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Energy-Dependent, Carrier-Mediated Extrusion of Carboxyfluorescein from *Saccharomyces cerevisiae* Allows Rapid Assessment of Cell Viability by Flow Cytometry

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Carboxyfluorescein diacetate is a nonfluorescent compound which can be used in combination with flow cytometry for vital staining of yeasts and bacteria. The basis of this method is the assumption that, once inside the cell, carboxyfluorescein diacetate is hydrolyzed by nonspecific esterases to produce the fluorescent carboxyfluorescein (cF). cF is retained by cells with intact membranes (viable cells) and lost by cells with damaged membranes. In this report, we show that *Saccharomyces cerevisiae* extrudes cF in an energy-dependent manner. This efflux was studied in detail, and several indications that a transport system is involved were found. Efflux of cF was stimulated by the addition of glucose and displayed Michaelis-Menten kinetics. A K_m for cF transport of 0.25 mM could be determined. The transport of cF was inhibited by the plasma membrane H^+-ATPase inhibitors *N,N'*-dicyclohexylcarbodiimide and diethylstilbestrol and by high concentrations of tetraphenylphosphonium ions. These treatments resulted in a dissipation of the proton motive force, whereas the intracellular ATP concentration remained high. Transport of cF is therefore most probably driven by the membrane potential and/or the pH gradient. The viability of *S. cerevisiae* was determined by a two-step procedure consisting of loading the cells with cF followed by incubation at 40°C in the presence of glucose. Subsequently, the fluorescence intensity of the cells was analyzed by flow cytometry. The efflux experiments showed an excellent correlation between the viability of *S. cerevisiae* cells and the ability to translocate cF. This method should prove of general utility for the rapid assessment of yeast vitality and viability.

Traditional enumeration of viable microorganisms by the plate count method takes several days. Hence, there is an interest in developing more rapid methods. In 1966, Rotman and Papermaster (20) reported the use of nonfluorescent precursor molecules which were taken up by mammalian cells and cleaved by intracellular enzymes to give a fluorescent product. This staining by fluorescein diacetate or related compounds is principally based on the assumption that only cells which have an intact plasma membrane are able to accumulate the fluorescent probe. Subsequently, this concept has been used by several others for the determination of viable microorganisms, yeasts, lymphocytes, and other eukaryotic cells (4–7, 26, 28). The fluorescence intensity of the individual cells can be measured by flow cytometry.

As clearly outlined by Shapiro (23), the criterion of membrane integrity for viability is only limited. The distinction between stained cells (which are considered viable) and cells which are not stained (considered nonviable) is not absolute. Cells which have lost the ability to reproduce but still have an intact membrane are nevertheless stained. In addition, the efflux of probe may frustrate the fluorescent viability staining, resulting in a decrease or loss of signal in viable cells. This excretion may be an energy-dependent process, as was shown for efflux of fluorescein and fluorescein derivatives in *Lactococcus lactis*, HeLa cells, and epithelial cells (1, 15, 16, 18).

At present, no satisfactory rapid method for the determination of yeast viability (defined as the ability of the yeast cell to reproduce) exists. The methylene blue exclusion test is used most often but has the disadvantage that it overestimates the viability (5). The use of intracellular glycogen or trehalose contents as an indication of viability is also limited since no unique correlation between the intracellular content of these compounds and the viability was found (25).

In this paper, we provide evidence that *Saccharomyces cerevisiae* extrudes cF in an energy-dependent manner, most likely via a secondary transport system. The efflux of cF from the cells was analyzed by flow cytometry, which provides a rapid cell-by-cell analysis. The implications of the accumulation and efflux of cF for the detection of yeasts and the rapid assessment of yeast viability and vitality (defined as the capacity of the yeasts to initiate metabolism rapidly after transfer from a nutrient-poor to a nutrient-rich environment [11]) by use of flow cytometry are discussed.

MATERIALS AND METHODS

Abbreviations. The following abbreviations are used in this report: cF, carboxyfluorescein; cFDA, carboxyfluorescein diacetate; TPP^+, tetraphenylphosphonium ion; DES, diethylstilbestrol; DCCD, *N,N'*-dicyclohexylcarbodiimide; HEPES, *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]; DNP, 2,4-dinitrophenol; $t_{1/2}$, time needed for 50% efflux of original probe content.

