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Transport of Lactate and Other Short-Chain Monocarboxylates in the Yeast *Saccharomyces cerevisiae*

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***Saccharomyces cerevisiae* IGC4072 grown in lactic acid medium transported lactate by an accumulative electroneutral proton-lactate symport with a proton-lactate stoichiometry of 1:1. The accumulation ratio measured with propionate increased with decreasing pH from ca. 24-fold at pH 6.0 to ca. 1,400-fold at pH 3.0. The symport accepted the following monocarboxylates (K_m values at 25°C and pH 5.5): D-lactate (0.13 mM), L-lactate (0.13 mM), pyruvate (0.34 mM), propionate (0.09 mM), and acetate (0.05 mM), whereas apparently a different proton symport accepted formate (0.13 mM). The lactate system was inducible and was subject to glucose repression. Undissociated lactic acid entered the cells by simple diffusion. The permeability of the plasma membrane for undissociated lactic acid increased exponentially with pH, and the diffusion constant increased 40-fold when the pH was increased from 3.0 to 6.0.**

Recently it was shown that the yeast *Candida utilis* is capable of transporting lactate by a proton symport (4, 9) and that the symport also accepts acetate, propionate, and pyruvate (9). No other information seems to have been published on transport mechanisms in yeasts for short-chain monocarboxylic acids. The subject is of interest since about one-half of the 500-odd yeast species which are currently recognized (1, 7) are able to use lactic acid as a carbon and energy source, and growing yeast on acetate medium is often used to relieve glucose repression or to induce sporulation (2). There is also a practical dimension to the question because yeasts have been proposed for single cell protein production from waste lactic acid and other monocarboxylic acids (6, 12, 13). Furthermore, yeasts may cause spoilage in brined and pickled vegetables by consuming lactic acid (11, 14).

Here we report results of a study on membrane transport of lactic acid and other monocarboxylic acids in a strain of the yeast *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Microorganism and growth conditions. *S. cerevisiae* IGC4072 used in the present study was originally isolated from a sample of Fermivin, an industrial wine yeast distributed by Rapidase, Selin, France. It was maintained on media containing glucose (2%, wt/vol), peptone (1%, wt/vol), yeast extract (0.5%, wt/vol), and agar (2%, wt/vol). For growth under conditions of glucose repression, a mineral medium with vitamins and 5.0% (wt/vol) glucose (15) was used at 25°C with mechanical shaking. Derepressed conditions were obtained by substituting 0.5% (wt/vol) DL-lactic acid for glucose in the medium described above.

Measurement of DL-lactic acid uptake rates. Cells were harvested in mid-exponential phase, centrifuged, washed twice with ice-cold distilled water, and resuspended in ice-cold distilled water, with a final concentration of about 40 mg (dry weight) per ml. To estimate uptake rates of labeled DL-lactic acid, 5-μl amounts of the yeast suspension were mixed in 10-ml conical centrifuge tubes with 35 μl of 0.1 M Tris buffer at the desired pH value. After 2 min of incubation at 25°C in a water bath, the reaction was started by the addition of 10 μl of an aqueous solution of DL-[1-^{14}C]lactic acid at the desired concentration and stopped by dilution with 5 ml of ice-cold water. Sampling times for DL-lactic acid-grown cells (active transport of lactate) were 0, 5, and 10 s. Sampling times for glucose-grown cells (simple diffusion of undissociated lactic acid) were 0, 1, and 2 min. The reaction mixtures were filtered immediately through Whatman GF/C membranes, washed on the filter with 10 ml of ice-cold water, and counted in a scintillation fluid that contained 10% (wt/vol) naphthalene, 0.7% (wt/vol) 2,5-diphenyloxazole (PPO), and 0.03% (wt/vol) 1,4-bis-[2]-(5-phenyloxazolyl)benzene (POPOP) in 1,4-dioxane. Radioactivity was measured in a Packard liquid scintillation counter.

For the proton-lactate symport, uptake rates were also estimated by measuring proton uptake with a standard pH meter (PHM 62; Radiometer, Copenhagen, Denmark) connected to a flatbed Perkin-Elmer 024 recorder. The pH electrode was immersed in a water-jacketed chamber of 10-ml capacity kept at 25°C and magnetically stirred. To the chamber were added 4.0 ml of Tris (10 mM) and 1.0 ml of yeast suspension. The pH was adjusted to the desired value, and a base line was obtained. The desired amount of DL-lactic acid (adjusted to the experimental pH value) was added, and the subsequent alkalinization was monitored in the recorder. The slope of the initial part of the pH trace was used to calculate the initial uptake rate. Calibration was performed with HCl.

