk1,2* mutant–*grey line/grey triangles*. *Dashed lines* represent linear regressions. Series of measurements repeated 2–3 times with independent cell suspensions on different days. Data represent arithmetic means

only a minor role in the characteristics of K^+-induced plasma membrane depolarization.

The plots of membrane potential vs. $[K^+]_{out}$ shown in Fig. 1 (cells energized by glucose) exhibit a biphasic response of cell membrane potential to the increasing concentration of extracellular potassium, reflecting an initial moderate depolarization at lower K^+ concentrations followed by a significantly steeper depolarization at higher K^+ concentrations. Moreover, the depolarization observed within the initial phase is practically negligible in cells lacking Trk1 and Trk2 transporters, in which the depolarization became apparent only for external K^+ concentrations exceeding 25–50 mM. Similar effects were also observed for cell suspensions deprived of external source of energy, except that the initial low-depolarization phase is practically absent in cells containing Trk1,2 transporters, whose K^+-titration curves can be fitted to straight lines, Fig. 2. In the mutants lacking these transporters the initial phase is still clearly manifested, and the onset of steep depolarization is shifted towards lower K^+ concentrations (about 10 mM) compared to experiments with cell suspensions containing glucose. Moreover, the K^+-induced depolarization in non-energized cells was more pronounced than in suspensions containing glucose.

Effect of staining period on the assessment of K^+ -induced depolarization of yeast cells

Conversion of diS-C_3(3) fluorescence spectra measured in yeast cell suspensions to data on membrane potential changes is only justified if the dye accumulation in the cells reached its equilibrium level (Plášek et al. 2012). With *R. glutinis*, the yeast used in the cited study, the steady-state level of dye accumulation had been reached in less than 20 min. Unfortunately, the course of diS-C_3(3) accumulation in *S. cerevisiae* cells is more complex. In particular, the steep increase of intracellular-to-extracellular diS-C_3(3) fluorescence ratio (B/A) observed immediately after adding the dye to cell suspensions is not followed by a true steady-state plateau. Instead, a slight but not negligible steady increase of intracellular dye accumulation can be observed for more than 60 min. For example, B/A ratios measured in the suspensions of wild type yeast cells increased by about 20 % on extending the length of cell staining from 20 to 40 min.

Therefore, we tested whether the absence of a true B/A plateau could affect the measurements of K^+ -induced cell depolarization in the *S. cerevisiae* strains used. The wild type and the *Δtrk1,2* mutant strains were chosen for this purpose. The K^+ induced depolarization plots based on diS-C_3(3) fluorescence spectra recorded sequentially in different times after adding the dye to cell suspensions are shown in Fig. 3. We found that doubling the period of diS-C_3(3) accumulation in the cells from 20 to 40 min had only a small influence on the pattern of observed differences between the wild type and the *Δtrk1,2* mutant strains. Moreover, this finding provides evidence that the methodology used in this study leads to reproducible and thus, reliable membrane potential data.

Na^+ induced depolarization of yeast cells

The Na^+ induced depolarization was measured in the wild type and the *Δtrk1,2* mutant strains only because the main goal of this experiment was to determine whether the two K^+ transporters may or may not mediate a significant Na^+ translocation across *S. cerevisiae* plasma membrane. Similar to K^+ titrations, the Na^+

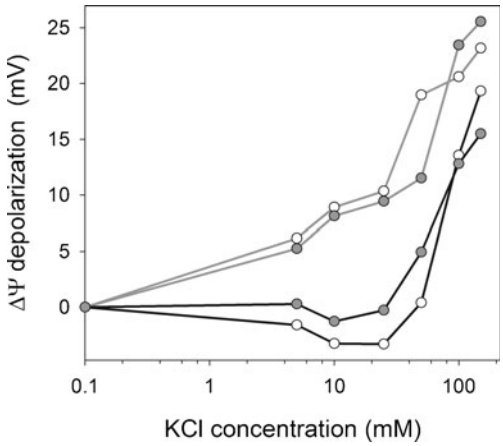

Fig. 3 Effect of staining period on the K^+ induced depolarization of yeast cells (early exponential phase) suspended in MES-TEA buffer (pH 6.2) with 100 mM glucose: *grey lines*–wild type; *black lines*–*Δtrk1,2* mutant; 20 min staining with dye (*empty circles*), and 40 min staining (*grey circles*). Series of measurements repeated 2 times with different cell suspensions on different days. Data represent arithmetic means

induced depolarization was measured two times on different days. An average cellular response to the changes of extracellular Na^+ concentration is shown in Fig. 4. For wild type cells supplied with glucose the Na^+ induced depolarization was comparable with the effect of K^+, both in size and in character. However, in contrast to the K^+ effect no significant difference was found between Na^+ induced depolarization of wild type and *Δtrk1,2* mutant cells. Another interesting difference between K^+ and Na^+ induced depolarization concerns the effect of glucose. In cell suspensions deprived of external energy source the Na^+ induced depolarization was less pronounced than in cells with glucose.