Organism and growth conditions. *S. cerevisiae* L115 was grown aerobically in Sabouraud broth at 30°C. Cells were harvested at the end of the exponential growth phase at a viable cell concentration of approximately 3×10^7 per ml of broth.

Loading of cells with cF. Washed cells were resuspended in McIlvaine buffer, which is composed of citric acid (100 mM)

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and disodium hydrogen phosphate dihydrate (200 mM) set to the desired pH (pH 4.0, unless indicated otherwise). cFDA (10 mg/ml in acetone) was added to a final concentration of 43 μM unless indicated otherwise. The suspension was incubated for 15 min at 40°C and placed on ice until further use.

Measurement of cF efflux. cF-loaded cells were washed twice and resuspended in ice-cold McIlvaine buffer, pH 4.0 or 7.3. At time zero, the tubes were placed in a water bath at 30°C, and samples (200 μl) were withdrawn and put in Eppendorf tubes, which were placed in ice. Cells were spun down immediately for 3 min in an Eppendorf centrifuge, and 150 μl of the supernatant was carefully pipetted off and diluted with 750 μl of McIlvaine buffer, pH 7.3. Inhibitor studies (see also below) were performed as described in the legends to the figures. McIlvaine buffer was replaced by an Na-HEPES buffer in experiments with the phosphate analog vanadate. Fluorescence was measured at excitation and emission wavelengths of 490 and 515 nm, respectively (at 4-nm slide width), in a Jobin Yvon JY3D spectrofluorometer supplied with a 200-W HBO mercury arc light source. Measurements were performed in the linear part of the calibration curve.

The intracellular cF concentration at the beginning of the experiment (time zero) was determined in duplicate by permeabilizing the cell suspension in McIlvaine buffer (pH 7.3) with 0.1% Triton X-100 plus 0.5 M KCl in combination with freezing and thawing (14). The cells were first washed either by repeated centrifugation and resuspension or by filtration on 0.4-μm-pore-size (diameter, 25 mm) polycarbonate membrane filters (Nuclepore) and subsequent washing with several volumes of ice-cold buffer. The permeabilized cells were centrifuged, and the cF concentration in the supernatant was determined as described above.

Flow cytometric analysis. Analysis of the fluorescence intensity of individual cells was performed by flow cytometry. *S. cerevisiae* suspensions were diluted 10-fold in McIlvaine buffer (pH 4.0 or 7.3; final cell concentration, approximately 10^7 per ml) in the absence or presence of glucose (10 mM). At time zero, the samples were placed in a water bath at 40°C. Time series were made by taking 60-μl aliquots, which were diluted into 3 ml of McIlvaine buffer (pH 7.3) and immediately analyzed with a flow cytometer (ChemFlow flow cytometer; Chemunex S.A., Maisons Alfort, France). The fluid system was composed of a closed laminar flow with a maximal flow rate of 0.4 ml/min. For flow cytometric enumeration, a sample volume of 0.20 ml was analyzed. The maximal concentration of fluorescent particles which could be measured by the ChemFlow flow cytometer was approximately 10^5 particles per ml. The instrument was supplied with a 100-W HBO mercury arc light source. The excitation wavelength was set between 450 and 490 nm by use of two bandpass filters. The emission wavelength of 515 nm was selected by a dichroic filter of 500 nm (eliminating signals of less than 500 nm) and an interferential filter (FI 515) at 515 nm.

The *y* axis of the histograms corresponds to the number of fluorescent particles. The *x* axis is divided into channels 0 to 255 relative to the fluorescence intensity of the particles. A logarithmic amplification (log 1 mode) of the incoming signal was applied to measure a wider dynamic range of signals in one histogram. The shift of the histograms was expressed by the change in the mean fluorescence of the fluorescent population. The instrument was calibrated at channel 230 in the log 1 mode with standard 2.355-μm, coumarin-6-labelled, fluorescent polystyrene latex spheres (Molecular Probes Inc., Eugene, Ore.).

