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Potassium ion efflux induced by cationic compounds in yeast

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Abstract

Potassium efflux in yeast induced by several cationic compounds showed different characteristics. All of the observed efflux required glucose as substrate at the concentrations used. For most of them, the phenomenon required binding of the cationic compound to the cell surface and increased with the negative cell surface charge, and for all the compounds tested, it depended on a metabolizable substrate. Efflux induced with terbium chloride appeared more likely due to the function of a K^+/H^+ antiporter. With DEAE-dextran and dihydrostreptomycin, potassium efflux was dependent on the cell potassium content and was also sensitive to osmotic changes of the medium. DEAE-dextran-provoked efflux was not due to cell disruption. Dihydrostreptomycin seemed to activate a potassium efflux system which could not be studied in isolation, but its inhibition of potassium uptake may also be involved. Except for cells treated with ethidium bromide, no appreciable cell disruption was observed. The potassium efflux observed appears to be a membrane phenomenon reversible after washing with magnesium chloride.

Keywords: Potassium ion efflux; Cationic compound; (*Saccharomyces cerevisiae*)

1. Introduction

Studies by Rothstein and Bruce [1,2] described a potassium efflux system in yeast. An energy-dependent efflux system for potassium ions in yeast was also demonstrated by Peña and Ramírez [3]. This efflux system was stimulated by glucose or ethanol; it was also stimulated, in a substrate-dependent manner, by cationic molecules like ethidium bromide and other cationic dyes [4], dihydrostreptomycin [3], diethylaminoethyldextran [5], and lanthanide cations and other trivalent cations such as Al^{3+} [3]. The efflux produced by terbium ions appeared to be mainly of electroneutral nature and ascribed to the operation of a K^+/H^+ exchanger [3].

Work by Gustin et al.[6] and Bertl and Slayman [7] based on patch-clamp analysis revealed the presence of both a mechanosensitive ion channel and an outward rectifying K^+ channel; the latter was identified as a pH-sensitive, depolarization-activated, outwardly-rectifying potassium channel, or TOK1 [8] or DUK1 [9] or YORK [10] or YKC1 [11] channel, with properties apparently different from those of the mechanosensitive ion channel [11].

Using yeast plasma membrane vesicles, Ramírez et al. [12] and Camarasa et al. [13] provided evidence for a K^+/H^+ exchanger, which may be involved in the regulation of the internal pH of the cells when

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they accumulate high concentrations of K^+ [12]. The J0909 gene from chromosome X reported by Miosga et al. [14] predicts an 873-residue protein with sequence similarity with the putative *Enterococcus hirae* Na^+/H^+ antiporter and the putative K^+/H^+ KefC antiporter of *Escherichia coli*. Yet, the sequence of a *Saccharomyces cerevisiae* gene on chromosome IV likely encodes a Na^+/H^+ antiporter [15].

These findings prompted studies on the potassium efflux mechanisms, regulation and energetic requirements of the efflux system. In this context, cationic dyes and other molecules [3,4] have been used as a tools to reveal the interactions governing potassium efflux. In the present work, we used four structurally unrelated cationic compounds to learn more about the mode of action of these compounds and their kind of interactions with yeast cells.

2. Materials and methods

2.1. Yeast and growth conditions

The yeast *Saccharomyces cerevisiae*, isolated as a single colony from a commercial product (La Azteca, Mexico) was used for this work. Cells were grown in 500 ml of YPD medium (1% yeast extract, 2% peptone and 2% glucose) during 24 h in a gyratory shaker placed in a controlled temperature room at 30°C. They were collected and washed twice with distilled water by centrifugation. The resulting pellet was resuspended in distilled water (10%, w/w) and aerated by incubation in the same gyratory shaker during 14 h. The cells were collected again, washed twice by centrifugation and resuspended in water to a ratio of 0.5 g per ml and kept on ice until used, during the course of the same day.

2.2. External potassium determinations

K^+ movements were measured by continuously recording its external concentration with a potassium-selective electrode. Data were captured using software specially designed for that use in our laboratory. Determinations were made in 10 ml of an incubation medium consisting of 2 mM MES–TEA (pH 6.0), 10 mM glucose and 25 mg of fresh cells.

2.3. Proton pumping activity

Proton pumping activity was continuously recorded using a pH electrode. Conditions were similar to those used for external potassium determinations.