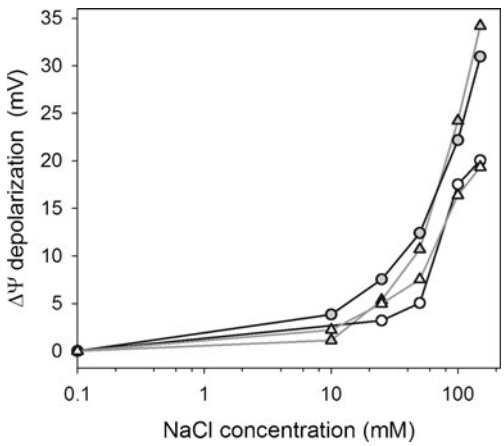

Fig. 4 Na^+ induced depolarization of yeast cells (early exponential phase) suspended in MES-TEA buffer (pH 6.2) in the presence of 100 mM glucose (*grey symbols*) and without glucose (*open symbols*). Wild type–*black line*/*circles*; *Δtrk1,2* mutant–*grey line*/*triangles*. Series of measurements repeated 2–3 times with different cell suspensions on different days. Data represent arithmetic means

Depolarization of yeast cells induced by changes in extracellular pH_o

The pH induced depolarization was measured in the wild type and the *Δtrk1,2* mutant strains for 6 different pH_o values ranging from 3 to 6.5. For each strain the measurements were carried out twice using two different yeast cultures grown on the same day. The mean values of depolarization data from these repeated experiments are shown in Fig. 5. The membrane potential of cells suspended at pH_o=6.5 was taken as the reference value since at this pH_o no significant H^+ concentration gradient across the plasma membrane exists. For cells supplied with glucose the scatter plots show a fairly strong and fairly linear relationship between the membrane potential and the pH_o of cell suspensions. Moreover, the respective linear regression curves for the wild type and the *Δtrk1,2* mutant cells are fairly identical. Without external source of energy the

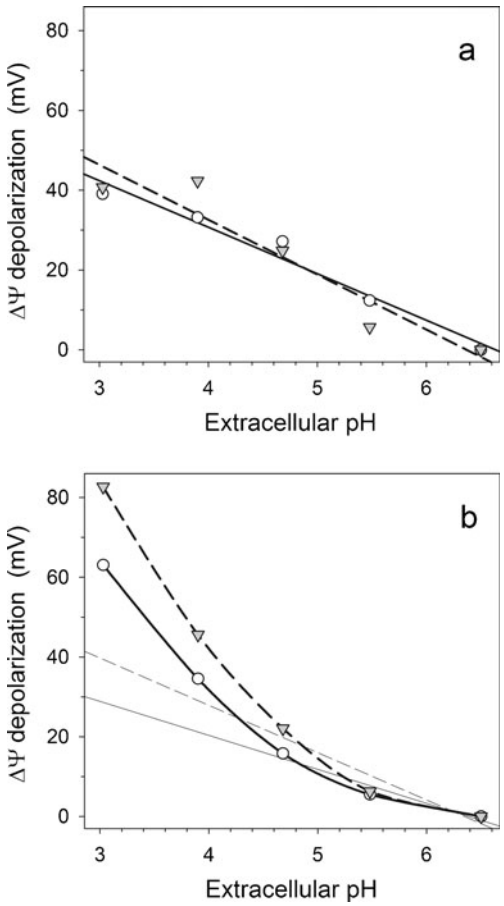

Fig. 5 pH induced depolarization of yeast cells (early exponential phase) suspended in MES-TEA buffer: *circles and full lines*–wild type; *triangles and dashed lines*–*Δtrk1,2* mutant. **a** cell suspensions with 100 mM glucose, **b** cell suspensions without glucose, with linear regression performed for the three highest pH_o values. The depolarization of plasma membrane is plotted as positive change of $\Delta\Psi$. The given data represent average values of two independent experiments. Series of measurements repeated 2 times with different cell suspensions on different days. Data represent arithmetic means