Measurement of the uptake rates of other monocarboxylic acids. Proton symport activity with respect to D-lactic acid, L-lactic acid, pyruvic acid, acetic acid, propionic acid, and formic acid was estimated by using lactic acid-grown cells and by measuring proton uptake as described above for DL-lactic acid.

Measurement of the accumulation of propionic acid. Preliminary experiments revealed that propionic acid was not metabolized by *S. cerevisiae* IGC4072. Since the organism

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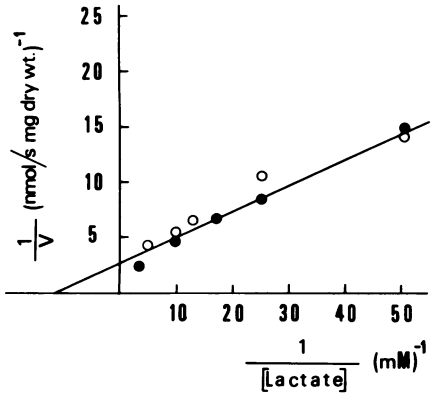

FIG. 1. Lineweaver-Burk plots of initial uptake rates at pH 5.5 of DL-lactic acid by lactic acid-grown *S. cerevisiae* 4072 cells as a function of the lactate concentration. Symbols: ●, initial uptake rates of labeled DL-lactic acid; ○, initial disappearance rates of protons after addition of DL-lactic acid.

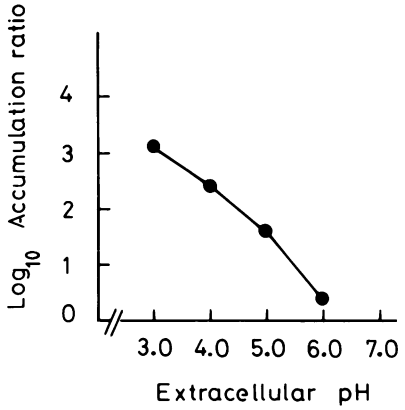

FIG. 3. Dependence on extracellular pH of the accumulation ratio of labeled propionic acid in lactic acid-grown *S. cerevisiae* 4072 cells.

was found to use the lactate-proton symport (see Results), propionic acid could be used to study the accumulative capacity of the symport without interference from metabolism. DL-Lactic acid-grown cells (40 μl; 40 mg [dry weight] per ml of water) were added to 120 μl of 0.1 M Tris buffer (adjusted to the desired pH value) and also to 120 μl of buffer (pH 5.5) containing carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and incubated at 25°C with magnetic stirring. The reaction was started by the addition of 40 μl of 5 mM [2-^{14}C]propionic acid (about 1,600 cpm/nmol). At appropriate times, 10-μl samples were taken from the reaction mixture and filtered immediately through Whatman GF/C membranes. The filters were washed three times with ice-cold water, and the radioactivity was counted as indicated above. The intracellular concentration of propionic acid was calculated assuming that 1 mg (dry weight) of the yeast contained 2.0 μl of intracellular water (5).

Estimation of glucose. Glucose was estimated by the glucose oxidase method (Test-Combination catalog no. 716 251; Boehringer Mannheim).

Calculation of concentrations. Concentrations of monocarboxylates and undissociated monocarboxylic acids were calculated by the use of the Henderson-Hasselbalch equa-

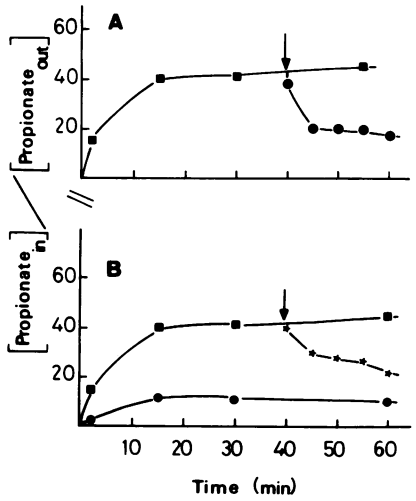

FIG. 2. Accumulation of labeled propionic acid by lactic acid-grown *S. cerevisiae* 4072 cells. The initial extracellular concentration of propionic acid was 0.64 mM. At the times indicated (arrows), one-half of the suspension received (A): ●, cold propionic acid to a final concentration of 0.2 M, or (B) ★, CCCP to a final concentration of 1 mM; ●, CCCP was added to the reaction mixture before the addition of labeled propionic acid.