2.4. Internal pH

This parameter was followed by measuring the fluorescence changes of pyranine (8-hydroxy-1,3,6-pyrene-thiosulfonic acid) introduced by electroporation, which was carried out with a Bio-Rad gene pulser, with a pulse controller, using a mixture of 0.7 ml of the cell suspension, plus 20 μl of 100 mM pyranine. A pulse of 1500 V with a capacitance of 25 μF and a resistance of 200 Ω was applied, lasting approximately 3 ms. Then the cells were centrifuged, washed three times in a Beckman microfuge for 10 s and resuspended to its original concentration of 0.5 g/ml. The fluorescence changes at 460–520 nm were followed in a DMX-1000 spectrofluorimeter (SLM Instruments, Urbana, IL), with a temperature-controlled sample compartment (30°C) [16].

2.5. Estimation of the membrane potential

Membrane potential was estimated by following the fluorescence changes of 0.25 μM of the cyanine $DiSC_3(3)$ (dithiacarbocyanine, Molecular Probes) at 540–590 nm [17] in the DMX-1000 spectrofluorimeter (SLM Instruments) with a cell compartment controlled at 30°C and an acquisition and processing system. 25 mg of cells were incubated in 2.0 ml of the following medium: 50 mM glucose, 10 mM MES–TEA (pH 6.0), 10 μM pentachlorophenol and 10 mM $CaCl_2$.

2.6. Rubidium uptake experiments

The experiments were carried out as follows: 50 mg of fresh cells were incubated in 1 ml of a medium containing 5 mM MES–TEA (pH 6.0) buffer, 50 mM glucose, and different concentrations of $^{86}RbCl$ ranging from 50 μM to 20 mM. 100-μl samples were withdrawn at 15, 30 and 45 s, 1, 2, 4 and 8 min, and filtered through a Millipore filter (0.45 μm mean pore size). Filters were washed twice with

20 mM KCl and then dried. Radioactivity was measured in a Beckman LS6500 scintillation counter. From these data plotted against time, the initial uptake rates were obtained. Inhibition experiments were performed as indicated previously, but the incubation medium contained 50 μM ethidium bromide (EB), 50 μM dihydrostreptomycin (DHS), 50 μg diethylaminoethyldextran (DEAE-D) or 20 μM terbium chloride additional to the rest of the components.

2.7. Surface charge determinations

Surface charge determinations were made according to Theuvenet et al. [18]. Two μM 9-aminoacridine was added to a medium containing 2 mM MES–TEA buffer (pH 6.0), 1 μM antimycin A, different concentrations of any of the cationic compounds (from 1 to 100 μM) and 25 mg of fresh cells. The cells were centrifuged immediately after mixing and the supernatant was collected for fluorometric determinations of the 9-aminoacridine using the wavelength pair for excitation and emission, 400 nm and 454 nm, respectively. The surface charge was calculated from the difference after measurement with 10 mM $MgCl_2$. 9-Aminoacridine binding versus concentration of the cationic compounds plots were built and from these plots the concentration of the cationic compound inhibiting half 9-aminoacridine binding was calculated.

2.8. Loading of cells with $^{86}Rb^+$

Six grams of fresh cells were diluted at 10% in a 10 mM glucose solution, 3 000 000 dpm of 100 mM ^{86}Rb were added, and the incubation mixture was transferred to Falcon tubes and incubated during 24 h with continuous shaking at 4°C. After this time, cells were centrifuged and washed twice with distilled water and resuspended at 50% w/v for use in the Rb^+ efflux experiments.

2.9. Rb^+ efflux determinations

Cells loaded with $^{86}Rb^+$ were used to determine the efflux of the cation. Fifty mg of $^{86}Rb^+$ preloaded cells were incubated in 1 ml of a medium containing 5 mM MES–TEA buffer (pH 6.0) and 50 mM glucose, with or without any of the following cationic compounds: 50 μM $TbCl_3$, 50 μM DHS, 50 μM EB or 50 μg DEAE-D. After an incubation at 30°C for 5 min, 100-μl samples were withdrawn and filtered through a millipore filter (0.45 μm mean pore size). Filters were washed twice with 20 mM KCl, dried in air and the radioactivity was measured in a Beckman LS6500 scintillation counter. $^{86}Rb^+$ loss was determined as the difference between the control without cationic compound and those with the cationic compounds added. $^{86}Rb^+$ content in the control remained unchanged after 30 min incubation.

2.10. Terbium binding determinations

Terbium binding determinations and maximal binding of terbium and dissociation constant estimations were made by measuring the free concentration of Tb^{3+} remaining after incubation of the cells as follows: 25 mg of fresh cells were placed in 5 ml of a medium containing 5 mM MES–TEA (pH 6.0) and different concentrations of terbium chloride, from 2 μM to 30 μM. Cells were incubated for 1 min and then centrifuged; the supernatant was used for terbium determinations. Terbium concentration was measured adding different aliquots of the supernatant to 20 μM dipicolinic acid, in 5 mM MES–TEA (pH 6.0), in a final volume of 5.0 ml. fluorescence was measured at 276–545 nm as the excitation and emission wavelengths, respectively. Fluorescence values were compared to a standard curve prepared with known concentrations of $TbCl_3$. The cation forms a fluorescent adduct with dipicolinic acid, and this property of the cation has been successfully used to measure its binding to biological molecules [19].