response of yeast plasma membrane potential to the acidification of cell suspensions turned to be nonlinear, with a moderate depolarization observed within pH=6.5−5, and a significantly steeper depolarization below pH_o 5. Again, the respective depolarization curves of the wild type and the *Δtrk1,2* mutant strains exhibited similar features, the magnitude of depolarization in the *Δtrk1,2* mutant cells being somewhat higher. The extrapolated regression lines of the high-pH parts of the depolarization curves within the higher range of pH_o (6.5−5) measured in suspensions without glucose correspond to the slopes of the depolarization straight lines for cells energized with glucose. Obviously, cells without external energy source are unable to compensate for massive proton influx at higher external H^+ concentrations.

Discussion

All three examined ions, i.e., K^+, Na^+, and H^+, caused a significant depolarization of *S. cerevisiae* plasma membrane upon increasing their concentrations in cell suspensions. The extent of potassium–induced depolarization was considerably reduced in yeast mutants lacking the Ttrk1,2 transporters. The availability of quantitative data on the extent of depolarization offers a new opportunity for interpretation of observed titration curves using an explicit theoretical model describing the relationship between plasma membrane potential and ion concentrations.

For the explanation of electrical membrane properties of living cells, in which ion translocation is mediated by specific ion channels and transporters, the equivalent-circuit equation (also known as Hodgkin-Horowicz equation) seems to be a more proper theoretical background than the alternative Goldman–Hodgkin–Katz equation, see e.g. (Jaffe 1974; Kotyk and Janacek 1977; Offner 1991). Within the framework of the equivalent circuit model under the assumption that active ion currents are negligible, the relationship between the transmembrane concentration gradients of the main players (K^+, Na^+, H^+) and the membrane potential $\Delta\Psi$ is given by

$$\Delta\Psi = \frac{g_K E_K + g_{Na} E_{Na} + g_H E_H}{g_K + g_{Na} + g_H} \quad (1)$$

where $E_K = (RT/F)\ \ln([K^+]_{out}/[K^+]_{in})$ and so forth are the Nernst-Donnan diffusion potentials corresponding to the K^+, Na^+ and H^+ transmembrane concentration gradients, and g_K, g_{Na} and g_H are membrane conductivities for K^+, Na^+ and H^+ ions, respectively, see e.g. (Johnston and Wu 1995).

Potassium concentrations measured in *S. cerevisiae* cells amount to 200–300 mM (Arino et al. 2010). Assuming that a perfect potassium homeostasis is maintained, E_K value is expected to change by about 190 mV for $[K^+]_{out}$ increasing from 0.1 to 150 mM, regardless of a particular $[K^+]_{in}$ value. Consequently, when increasing $[K^+]_{out}$ from 0.1 mM to 150 mM E_K varies from greatly negative toward much less negative values, which may eventually become close to zero at $[K^+]_{out} \approx [K^+]_{in}$. The fairly linear regression fits of $\Delta\Psi$ vs. $[K^+]_{out}$ semi-logarithmic plots measured with starved cells of wild type and *Δtok1* mutant (both containing Trk1,2) indicated that in this case the effect of increasing K^+ concentration can be interpreted in terms of the simple equivalent circuit model as a result of passive inward K^+ diffusion. At this point it is worth reminding that choline chloride was added to all KCl (as well as to NaCl) series of experiments to keep both the ionic strength of cell suspensions and the extracellular chloride concentration constant. In this way, the risk of unwanted artefacts due to osmotic phenomena and/or chloride currents was minimized.

The considerably lower extent of cell depolarization found in cells lacking Trk1,2 transporters reveals an expected fact that the two transporters play a major role in enabling the depolarizing inward flow of K^+ ions, though perhaps with a contribution of non-selective cation transport systems, such as Nha1 or Nsc1. The latter two are possibly also responsible for the K^+ induced membrane depolarization in strains lacking both Trk transporters. These results are consistent with previous findings that the plasma membrane potential of yeast is inversely correlated with Trk1,2 activity (Mulet et al. 1999; Serrano 1991). The clearly biphasic profile of K^+-titration curves in *Δtrk1,2* mutant cells energized by added glucose indicates that for a certain range of low $[K^+]_{out}$ concentrations the Pma1 proton pump is able to compensate the K^+-induced loss of intracellular negative charge by pumping H^+ out of the cells. Comparison of biphasic K^+-titration curves measured in three different situations (wild type cells and *Δtrk1,2* cells in buffers with glucose, Fig. 1, and starving *Δtrk1,2* cells, Fig. 2, triangles) reveals that this capacity of Pma1 to compensate for the depolarizing effect of K^+ influx depends on the proportion between the energy-dependent Pma1 activity and the membrane permeability defining the inward flow of K^+. The $[K^+]_{out}$ limit value below which Pma1 was able to compensate for K^+ influx in *Δtrk1,2* cells was about 25–50 mM . Interestingly, the reported limited growth of *Δtrk1,2* cells was released at comparable external K^+ concentrations (20–50 mM) (Navarrete et al. 2010).