Determination of proton pump activity of *S. cerevisiae*. A suspension of approximately 5×10^7 cells per ml was preincubated for 15 min at 40°C in McIlvaine buffer (pH 4.0), washed, and then resuspended in 5 mM Na-HEPES buffer (pH 7.3). Cell suspensions were stirred in a water-jacketed vessel (35°C), and the pH was recorded continuously with a pH electrode. The proton pump activity (in nanomoles of H^+ per minute per milligram of protein) was determined from the slope directly after the addition of glucose (10 mM). Inhibitor studies were performed by incubating the cells with vanadate (1 mM), DES (150 μM), or DCCD (200 μM), whereafter proton pumping was measured upon the addition of glucose.

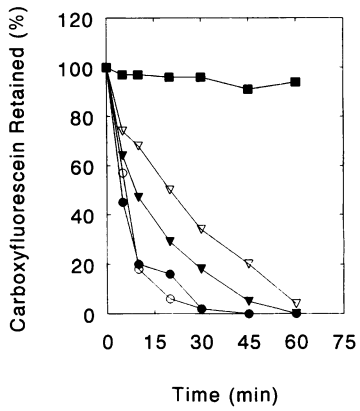

FIG. 1. Retention of cF in *S. cerevisiae* L115 under different conditions. The cells were loaded with cF by incubation at 40°C with 0.22 mM cFDA. cF efflux at pH 7.3 was measured at 0°C (■) and at 30°C in the absence (▽) and presence (▼) of glucose (10 mM). cF efflux at pH 4.0 was measured at 30°C in the absence (○) and presence (●) of glucose (10 mM).

Measurement of intracellular ATP concentration. The ATP concentration was measured in a Lumac/3M biocounter M 2010, using the Lumac luciferin-luciferase enzyme assay. The samples (20 μl) were mixed with dimethyl sulfoxide (80 μl) and diluted with 5 ml of nanopure filtered water. The luciferin-luciferase mixture (100 μl) was added to 200 μl of this diluted sample, after which the luminescence was measured.

Chemicals. cF, DES, sodium orthovanadate, DNP, benzoic acid, TPP^+, dimethyl sulfoxide, and DCCD were obtained from Sigma Chemical Co., St. Louis, Mo. cFDA was obtained from Boehringer, Mannheim, Germany. Lumit-PM (luciferin-luciferase mixture) and Lumit buffer were obtained from Perstop Analytical, Oud-Beyerland, The Netherlands.

RESULTS

Accumulation and retention of cF in *S. cerevisiae*. *S. cerevisiae* cells were loaded with cF at pH 4, and subsequently the retention of cF was studied under different conditions. cF was well retained in cells stored on ice, but at 30°C a rapid efflux of cF occurred (Fig. 1). This efflux was pH dependent and more rapid at lower pH. At pH 4 and 7.3, the $t_{1/2}$ values were approximately 7 and 21 min, respectively. Addition of glucose to the cells resulted in a significant stimulation of the cF efflux at pH 7.3 ($t_{1/2}$, 10 min), while efflux at pH 4.0 was only slightly stimulated ($t_{1/2}$, 6 min). cF efflux was also stimulated in the presence of fructose and mannose, while galactose and glycerol had no effect. Stimulation of cF efflux in the presence of metabolizable substrates suggests that cF extrusion is an energy-dependent process.

Efflux of cF against a concentration gradient. Further evidence for the involvement of metabolic energy in cF extrusion can be obtained by studying cF efflux in the presence of a