2.11. Data analysis

All experiments were repeated at least three times, without significant differences. In most cases, typical tracings are shown. For kinetic data, values ± standard deviation are given. In the case of K^+ movements and pH changes, representative experiments are presented. Values of K_m and K_d were obtained by nonlinear regression (Inplot program) and fitted best to a rectangular hyperbola with one component. Dispersion of the data gave a maximum value of

15%. K_d is the concentration value of terbium chloride necessary to attain 50% of the maximum binding.

3. Results

The efflux of potassium ions to a potassium-free medium is a common response of the cells to the presence of some cationic compounds [3,4]. This can be observed only if an adequate substrate is present in the medium (Fig. 1A). As Fig. 1B shows, in the absence of glucose, at the concentrations of the cationic agents used, no potassium efflux was detected in the presence of DHS, EB, DEAE-D or $TbCl_3$. In the presence of glucose, at the concentrations used, this efflux showed in some instances a variable delayed response of 20 to 30 s, and after this time a steep efflux was observed. The response consisted of a transient inhibition of the potassium efflux in the presence of glucose, more or less coincident with the building of the membrane potential. It is worthwhile to mention that a dependence exists between the extent of potassium efflux and the time of fasting of the cells, which modifies the internal content of potassium (see below).

Potassium efflux induced by the addition of cationic compounds was tested for its dependence on glucose metabolism or a related event thereafter, and it was found dependent on this substrate. The efflux was not observed using 2-deoxy-D-glucose or 6-deoxy-D-glucose instead of glucose which can not be metabolized by yeast. Fig. 2A, shows the results with DHS, which were similar with the other agents (data not shown). No effect was also observed when using a poorly utilized substrate (xylose); results of Fig. 2B also show this fact. None of the substitutes for glucose used could support the efflux of potassium to the extent observed when using this substrate, indicating a metabolism-derived link as responsible for this efflux. Similar results were obtained when DEAE-D was used instead of DHS.

As Fig. 3 shows, an increase in the efflux of potassium with most cationic compounds was observed in the presence of EDTA. In the presence of this chelator, which increases the negative surface charge of the cells, potassium efflux was increased with EB and DHS. On the other hand, no change in the efflux of potassium promoted by DEAE-D was observed. The addition of EDTA, on the other hand, prevented potassium efflux by Tb^{3+}, probably because of the complexation of the cation by EDTA.

These results indicated that a negative surface charge might be involved in the efflux of potassium

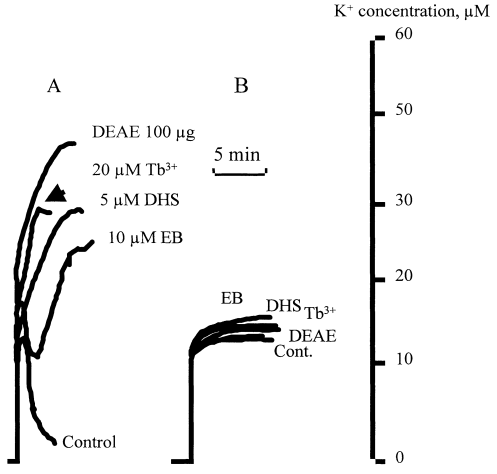

Fig. 1. Potassium efflux produced by different cationic agents; glucose requirement. Incubation conditions: 2 mM MES–TEA buffer (pH 6.0), 10 mM glucose. Final volume was 10.0 ml. 25 mg of yeast (wet weight) was added after a few seconds of incubation. In B, glucose was omitted. K^+ concentration was followed with a K^+-selective cationic electrode.

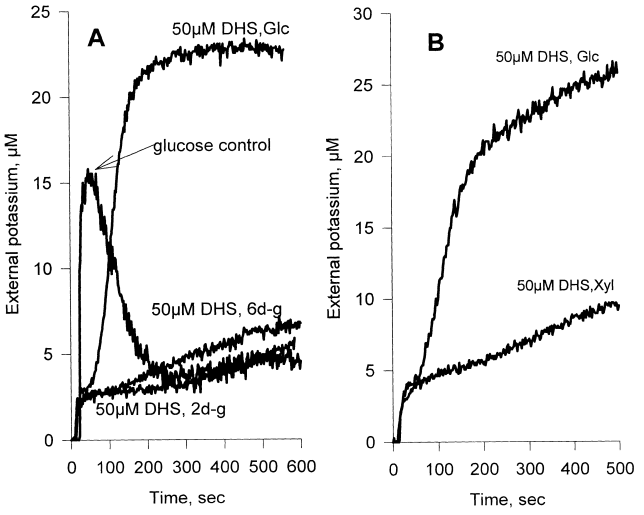

Fig. 2. K^+ efflux produced by DHS with different substrates. Potassium efflux was determined with a potassium-selective electrode. Incubation medium: 10 mM glucose, 2 mM MES–TEA buffer (pH 6.0); final volume, 10.0 ml. Where indicated, 10 mM 2-deoxyglucose and 6-deoxyglucose (A) and 10 mM xylose (B) were added instead of glucose.