The linear $\Delta\Psi$ vs. $[K^+]_{out}$ semi-logarithmic plots observed in starved cells containing Trk1,2 (both wild type and *Δtok1* mutant) make possible to estimate the relative K^+ conductivities for passive ion flow in these cells. Their values, $g_K/(g_K + g_{Na} + g_H)$, which can be derived from the slopes of linear fits shown in Fig. 2, were 0.23 in wild type cells, and 0.17 in *Δtok1* cells. Such low values indicate that Na^+ and H^+ conductivities of *S. cerevisiae* plasma membranes are not negligible as compared to K^+ conductivity albeit the latter is mediated by the Trk1,2 transporters. The decrease of $g_K/(g_K$

+ g_{Na} + g_H) value caused by Δtok1 deletion indicates that the potassium channel Tok1 contributes to plasma membrane permeability for K^+ in a not negligible extent.

Similar to potassium, adding sodium ions to yeast suspensions caused also significant plasma membrane depolarization upon increasing their extracellular concentration. However, the backgrounds of the effects of K^+ and Na^+ differ not only because of different sets of transporters involved, but also because of different properties of the respective Nernst-Donnan potentials (E_K vs. E_{Na}). Note, $[Na^+]_{in}$ is around 20 mM for $[Na^+]_{out}$ < 250 mM (Sunder et al. 1996). Hence, $E_{Na} = (RT/F)\ln([Na^+]_{out}/[Na^+]_{in})$ can be negative inside only for $[Na^+]_{out} < [Na^+]_{in}$ while for $[Na^+]_{out} > [Na^+]_{in}$ it turns to be positive inside. A correspondingly low extracellular sodium concentration $[Na^+]^R_{out}$ (reversal point) must therefore exist for which the sodium Nernst-Donnan potential E^R_{Na} is equal to ΔΨ, and the direction of passive sodium transmembrane fluxes reverses from inward to outward. Since the inward and outward sodium fluxes are not symmetrical, the actual sodium conductivity $g_{Na}/(g_K + g_{Na} + g_H)$ values cannot be the same for low and high ranges of $[Na^+]_{out}$. Under these conditions the Na^+-titration curves must have a biphasic character even without a significant contribution of Pma1 and/or other actively driven Na^+ fluxes. Thus, the Na^+-titration curves comprise a rather flat part related to the decreasing negative E_{Na} value in the range of $[Na^+]_{out}$ increasing towards the $[Na^+]^R_{out}$, and another more steep part related to the increasing positive E_{Na} value observed when $[Na^+]_{out} > [Na^+]^R_{out}$. Further important conclusion concerns the ion specificity of the Trk1,2 transporters. The observed compliance of the titration curves in *wt* and in Δ*trk1,2* cells indicates that the two Trk transporters do not mediate any significant Na^+ translocation across *S. cerevisiae* plasma membrane.

The Na^+-induced depolarization of *S. cerevisiae* plasma membrane is stronger in cells metabolizing glucose than in starving cells, Fig. 4. This finding is consistent with the fact that in starving cells the electro neutral $Na^+(K^+)/H^+$-antiporter, Nha1, is the prevailing system for Na^+ translocation across the plasma membrane, while the $Na^+(K^+)$-ATPase, Ena1, the major Na^+-extrusion system of *S. cerevisiae* (Serrano et al. 1999), operate only with a limited efficiency. The less efficient efflux of Na^+ from starving cells obviously results in a higher steady-state $[Na^+]_{in}$ level than in cells with enough energy to fuel Ena1. As a result of the increased steady-state $[Na^+]_{in}$ in energized cells, the sodium Nernst-Donnan potentials, E_{Na}, at higher $[Na^+]_{out}$ must be considerably lower for the starving cells than for cells metabolizing glucose. Since E_{Na} is positive inside for concentrations $[Na^+]_{out} > [Na^+]^R_{out}$, i.e., polarized opposite to ΔΨ, the plasma membrane should be, according to Eq. 1, more depolarized in cell metabolizing glucose. The existence of a significant depolarization caused by NaCl stress was also predicted by the integrative mathematical model of ion regulation in *S. cerevisiae* (Ke et al. 2013).