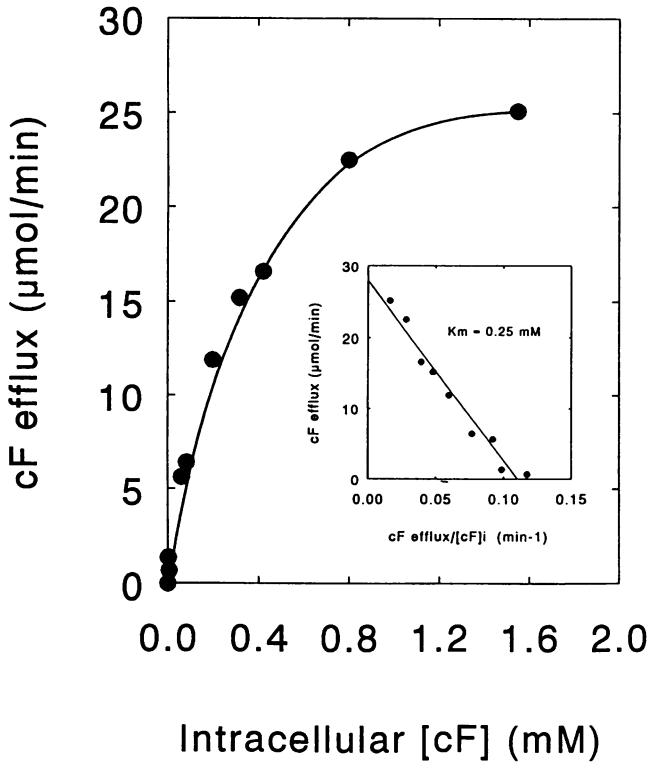

FIG. 2. Effect of intracellular cF concentration on initial rate of cF efflux. *S. cerevisiae* L115 cells were loaded with cF by incubation at 40°C with different concentrations of cFDA in McIlvaine buffer (pH 4). The cells were washed and resuspended in McIlvaine buffer (pH 7.3), and cF efflux was measured at 30°C in the presence of glucose (10 mM).

high extracellular cF concentration (1 mM). The initial intracellular cF concentration was approximately 0.5 mM. In the absence of glucose, efflux occurred at a low rate, while in the presence of glucose as an energy source, efflux was observed to proceed against a concentration gradient (data not shown). The results suggest that cF efflux in *S. cerevisiae* is mediated by an active transport system.

Kinetics of cF efflux. Cells were loaded with different amounts of cF, and the initial rate of efflux was determined (Fig. 2). The cF efflux was saturable at high internal concentrations and obeyed Michaelis-Menten kinetics. The results were analyzed in an Eadie-Hofstee plot, and a K_m of 0.25 mM for cF transport was determined, which further supports the previous observations (see above) and provides strong evidence that cF translocation occurs via a carrier-mediated process.

Temperature dependence of cF efflux. The rate of cF efflux from cells is dependent on the temperature (Fig. 1). At initial intracellular concentrations of cF considerably greater than the determined K_m, a zero-order efflux rate constant could be determined. The rate constants were plotted in an Arrhenius plot (Fig. 3), and from the slope an activation energy of 50 kJ/mol was calculated, indicative of a carrier-mediated process.

Inhibition of cF efflux. To study the mechanism of transport, the effect of various compounds on cF efflux was investigated.

(i) Effect of DNP and benzoic acid. Additional arguments for energy-dependent efflux can be obtained by inhibition of cF efflux in deenergized cells. At low external pH, yeasts can be (partly) depleted of ATP with protonophores or weak acids

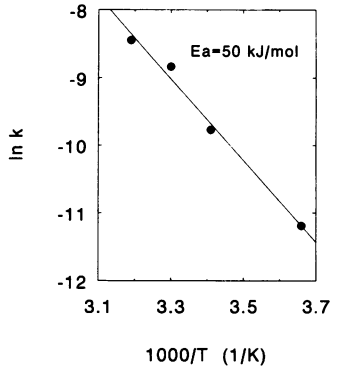

FIG. 3. Arrhenius plot of cF efflux from *S. cerevisiae*. Loading of cells with cF and subsequent analysis of cF efflux at different temperatures are described in Materials and Methods. The activation energy was calculated from the slope of the fitted line.

(22, 30, 31). Cells were preincubated with DNP (1 mM) or benzoic acid (10 mM) for 20 min at pH 4.0 and 40°C. After washing, the cells were resuspended in buffer (pH 7.3) and efflux was analyzed (Fig. 4). cF efflux was severely reduced in cells treated with DNP or benzoic acid. Control cells had lost approximately 100% of the original amount of cF in 60 min, while 14 and 58% was excreted from cells treated with DNP or benzoic acid, respectively. Apparently, under the conditions used, endogenous energy was more efficiently dissipated with DNP than with benzoic acid. In additional experiments, cF efflux was studied in the presence of glucose, and now both control cells and DNP-benzoic acid-treated cells extruded cF at a higher rate (data not shown). These results support the presence of an energy-dependent cF extrusion system.