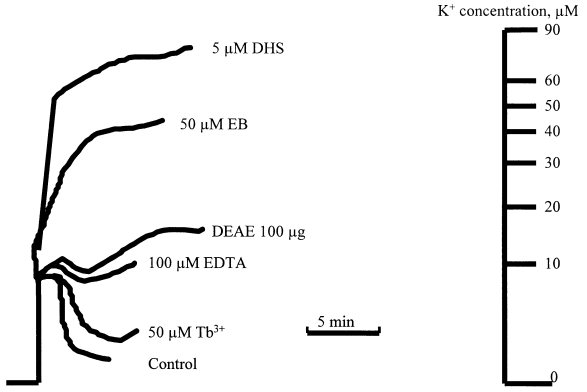

Fig. 3. Efflux of potassium produced by different cationic compounds in the presence of 100 μM EDTA. Incubation medium: 10 mM glucose, 2 mM MES–TEA buffer (pH 6.0); final volume, 10.0 ml.

when treating cells with cationic compounds; they agree with those earlier reported by Elferink and Booij [20]. Results in Table 1 agree with those of Fig. 3, in the sense that cationic compounds may be binding to negative surface charges, as shown by their ability to displace 9-aminoacridine from binding to the cells. We measured this capacity as the concentration of the cationic compound necessary to inhibit 50% binding of the dye to the cell surface; a correlation was found between the affinity of the cationic compound for the negative surface charge and the extent of potassium efflux they promoted. However, DEAE-D showed a low affinity for the negative groups on the cell surface; as a consequence, with this agent, no differences in the efflux of potassium were expected with an increased cell surface charge due to EDTA presence (Fig. 3).

From these results we could expect that if the negative cell surface charge was blocked, then no efflux of potassium would result by treatment of the cells with cationic compounds. Results of Table 2 are consistent with this view; if 1 mM $MgCl_2$ was present at the same time as the cationic compound, less potassium efflux was promoted by the cationic compound. It is important to mention that 1 mM $MgCl_2$ did not significantly affect potassium efflux or uptake in control experiments.

Measurements of terbium ion binding in the presence and absence of glucose were carried out, to define if the presence of the substrate could increase either the number or affinity of negative surface charges of the cells for the cations. Results showed no differences due to the presence of glucose. Maximal binding of terbium, and its dissociation constant in the absence of glucose were 2.15 ± 0.57 nmol/mg of fresh cells and 9.49 ± 0.42 μM, respectively, when terbium was preincubated for 1 min. In the presence of glucose and after incubating terbium during 1 min maximal binding of terbium was 2.08 ± 0.46 nmol/mg (wet weight) of cells, with a dissociation constant of 8.48 ± 1.35 μM. These results indicate that glucose does not promote any appreciable change in cell surface charge that could be detected with this method, based on the determination of residual terbium with dipicolinic acid [19].

In order to define if the cationic compounds could be removed from the cells, the experiment shown on Table 3 was carried out. The cells were incubated for 5 min with the respective cationic compound; after this time cells were collected by centrifugation, then

Table 1
Concentrations of cationic compounds inhibiting 50% of 9-aminoacridine binding

Addition	ID_{50}
Dihydrostreptomycin	5 ± 2.3 μM
Terbium chloride	10 ± 6.1 μM
DEAE-dextran	300 ± 56 μg/ml

The binding of 9-aminoacridine to the cells was determined according to Theuvenet et al. [18]. 2 μM 9-aminoacridine was added to a medium containing 2 mM MES–TEA (pH 6.0); 1 μM antimycin A, different concentrations of any of the cationic compounds (1 to 100 μM) and 25 mg of fresh cells. The cells were centrifuged immediately after mixing with 9-aminoacridine and the supernatant was collected for the spectrofluorimetric determination of the free dye.