Hydrogen ions turned out to be the most effective ion species to depolarize *S. cerevisiae* plasma membrane. As expected, plasma membrane depolarization by H^+ was independent of the presence or absence of the two K^+ transporters Trk1,2. Whereas in cells energized with glucose the titration curves were fairly linear over the whole pH-range tested, in starving cells they were linear at higher pH_o values only. As the extracellular H^+ concentration further increased (pH ≤ 4) membrane depolarization in starving cells became stronger. In this respect it should be taken into account that contrary to both K^+ and Na^+ the Nernst-Donnan potential of H^+ ($H_H = (RT/F)\ln([H^+]_{out}/[H^+]_{in})$ across *S. cerevisiae* plasma membrane is positive over the whole range of extracellular pH_o applied in this study (pH_o 6.5 was chosen as the reference H^+_{out} concentration since at this pH_o the H^+ concentration gradient across the plasma membrane is close to zero). Hence, at lower pH_o the plasma membrane ATPase, Pma1, in starving cells cannot resolve the high electrochemical proton gradient and consequently, the plasma membrane becomes stronger depolarized. The transition from linear to rather exponential character of plasma membrane depolarization was unlike K^+ or Na^+ effects smooth demonstrating certain non-specific passive plasma membrane permeability for H^+.

The results presented in this paper showed the true extent of *S. cerevisiae* plasma membrane depolarization in the scale of mV. By this the possibility has been provided for future studies quantifying changes in the overall driving force for membrane transport upon varying physiological conditions of the cells.

Acknowledgments The generous provision of *S. cerevisiae* mutant strains by Dr. Hana Sychrová has been greatly appreciated. This work was supported by the Czech Science Foundation grant 205/10/1121, as well as by German Federal Ministry for Education and Research under the SysMO (System Biology of Microorganisms) ERA-Net grant 0315786B.

References

Arino J, Ramos J, Sychrova H (2010) Alkali metal cation transport and homeostasis in yeasts. Microbiol Mol Biol Rev 74:95–120

Banuelos MA, Sychrova H, Bleykasten-Grosshans C, Souciet JL, Potier S (1998) The Nha1 antiporter of *Saccharomyces cerevisiae* mediates sodium and potassium efflux. Microbiology 144:2749–2758

Bertl A, Bihler H, Reid JD, Kettner C, Slayman CL (1998) Physiological characterization of the yeast plasma membrane outward rectifying K+ channel, DUK1 (TOK1), in situ. J Membr Biol 162:67–80

Brachmann CB, Davies A, Cost GJ, Caputo E, Li JC, Hieter P, Boeke JD (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14:115–132

Brett CL, Tukaye DN, Mukherjee S, Rao RJ (2005) The yeast endosomal $Na^+(K^+)/H^+$ exchanger Nhx1 regulates cellular pH to control vesicle trafficking. Mol Biol Cell 16:1396–1405

Corratge-Faillie C, Jabnoune M, Zimmermann S, Very AA, Fizames C, Sentenac H (2010) Potassium and sodium transport in non-animal cells: the Trk/Ktr/HKT transporter family. Cell Mol Life Sci 67: 2511–2532

Evans HJ, Wildes RA (1971) Potassium and its role in enzyme activation. In: Proceedings of the 8th Colloquium of the International Potash Institute. International Potash Institute, Berne, pp 13–39

Gášková D, Brodská B, Heřman P, Večeř J, Malínský J, Sigler K, Benada O, Plášek J (1998) Fluorescent probing of membrane potential in walled cells: diS-C_3(3) assay in *Saccharomyces cerevisiae*. Yeast 14:1189–1197

Jaffe LF (1974) The interpretation of voltage-concentration relations. J Theor Biol 48:11–18

Johnston D, Wu SM-S (1995) Foundations of cellular neurophysiology. MIT Press, Cambridge, Chapt. 2