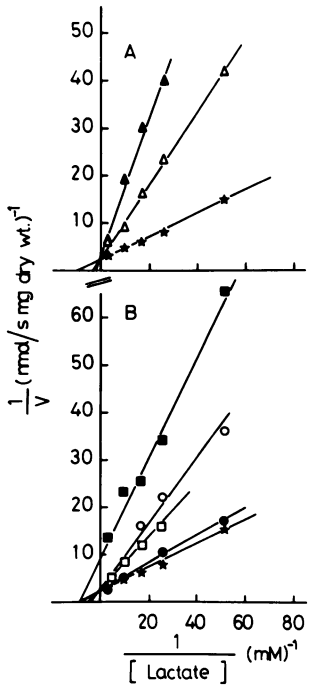

FIG. 4. Lineweaver-Burk plots of initial uptake rates at pH 5.5 of labeled DL-lactic acid by lactic acid-grown *S. cerevisiae* 4072 cells as a function of the lactate concentration. (A) Symbols: ★, uptake in the absence of other monocarboxylic acids; △, uptake in the presence of 1.0 mM L-lactic acid; ▲, uptake in the presence of 1.0 mM D-lactic acid. (B) Symbols: ★, uptake in the absence of other monocarboxylic acids; ●, uptake in the presence of 1.5 mM propionic acid; □, uptake in the presence of 0.6 mM pyruvic acid; ○, uptake in the presence of 1.0 mM acetic acid; ■, uptake in the presence of 1.0 mM formic acid.

tion with the following pK values: DL-lactic acid, 3.83; L-lactic acid, 3.79; pyruvic acid, 2.49; propionic acid, 4.87; acetic acid, 4.74; and formic acid, 3.77.

RESULTS AND DISCUSSION

Characterization of the lactate transport system of *S. cerevisiae*. Proton signals were observed when DL-lactic acid was added to suspensions, in weak buffer (pH 5.5), of cells that had been grown in lactic acid medium. Lineweaver-Burk plots of the initial DL-lactic acid uptake rates, calculated from the slopes of the proton signals, as well as of the initial rates of uptake of labeled DL-lactic acid, were linear and coincident as a function of lactate concentration (Fig. 1), which revealed that one proton was taken up for each lactate molecule transported. Since the relative concentration of undissociated lactic acid at pH 5.5 is low (less than 2.5%), it was unlikely that the alkalinization of the external medium was due to facilitated diffusion of undissociated acid. Rather, it appeared that the transport of lactic acid at pH 5.5 represented an electroneutral proton-lactate symport with a stoichiometry of 1:1.

The transport of labeled propionic acid, a nonmetabolizable lactate analog, was accumulative (Fig. 2). The accumulation was prevented by the uncoupler CCCP (Fig. 2b), whereas cold propionic acid (Fig. 2a) or CCCP (Fig. 2b) induced the efflux of accumulated free propionic acid.

When a proton-substrate symport is electroneutral, the accumulation ratio of the substrate should depend exclusively on the ΔpH across the plasma membrane and should in the present case obey the following relation (3): $[\text{Lactate}]_{in}/[\text{Lactate}]_{out} = [H^+]_{out}/[H^+]_{in}$. Taking the logarithm and rearranging, we obtain the following relation: $\log_{10}$ accumulation ratio $= pH_{in} - pH_{out}$.

TABLE 1. Kinetic parameters of the monocarboxylate-proton symport of *S. cerevisiae* 4072 at 25°C and pH 5.5

Monocarboxylate	V_{max} (nmol s^{-1} mg [dry wt]$^{-1}$)	K_m (mM)
L-Lactate	0.34	0.13
D-Lactate	0.48	0.13
Pyruvate	0.90	0.34
Propionate	0.50	0.09
Acetate	0.75	0.05
Formate[a]	0.21	0.13

[a] Apparently transported by another proton symport.

According to this equation, if pH_{in} is a constant, the $\log_{10}$ accumulation ratio against the extracellular pH value should be linear with a slope of 1. The accumulation ratio increased with increasing pH from about 24-fold at pH 6.0 to about 1,400-fold at pH 3.0 (Fig. 3). However, the relation was not linear, and the slope of the logarithm plot decreased with decreasing pH. This behavior can be explained in terms of a decrease of the intracellular pH when the extracellular pH is lowered (10). The observed accumulation behavior is consistent with transport by an electroneutral proton-lactate symport but does not constitute final proof because simple or facilitated diffusion of undissociated lactic acid in the absence of lactate transport might display similar accumulation.