Table 2
Effect of $MgCl_2$ on K^+ efflux produced by several cationic compounds

Treatment	Total efflux, nmol
Control	95 ± 23
1 mM $MgCl_2$	110 ± 32
50 μg DEAE-D	260 ± 42
50 μg DEAE-D, 1 mM $MgCl_2$	45 ± 12
50 μM DHS	315 ± 45
50 μM DHS, 1 mM $MgCl_2$	78 ± 23

K^+ efflux was measured by means of a potassium-selective electrode in a medium containing 25 mg (wet weight) of cells, 2 mM MES–TEA (pH 6.0), 10 mM glucose and where stated, 1 mM $MgCl_2$ and the respective concentration of the cationic compound. Final volume was 10 ml.

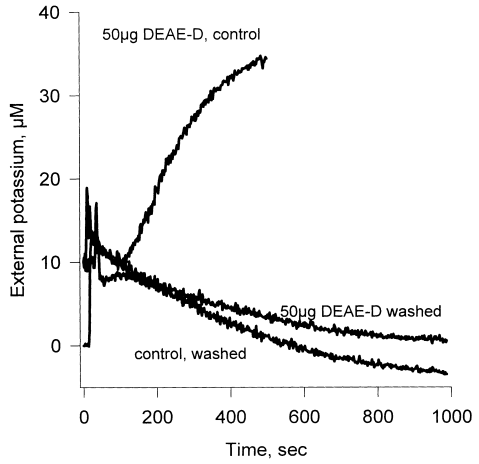

Fig. 4. Reversibility of the effect of DEAE-D by washing of the cells with $MgCl_2$. 50 mg (wet weight) of cells previously incubated with DEAE-D during 5 min were washed twice with 1 mM $MgCl_2$ and then washed with distilled water, and used for potassium efflux determinations with a potassium-selective electrode. Incubation conditions were the same as used in Fig. 1.

washed once with 1 mM $MgCl_2$ and twice with distilled water; the cells so treated were used for continuous recording of potassium in the presence of 10 μM KCl. The effect of DEAE-D disappeared after washing (Fig. 4). To a minor extent, treatment with dihydrostreptomycin could also be blocked as a result of washing. In this case the recordings also show a slow uptake of the potassium externally added. On the contrary, washing the cells treated with ethidium bromide or Tb^{3+} did not produce the disappearance of the effect promoted by the cationic compounds.

Table 3
Reversion of the effects of cationic compounds by washing with magnesium chloride

Treatment	External potassium (μM)
Control, washed	3.5 ± 1.3
20 μM $TbCl_3$	30 ± 8.4
20 μM $TbCl_3$, washed	12.5 ± 4.2
DEAE-D, 50 μg/ml	28 ± 13
DEAE-D, 50 μg/ml, washed	4 ± 1.9
50 μM DHS	33 ± 14
50 μM DHS, washed	13 ± 5.6
50 μM EB	40 ± 15
50 μM EB, washed	24 ± 7.3

25 mg (wet weight) of cells were treated with the respective concentrations of the cationic compounds in a medium containing 2 mM MES–TEA (pH 6), 10 mM glucose, during 5 min. After this time, the cells were collected by centrifugation in a microfuge, suspended in 1 mM $MgCl_2$, centrifuged and washed twice with distilled water. The cells were then incubated in 10 ml of medium containing 2 mM MES–TEA (pH 6), 10 mM glucose and 10 μM KCl for continuous recording of external potassium with a potassium-selective electrode. Values presented were obtained after 10 min of this second incubation.

The efflux of potassium might be attributed to the operation of a K^+/H^+ exchanger and also the mechanosensitive ion channel that can transport K^+ and H^+ [3,6]. This prompted the necessity of assaying the effect of cationic compounds on the internal pH and proton pumping activity. The method developed by Peña et al. [16] was used to measure the internal pH. Results on Table 4 show that DEAE-D and DHS both promoted a negligible, if any, increase in the internal pH, showing small differences between the two agents tested. On the contrary, $TbCl_3$ promoted a high internal acidification of the cell. That is, cationic compounds showed a differential effect on the cell pH; in spite of the fact that potassium efflux was the common element.

In agreement with the results of Table 4, the increase of the cell pH appeared to correspond to a proton pumping activity. Fig. 5 shows that both DEAE-D and DHS promoted a negligible increase of the proton pumping activity and this results agree with those of Table 4 in the sense that the increase in cell pH is the result of a higher proton pumping activity. On the other hand, terbium chloride diminished proton pumping activity (data not shown), in agreement with the decrease found of the internal pH value and previous results [3] (Table 4).

Table 4
Effect of the cationic compounds on internal cell pH using pyranine, introduced by electroporation as an indicator

Treatment	Internal pH
Control	6.5
DHS, 50 μM	6.6
DHS, 100 μM	6.65
$TbCl_3$, 50 μM	6.15
$TbCl_3$, 100 μM	6.12
DEAE-D, 25 μg/ml	6.7
DEAE-D, 50 μg/ml	6.75

0.7 ml of a 50% cell suspension was electroporated in the presence of 20 μl of 100 mM pyranine with a pulse of 3 ms of 1500 V at 25 μF and 200 Ω. Electroporated cells were washed three times with distilled water. Fluorescence at 460–520 nm was measured, using 25 mg (wet weight) of cells in a medium containing 2 mM MES–TEA (pH 6), 10 mM glucose and the indicated concentrations of each of the cationic compounds.