(ii) Effect of TPP^+. Is cF transport mediated by an ATP-dependent transport system or by a secondary transport system which is driven by the proton motive force? Attempts to measure the membrane potential with low concentrations of the lipophilic cation TPP^+ (final concentration, 4 µM) failed, which might be due to a low uptake rate of TPP^+ (3). However, the membrane potential in yeasts can be abolished

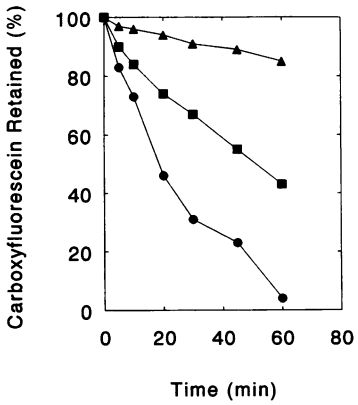

FIG. 4. Effect of benzoic acid and DNP on cF efflux in *S. cerevisiae* L115. Cells were preincubated for 20 min at 40°C in McIlvaine buffer (pH 4) in the absence (●) or the presence of 1 mM DNP (▲) or 10 mM benzoic acid (■). After washing, the cells were loaded with cF by incubation at 40°C with 0.043 mM cFDA in McIlvaine buffer (pH 4), washed, and resuspended in McIlvaine buffer (pH 7.3). cF efflux was determined at 30°C in the absence of glucose.

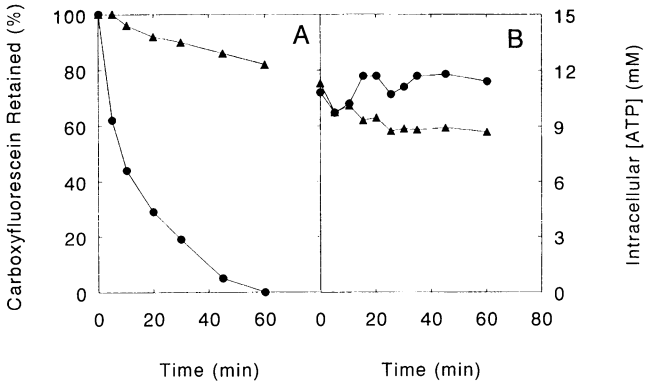

FIG. 5. Effect of TPP^+ (10 mM) on cF efflux (A) and intracellular ATP concentrations (B) in *S. cerevisiae* L115. Cells were loaded with cF at 40°C with 0.043 mM cFDA in McIlvaine buffer (pH 4.0), washed, and resuspended in McIlvaine buffer (pH 7.3). cF efflux and intracellular ATP concentrations were determined at 30°C in glucose-energized cells in the absence (●) and presence of (▲) 10 mM TPP^+.

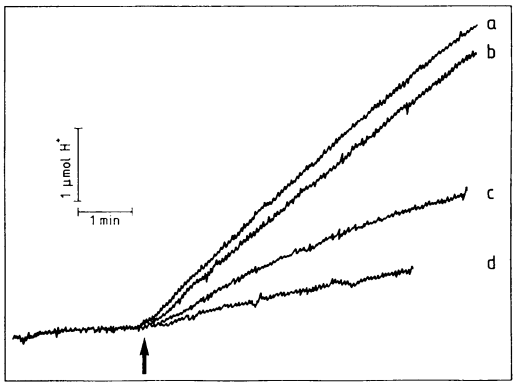

FIG. 6. Effect of plasma membrane ATPase inhibitors on H^+-pumping capacity in *S. cerevisiae* L115. Cells were loaded with cF by incubation with 0.043 mM cFDA in McIlvaine buffer (pH 4.0) at 40°C. Cells were washed and resuspended in 5 mM Na-HEPES buffer (pH 7.3). Glucose (10 mM) was added at the time indicated by the arrow. The assay was performed at 35°C. The following additions were made: a, none; b, vanadate (1 mM); c, DCCD (200 μM); d, DES (150 μM).

by adding high concentrations (e.g., 10 mM) of TPP^+ (10). Nigericin and valinomycin are not very effective on the plasma membrane of yeasts (12), and we therefore decided to use TPP^+ to dissipate the membrane potential in *S. cerevisiae*. cF efflux from cells in the presence of glucose was almost completely inhibited when TPP^+ was added to a final concentration of 10 mM (Fig. 5A). However, the intracellular ATP levels were only slightly reduced under these conditions and were very similar to those in the control cells (Fig. 5B). These results suggest that cF efflux is driven by the proton motive force.