It was reported earlier that the proton-lactate symport of human erythrocytes (3) was specific for L-lactate, whereas both enantiomers were transported by the lactate-proton symport of the yeast *C. utilis* (9). Our results with respect to the yeast *S. cerevisiae*, showed that, as in *C. utilis*, either enantiomer was a competitive inhibitor of the transport of labeled DL-lactate (Fig. 4a). Both were transported by a

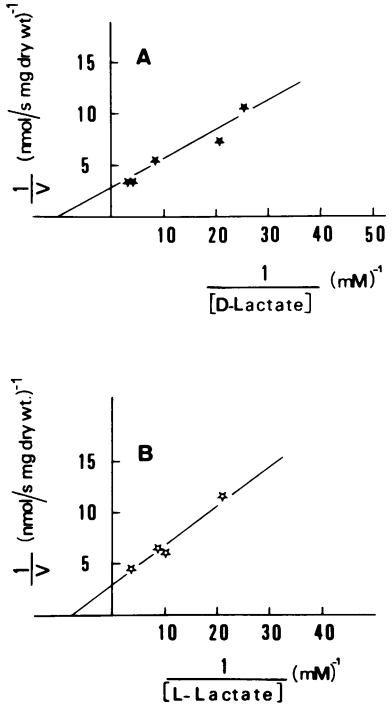

FIG. 5. Lineweaver-Burk plots of initial uptake rates at pH 5.5 of protons by lactic acid-grown *S. cerevisiae* 4072 cells as a function of the lactate concentration. (A) D-Lactate. (B) L-Lactate.

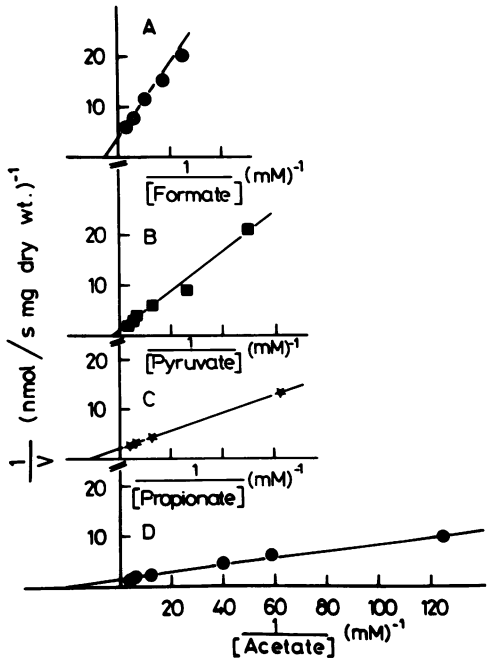

FIG. 6. Lineweaver-Burk plots of initial uptake rates at pH 5.5 of protons by lactic acid-grown *S. cerevisiae* 4072 cells as a function of monocarboxylate concentration. (A) Formic acid. (B) Pyruvic acid. (C) Propionic acid. (D) Acetic acid.

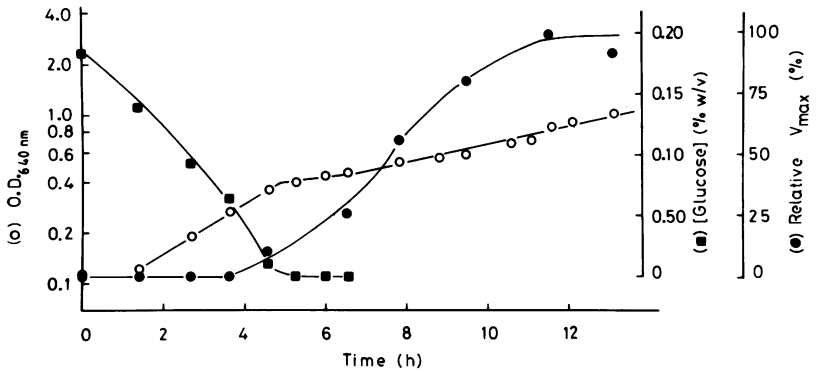

FIG. 7. Growth of *S. cerevisiae* 4072 at pH 5.5 in a stirred mineral medium with vitamins, glucose (0.2%, wt/vol), and DL-lactic acid (0.5%, vol/vol). Symbols: ■, glucose concentration (%, wt/vol); ○, cell density (OD_{640} 10^1); ●, relative activity of the proton-lactate symport measured at a saturating concentration of DL-lactic acid.

proton symport (Fig. 5), and displayed identical kinetic parameters (Table 1).