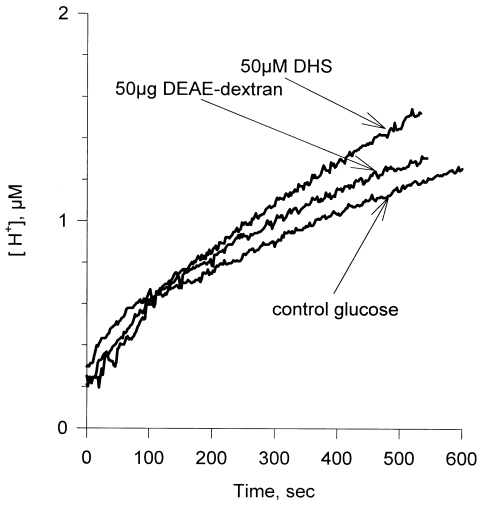

Fig. 5. Effects of DEAE-D and DHS on proton pumping by yeast. H^+ concentration was continuously recorded with a pH electrode in a medium similar to that of Fig. 1, with 50 mg of fresh cells. Where indicated, 50 μM DHS or 50 μg DEAE-D was added to the medium.

The efflux of potassium induced by DEAE-D and DHS also showed to be sensitive to changes in the osmotic pressure of the medium or to differences of osmotic pressure between the internal and external compartments (Fig. 6). When the cells were incubated with 0.8 M sorbitol, the efflux of potassium induced with 50 μg of DEAE-D was abolished; also glucose-induced potassium efflux was abolished completely. However, with DHS, only half of the potassium efflux was abolished in the presence of 0.8 M sorbitol. Similar results were obtained when 0.8 M galactose, a nonmetabolizable sugar by these cells was used instead of sorbitol, and no differences with the results of Fig. 6 could be noted; that is, galactose also abolished totally the efflux of potassium induced by DEAE-D and partially that produced with DHS. The measurement of respiratory rates by our starved cells (data not shown) showed that sorbitol was not used as a substrate by yeast and also did not interfere with glucose utilization. With these two agents, also changes in the transport kinetics of $^{86}Rb^+$ uptake were observed; DEAE-D affected V_{max} for Rb^+ uptake, but not K_m; V_{max} was 18.3 ± 3.2 in its absence, and 8.0 ± 0.96 nmol/mg fresh cells/min in the presence of 50 μg of DEAE-D and the Km varied only from 0.8 ± 0.32 to 0.5 ± 0.2 mM. DHS also diminished the V_{max} for Rb^+ uptake to 4.96 ± 1.96 nmol/mg fresh cells/min with 50 μM of DHS and the K_m (0.77 ± 0.28 mM) did not show any increase, in agreement with previous results [21].

Cell disruption was assessed by two methods: by measuring absorbency at 260 nm of material leaking out of the cells, using as the control 200 μM CTAB (data not shown) (this method was not used with ethidium bromide, because of its absorbency at the wavelength used). We also measured $^{86}Rb^+$ efflux in cells preloaded with $^{86}RbCl$ during 24 h; after this time $15 \pm 4.5\%$ of the total cell potassium had been replaced with $^{86}Rb^+$. An efflux in the range of 1–3% was observed when incubating the cells for 5 min 50 μM DHS, 50 μg of DEAE-D or 50 μM $TbCl_3$. However, incubation with 50 μM ethidium bromide under the same conditions promoted a $30 \pm 5.6\%$ loss of ^{86}Rb. It is important to point out that discrimination for K^+ over $^{86}Rb^+$ exists, judging from the failure of cationic compounds to promote a higher efflux of ^{86}Rb. In order to define if the effect of EB on K^+ efflux was only due to cell disruption or also to the competitive inhibition of monovalent cation transport by this agent, we measured the effect of this dye on Rb^+ transport: The K_m for the transport of the monovalent cation in the presence of EB was 3.6 ± 1.7 mM, as compared to 0.8 ± 0.32 mM for the