(iii) Effect of ATPase inhibitors. Further arguments for the involvement of the proton motive force in carrier-mediated cF efflux were obtained by investigating the effect of plasma membrane H^+-ATPase inhibitors. Experiments in cells and in isolated yeast plasma membranes have shown that the H^+-extruding ATPase is inhibited by vanadate, DES, and DCCD (9, 21, 24). The effect of these compounds on proton pumping activities in glucose-energized cells of *S. cerevisiae* is shown in Fig. 6. In the presence of glucose only (10 mM), the initial H^+ extrusion was approximately 540 nmol of H^+/min/mg of protein. In the presence of DCCD (200 μM) or DES (150 μM), H^+ pumping was reduced to 330 and 122 nmol of H^+/min/mg of protein, respectively (40 and 80% inhibition with DCCD and DES, respectively). In the presence of the phosphate analog vanadate (24), H^+ extrusion was only slightly affected (Fig. 6). Longer preincubation of the cells (45 min) with DCCD or vanadate did not result in increased inhibitory effects.

The inhibition of the proton pump was correlated with the inhibition of cF efflux. In energized cells, DES strongly inhibited the efflux of cF, while DCCD showed a smaller inhibitory effect (Fig. 7A). The intracellular ATP concentration was not much influenced by DES or DCCD (Fig. 7B). Vanadate (1 mM) did not have an effect on cF efflux (data not shown), which is in line with the previous observation that it did not affect H^+ pumping (Fig. 6). These results show that dissipation of the proton motive force or inhibition of the proton motive force generation by the plasma membrane H^+-ATPase results in a strong reduction of cF efflux. Since under these conditions the intracellular ATP concentrations remained high (Fig. 6B and 7B), it is unlikely that cF is extruded via an ATP-dependent transport system. The results favor a secondary transport system for cF.

Flow cytometric analysis of efflux. The accumulation of cF in yeast cells is principally based on membrane integrity. However, "vital" cells translocate cF to the external environment (see above). A relatively simple method to determine efflux of fluorescent probes from individual cells is flow cytometry. As illustrated in Fig. 8, the cF fluorescence of individual cells shown in the histograms was quite heterogeneous. The mean intracellular cF concentration of a *S. cerevisiae* culture loaded by using cFDA was about 0.1 to 0.4 mM.

Fig. 8A-1 shows a typical shift to the left of the histograms due to the loss of fluorescence at 40°C by yeast cells previously loaded with cF. As expected from the results presented above, addition of glucose caused a significant extra shift in the fluorescence intensity of the cells as a function of time (Fig. 8B-1). In a subsequent series of experiments, cF retention was analyzed in *S. cerevisiae* cells which were stressed in different ways: (i) a short incubation at high temperature (1.5 min at 60°C); (ii) preincubation with DNP (1 mM); or (iii) preincu-

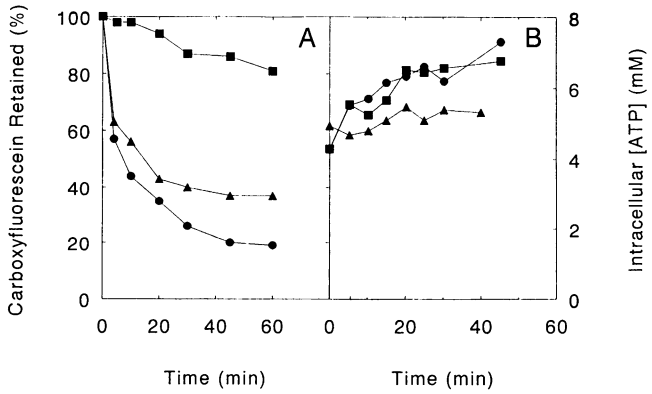

FIG. 7. Effect of plasma membrane ATPase inhibitors on cF efflux (A) and intracellular ATP concentrations (B) in *S. cerevisiae* L115. Cells were loaded with cF at 40°C with 0.043 mM cFDA in McIlvaine buffer (pH 4.0), washed, and resuspended in McIlvaine buffer (pH 7.3). The assay was performed at 30°C in the presence of glucose (10 mM). The following additions were made: ●, none; ■, DES (150 μM); ▲, DCCD (100 μM).