Acetic acid, pyruvic acid, and propionic acid were competitive inhibitors of DL-lactate transport at pH 5.5 (Fig. 4b), suggesting that these acids, if transported at all, are also accepted by the proton-lactate symport as monocarboxylates. The three acids were indeed transported by lactate-grown *S. cerevisiae* cells, displayed Michaelis-Menten kinetics and were transported by a proton symport which, in all likelihood, is the proton-lactate symport (Fig. 6; Table 1). Formic acid also appeared to be accepted by a proton symport (Fig. 6b; Table 1). However, the inhibition of DL-lactate transport at pH 5.5 by formic acid was noncompetitive (Fig. 4b), suggesting that formate was transported by a proton symport distinct from the proton-lactate symport.

The parameters in Table 1 were obtained at pH 5.5. From Lineweaver-Burk plots obtained at various pH values, it was learned that the affinity of the proton-lactate symport for DL-lactate, expressed as K_m, as well as the capacity of the system, expressed as the maximum transport velocity, were not significantly affected by pH over the range (3.0 to 6.0) used in the experiments (data not shown).

S. cerevisiae cells grown in media with either ethanol (0.5%, wt/vol) or glycerol (0.5%, wt/vol) as the carbon source did not display proton-lactate symport activity. Growth in a medium containing glucose (0.2%, wt/vol) and DL-lactic acid was diauxic. Proton-lactate symport activity became detectable only after the glucose had been consumed (Fig. 7). On the basis of these observations, we concluded that, as in the case of the proton-lactate symport of the yeast *C. utilis*, the proton-lactate symport of *S. cerevisiae* was also inducible and subject to glucose repression.

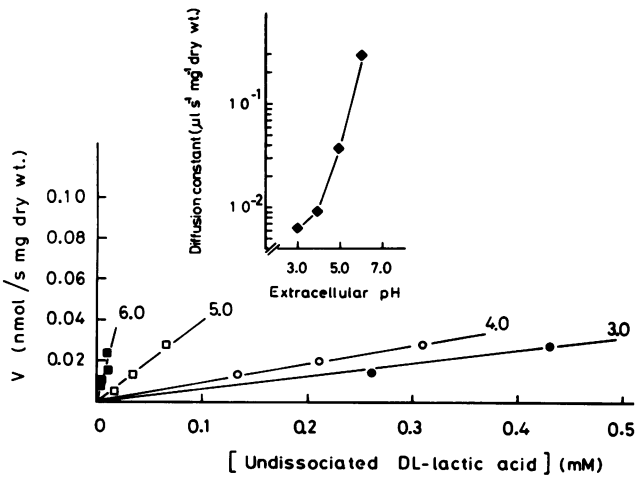

FIG. 8. Initial uptake rates of undissociated lactic acid as a function of its concentration by glucose-repressed *S. cerevisiae* 4072 cells. Numbers indicate pH values. Inset: dependence on pH of the diffusion constants calculated from the slopes.

Diffusion of undissociated lactic acid. Cells that had been grown in glucose medium, which therefore lacked the proton-lactate symport, were used to study the uptake kinetics of undissociated DL-lactic acid at several pH values. The plots of the initial uptake rates of DL-lactic acid against the concentration of undissociated acid were linear, which is consistent with undirectional diffusion kinetics of lactic acid across the plasma membrane (Fig. 8). In turn, the free diffusion of lactic acid indicated that the form that entered the glucose-grown cells was indeed uncharged.

Estimates of the diffusion constants were obtained from the slopes of the linear plots at the various pH values. The diffusion constant, expressed in the units of the plots of Fig. 8, has the dimension of volume (microliters), reciprocal time ($seconds^{-1}$), and reciprocal biomass ($milligrams^{-1}$). The values of the diffusion constant increased exponentially with pH, i.e., linearly with increasing extracellular proton concentration (Fig. 8 inset). Thus, the passive diffusion of undissociated lactic acid across the plasma membrane of *S. cerevisiae* is subject to opposing pH influences—increase due to the relative increase of undissociated acid with decreasing pH and decrease due to decreasing permeability with decreasing pH. A similar behavior was observed earlier with respect to the passive proton diffusion across the plasma membrane of *S. cerevisiae* (8) and with respect to the passive diffusion of undissociated lactic acid across the plasma membrane of *C. utilis* (9).

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