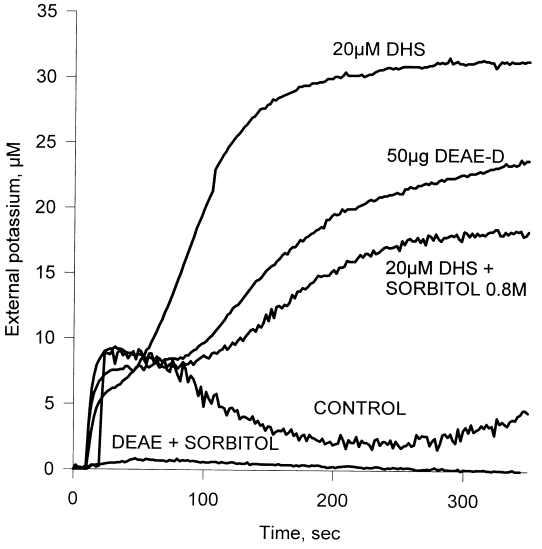

Fig. 6. Effects of 0.8 M sorbitol on potassium efflux produced by DEAE-D and DHS. Potassium efflux was followed continuously with a potassium-selective electrode in a medium and conditions similar to those of Fig. 1. Where indicated, 0.8 M sorbitol and/or 20 μM DHS and 50 μg of DEAE-D were present in the medium.

control, and the V_{max} of 13.8 ± 2.7 and 18.3 ± 3.2 nmol/mg of fresh cells/min in the presence 50 μM of EB or in its absence, respectively.

Measurements of membrane potential using a fluorescent cyanine as a probe [17] showed no significant changes of this parameter using cationic compounds at concentrations of 20–50 μM which promoted potassium efflux. Only concentrations of $TbCl_3$ 50-fold higher (2.5 mM) quenched 28 ± 3.3% the fluorescence of the control; under similar conditions, 2.5 mM DHS only produced a decrease of 11 ± 5%. DEAE-D used at the same concentration inducing potassium efflux (50 μg) only quenched 4% of the fluorescence, compared with 20 mM of KCl that quenched 16 ± 3.9% of the same control.

The efflux of potassium induced by cationic compounds showed a response depending on the cellular potassium concentration; when cell potassium decreased, a decreased efflux of potassium by DEAE-D and $TbCl_3$ was observed (data not shown). Our results showed that at a cell K^+ content of 256 mM, treating the cells for 5 min with 100 μg of DEAE-D an efflux of 11% of the cell potassium was produced; when cellular potassium was 116 mM, no efflux of potassium could be measured. For 100 μM $TbCl_3$, with a cellular potassium concentration of 215 mM, an efflux of 12% was observed, and with a cellular potassium concentration of 164 mM no efflux of potassium was observed. With EB and DHS, the response was independent of the cellular potassium. With these agents, no changes in the efflux were found in relation to cell potassium: with 100 μM DHS, for a cellular potassium of 271 and 153 mM, 16% and 13% of efflux was produced, respectively.

4. Discussion

The results presented here show that the several cationic compounds used promoted efflux of potassium with certain particularities, which probably derived from the kind of interaction with yeast cells and response elements involved in the phenomenon. For the agents used, the phenomenon appears to be unrelated to their structure, and mainly related to their charge. However, the mechanisms by which they produced K^+ efflux appear to be different; in fact, some other cations which have been tested, containing in their structure a guanido group, sulfaguanidine and arginine, for instance, did not produce a potassium efflux (data not shown), but alkylguanidines do [21]. This may indicate that the potassium efflux induced by cationic compounds is not derived from a generalized effect due to their interaction with cell surface negative charges; it appears to depend on different mechanisms characteristic to each agent, although for most of them the effect appears to involve the negative charges on the cell surface.

The experiments showed that binding of most of the cationic compounds to the negative surface charges of the cell was necessary for the phenomenon to occur, since some of the agents could be removed from the surface of the cell by washing with $MgCl_2$, showing that the efflux of potassium occurred at the level of the cell membrane. Our experiments also show that although a generalized increase of surface charge produced by EDTA favored the cationic compound binding, this is not, in all cases, a factor defining the efflux of potassium, since with DEAE-D, the presence of the chelator prevented its effect.

As previously reported [3], the efflux of potassium induced by cationic compounds showed glucose dependence as a common characteristic to all those tested, as well as to others already reported [4,21]. This dependence was not only due to transport of the substrate, because it was not observed when two transportable nonmetabolizable sugar analogues were used instead of glucose. It was also not due to a decrease of the internal pH as a result of phosphorylation of glucose or the nonmetabolizable analogue 2-deoxyglucose; if this were the case, potassium efflux should have been observed with this glucose derivative, which is phosphorylated. The efflux of potassium appears to depend on a glucose-induced establishment of a membrane potential difference; this may be inferred because when the cells were treated with DEAE-D, DHS and EB, these compounds induced a delayed response of glucose-dependent potassium efflux, approximately similar to that required for the establishment of a membrane potential, as shown by the use of the fluorescent cyanine $DiSC_3(3)$ [17]. However, it is possible that some other event related to metabolism of glucose may be involved.