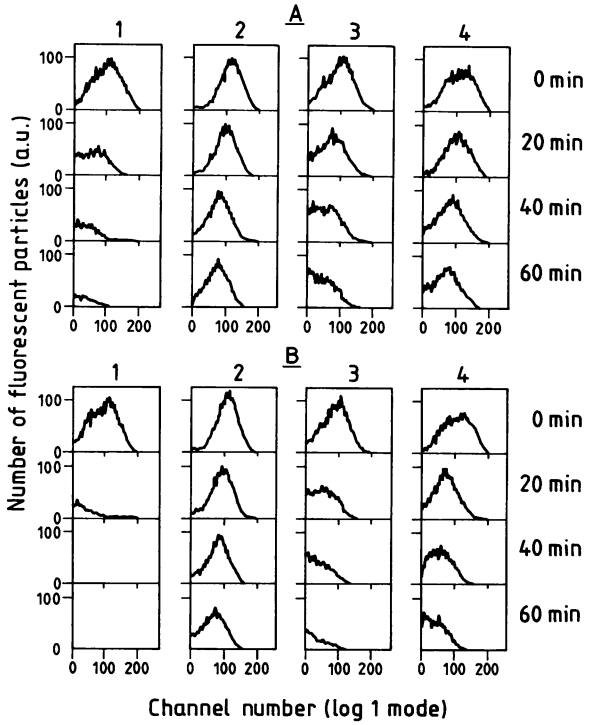

FIG. 8. Histograms of an *S. cerevisiae* population. Cells were loaded with 0.043 mM cFDA at 40°C. Subsequently, loss of fluorescence at 40°C after 0, 20, 40, and 60 min was measured with a flow cytometer in nonenergized cells (A) and in cells energized with glucose (10 mM) (B). Cells were pretreated as follows: 1, no treatment; 2, heat treatment at 60°C for 90 s; 3, preincubation with benzoic acid (10 mM); and 4, preincubation with DNP (1 mM). a.u., arbitrary units.

bation with benzoic acid (10 mM). The histograms show that neither the initial number of fluorescent particles nor the initial fluorescence intensity of cF-loaded cells was significantly decreased (Fig. 8A). However, the decrease in viable cells as determined from the decrease in CFU after the temperature treatment of the *S. cerevisiae* suspension was more than 4 log units. In these temperature-treated cells, the shift to the left of the histogram appeared to be strongly reduced (Fig. 8A-2), and upon addition of glucose no additional shift in the histogram was observed, which indicates that the cells were not vital (Fig. 8B-2). In contrast, washed cells preincubated with benzoic acid did not show any decrease in the viable plate count. The histograms showed a reduction in the shift of the histogram, indicating that the cells were stressed (Fig. 8A-3). However, subsequent addition of glucose again induced a significant decrease in the fluorescence intensity, which indicates that the cells were indeed still vital. After preincubation with DNP, the cells were severely stressed and a slight decrease in viable counts was observed (data not shown). The limited shift in the absence of glucose coincides with this observation (Fig. 8A-4), but the shift upon addition of glucose indicated that the largest part of the population was still vital (Fig. 8B-4).

The extent to which a yeast population loses its fluorescence was correlated in flow cytometry experiments with yeast "viability." Viable and nonviable cells (determined by the plate count method) were mixed at different ratios. As demonstrated in Fig. 9, an excellent correlation was found between the viable counts as determined by the plate count method (counting after 5 days of incubation at 30°C on Sabouraud agar) and the

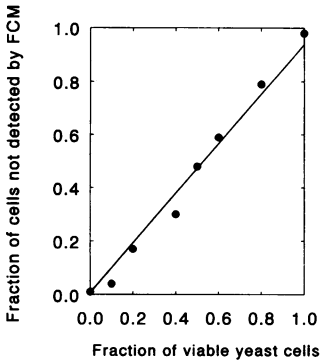

FIG. 9. Comparison of the fraction of viable cells and the fraction of *S. cerevisiae* L115 cells which lost all fluorescence. The viable cell counts were determined by the plate count method. The cells were loaded with cF by incubation with 0.043 mM cFDA in McIlvaine buffer (pH 4.0), washed, and resuspended in McIlvaine buffer (pH 7.3). The efflux assay was performed at 40°C. The fluorescent cells were counted at time zero and after 30 min. FCM, flow cytometry.

fraction of yeast cells which had lost approximately 100% of cF after incubation for 30 min at 40°C, such that they could not longer be detected with the flow cytometer.