Ke RA, Ingram PJ, Haynes K (2013) An integrative model of ion regulation in yeast. PLoS Comput Biol 9:e1002879. doi:10.1371/journal.pcbi.1002879

Kotyk A, Janacek K (1977) Membrane transport: an interdisciplinary approach. Plenum Press, New York

Leigh RA, Jones RGW (1984) A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. New Phytol 97:1–13

Madrid R, Gomez MJ, Ramos J, Rodriguez-Navarro A (1998) Ectopic potassium uptake in trk1 trk2 mutants of Saccharomyces cerevisiae correlates with a highly hyperpolarized membrane potential. J Biol Chem 273:14838–14844

Maresova L, Urbankova E, Gaskova D, Sychrova H (2006) Measurements of plasma membrane potential changes in *Saccharomyces cerevisiae* cells reveal the importance of the Tok1 channel in membrane potential maintenance. FEMS Yeast Res 6: 1039–1046

Maresova L, Muend S, Zhang YQ, Sychrova H, Rao R (2009) Membrane hyperpolarization drives cation influx and fungicidal activity of amiodarone. J Biol Chem 284:2795–2802

Miesenbock G, De Angelis DA, Rothman JE (1998) Visualizing secretion and synaptic transmission with pH-sensitive green fluorescent proteins. Nature 394:192–195

Mulet JM, Leube MP, Kron SJ, Rios G, Fink GR, Serrano R (1999) A novel mechanism of ion homeostasis and salt tolerance in yeast: the Hal4 and Hal5 protein kinases modulate the Trk1-Trk2 potassium transporter. Mol Cell Biol 19:3328–3337

Nakayama H, Yoshida K, Shinmyo A (2004) Yeast plasma membrane Ena1p ATPase alters alkali-cation homeostasis and confers increased salt tolerance in tobacco cultured cells. Biotechnol Bioeng 85:776–789

Navarrete C, Petrezselyova S, Barreto L, Martinez JL, Zahradka J, Arino J, Sychrova H, Ramos J (2010) Lack of main K plus uptake systems in *Saccharomyces cerevisiae* cells affects yeast performance in both potassium-sufficient and potassium-limiting conditions. FEMS Yeast Res 10:508–517

Offner FF (1991) Ion flow through membranes the resting potential of cells. J Membr Biol 123:171–182

Pena A, Uribe S, Pardo JP, Borbolla M (1984) The use of a cyanine dye in measuring membrane potential in yeast. Arch Biochem Biophys 231:217–225

Pena A, Sanchez NS, Calahorra M (2010) Estimation of the electric plasma membrane potential difference in yeast with fluorescent dyes: comparative study of methods. J Bioenerg Biomembr 42: 419–432

Petrezselyova S, Zahradka J, Sychrova H (2010) Saccharomyces cerevisiae BY4741 and W303-1A laboratory strains differ in salt tolerance. Fungal Biol 114:144–150

Petrezselyova S, Ramos J, Sychrova H (2011) Trk2 transporter is a relevant player in K^+ supply and plasma-membrane potential control in *Saccharomyces cerevisiae*. Folia Microbiol 56:23–28

Plášek J, Gášková D, Lichtenberg-Frate H, Ludwig J, Höfer M (2012) Monitoring of real changes of plasma membrane potential by diS-C_3(3) fluorescence in yeast cell suspensions. J Bioenerg Biomembr 44:559–569

Rodriguez-Navarro A (2000) Potassium transport in fungi and plants. Biochim Biophys Acta 1469:1–30

Serrano R (1991) Transport across yeast vacuolar and plasma membranes. In: Broach JR, Jones EW, Prigle JR (eds) The molecular and cellular biology of the yeast Saccharomyces. Genomic dynamics, protein synthesis and energetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 523–585

Serrano R, Mulet JM, Rios G, Marquez JA, de Larrinoa IF, Leube MP, Mendizabal I, Pascual-Ahuir A, Proft M, Ros R, Montesinos C (1999) A glimpse of the mechanisms of ion homeostasis during salt stress. J Exp Bot 50:1023–1036

Sunder S, Singh AJ, Gill S, Singh B (1996) Regulation of intracellular level of Na^+, K^+ and glycerol in *Saccharomyces cerevisiae* under osmotic stress. Mol Cell Biochem 158:121–124

Zahradka J, Sychrova H (2012) Plasma-membrane hyperpolarization diminishes the cation efflux via Nha1 antiporter and Ena ATPase under potassium-limiting conditions. FEMS Yeast Res 12:439–446