Regarding the requirement of glucose to observe the effects of the cationic agents tested, another pos-

sibility was explored: a glucose-dependent increase of the negative surface charge. However, this possibility does not apply to all the cationic compounds tested, because when the surface charge was increased by EDTA treatment the effect of DEAE-D diminished, indicating that for this agent, other mechanisms are involved, although a cell surface increase should favor DEAE-D binding over the entire cell surface, because of its cationic nature. On the other hand, the effect of this compound was greatly diminished by the presence of $MgCl_2$. Also, the presence of glucose did not appear to modify the negative surface potential of the cells (see below).

Another likely explanation to reconcile these apparently contradictory results may be that glucose promotes the accessibility of particular negative charges in the vicinity of potassium transporters, making them accessible to the cationic compounds, some of which have been shown to produce an effect upon the kinetics of ion transport [22]. Except for the case of DEAE-D, this was supported by the enhanced effects of the agents in the presence of EDTA, that promotes a generalized increase of negative surface charges. Differences of DEAE-D against DHS and EB could be attributable to differences in affinity for surface charges, or other factors involved in DEAE-D binding, perhaps due to its sugar nature.

Measurement of the surface charge with 9-aminoacridine showed that, in fact, the different agents used displaced this dye from the negative charges to the surface, but again, DEAE-D was the exception. In the presence of glucose, the measurement of surface charge with this dye is difficult because the dye is taken up by the cells in the presence of glucose, as revealed by clear changes of its fluorescence during longer incubations (data not shown). However, measurement of terbium ion binding can be taken as an indication of the surface charge of the cells, and it showed no differences in the presence or absence of glucose. Neither maximal binding of terbium, nor its dissociation constant were modified in the presence of glucose as compared with control cells. These results indicate that glucose does not promote any appreciable change in cell surface, but, as mentioned before, discrete changes in the vicinity of transporters which may not have been detected with our method cannot be ruled out.

An additional finding of the experiments is that Tb^{3+} did not appear to be significantly washed from the cells, and it is not transported into the cells (data not shown). This opens the possibility to use this cation to measure the negative surface charge of the cells under different conditions, in a similar way as has been used to measure its binding to biological molecules [19]. This procedure is currently under further study in our laboratory.

Previous reports showed that cationic compounds like mercury ion [23] methylene blue [24], ethidium bromide [25] and some other compounds [26] induced an all-or-none effect in the cells, attributing potassium efflux to cell disruption. In this respect, it was confirmed that EB promoted cell disruption, but the rest of the cationic compounds tested, at the concentrations used, did not promote the disruption of the cells, as revealed by the amount of material absorbing at 260 nm as an indication of nucleotides leaking from the cells together with K^+. Also, disruption of the cells should have produced a leak of $^{86}Rb^+$, and no reversion of the effect on potassium efflux after washing should have been seen. Moreover, glucose dependence of potassium efflux and the increase of cell pH induced by the treatment are inconsistent with this mechanism. In summary, of the cations tested here, only EB, in agreement with other authors [23–25], seemed to produce cell disruption. With this agent, as well as in the case of DHS and DEAE, another factor may be involved; that is, a competitive inhibition of Rb^+ uptake [27].

Potassium efflux, especially with DEAE-D and DHS, was found to be modified by changes in the osmotic pressure of the medium with several sugars, and no efflux could be produced when the cells had a diminished cell potassium content. This is important to note, in view of previous reports [5] ascribing to DEAE-D a selective disruption of the plasma membrane, leaving intact the internal membranes of the cells in the presence of sorbitol. This behavior of the cells was not confirmed in our experiments, which is interesting, and may be due to the strain of yeast cells used.

DEAE-D, and DHS to a lesser extent, both induced potassium efflux through a mechanism apparently not related to the operation of a K^+/H^+ exchanger, but to another mechanism that can produce the efflux of potassium ions. Several possibilities exist, one of which could be the activation of an ion

channel [6]; however, from our results it is not evident that a voltage-dependent potassium channel [7–11] may be activated as a result of binding or the efflux of K^+ produced by the cationic compounds, because no appreciable changes on membrane potential were observed after binding.

Perhaps the most interesting results were those obtained with terbium chloride, which are consistent with a stimulating effect of this cationic compound on a putative K^+/H^+ antiporter [12,13] producing the extrusion of K^+ ions; this agent promoted potassium efflux concomitantly with cell acidification and had no appreciable effect on the membrane potential. None of the other cationic compounds tested showed this stimulating effect over a putative K^+/H^+ antiporter.

It is also important to point out that, except for EB, which produced the disruption of the cells, some specificity of the cation efflux system exists, since all other compounds produced a high efflux of K^+, and only a very small one for $^{86}Rb^+$.

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