DISCUSSION

This report shows that *S. cerevisiae* actively extrudes cF via a transport system. Passive transport of cF is not very likely since at physiological pH cF has predominantly a threefold negative charge and can thus be considered practically membrane impermeable (13). Furthermore, it has previously been shown that cF leaks indeed only very slowly from artificial membrane vesicles (31). The activation energy found for cF efflux (50 kJ/mol) is more in agreement with a carrier-mediated process, which is in the range of 30 to 84 kJ/mol (16), than with passive diffusion. In addition, the cF efflux was saturable and obeyed Michaelis-Menten kinetics. Also, the inhibition of cF efflux by high concentrations of TPP^+ and by the H^+-ATPase inhibitors DCCD and DES indicates that cF is extruded in an energy-dependent manner. Since in the presence of these inhibitors the proton motive force was dissipated while the intracellular ATP concentrations remained high, the cF extrusion system is most likely correlated with the magnitude of the proton motive force and not directly with ATP or a related compound.

Is transport of cF driven by the membrane potential (negative inside) and/or the proton gradient (ΔpH, alkaline inside)? The activation of the H^+-ATPase in *S. cerevisiae* by glucose is well documented, although the mechanism is still a matter of discussion (2, 19, 22, 24, 27), and results in the generation of a membrane potential (negative inside) and a pH gradient (alkaline inside). The increased rate of cF efflux at pH 4 versus pH 7.3 might be indicative of the involvement of the pH gradient (ΔpH). The higher efflux rate at pH 4.0 is unlikely to be the result of an increased passive efflux, because addition of DNP or benzoic acid at pH 4 (inducing a decrease of the pH_i) does not stimulate but rather severely inhibits the efflux of cF.

We suggest that there may exist in *S. cerevisiae* a transport system for anionic compounds which is most likely driven by the proton motive force via a solute/proton-antiport or uniport mechanism. This transport system might be involved in the extrusion of (toxic) compounds from the cell (16). However, substrates for described (bacterial) multidrug resistance-like solute/proton-antiport systems are not negatively, but mostly

positively, charged molecules (17). Extrusion of cF in *S. cerevisiae* is not inhibited by reserpine, a typical inhibitor of multidrug resistance systems (1). The physiological role of this efflux system therefore remains to be elucidated.

S. cerevisiae cells can be loaded with cF by incubation with cFDA. The final fluorescence intensity of the loaded cells will depend on the membrane integrity, the intracellular esterase activity, the intracellular pH, and the loss of the fluorescent dye during the loading. Hence, the fluorescence intensity is not necessarily reflecting the viability as such. The only reasonable assumptions which can be made are that stained cells do not have severely damaged membranes and contain esterase activity. However, information about the energy status in vivo can be obtained by the efflux properties of a cell population loaded with cF which can easily be determined with flow cytometry. The histogram will shift to the left side of the x axis as a result of the loss of intracellular fluorescence, and this phenomenon is apparently correlated to the ability of the cell to synthesize ATP and subsequently generate a proton motive force.

At 40°C, the cF efflux from the viable yeasts within 30 min was excellently correlated with the viability determined by the plate count method (Fig. 9). However, the test conditions (loading procedure, temperature and pH during efflux, glucose concentration, and the time interval for the measurement) play a key role in the final shift of the histogram. Hence, for each application, validation and standardization of the different parameters will be necessary.

In conclusion, we suppose that the determination of the cF efflux rate potentially allows a rapid and quantitative determination of the change in metabolizing capacity of a cell population upon addition of a metabolizable substrate, i.e., measurement of yeast vitality. This method should prove of general utility for the rapid assessment of yeast viability and vitality, especially for the determination of pitching yeast quality.

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