ISSN 0006-2979, Biochemistry (Moscow), 2006, Vol. 71, No. 10, pp. 1161-1169. © Pleiades Publishing, Inc., 2006.
Original Russian Text © D. A. Aliverdieva, D. V. Mamaev, D. I. Bondarenko, K. F. Sholtz, 2006, published in Biokhimiya, 2006, Vol. 71, No. 10, pp. 1430-1440.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM06-133, September 10, 2006.

Properties of Yeast *Saccharomyces cerevisiae* Plasma Membrane Dicarboxylate Transporter

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Received May 11, 2006
Revision received June 23, 2006

Abstract—Transport of succinate into *Saccharomyces cerevisiae* cells was determined using the endogenous coupled mitochondrial succinate oxidase system. The dependence of succinate oxidation rate on the substrate concentration was a curve with saturation. At neutral pH the K_m value of the mitochondrial "succinate oxidase" was fivefold less than that of the cellular "succinate oxidase". O-Palmitoyl-L-malate, not penetrating across the plasma membrane, completely inhibited cell respiration in the presence of succinate but not glucose or pyruvate. The linear inhibition in Dixon plots indicates that the rate of succinate oxidation is limited by its transport across the plasmalemma. O-Palmitoyl-L-malate and L-malate were competitive inhibitors (the K_i values were 6.6 ± 1.3 μM and 17.5 ± 1.1 mM, respectively). The rate of succinate transport was also competitively inhibited by the malonate derivative 2-undecyl malonate (K_i = 7.8 ± 1.2 μM) but not phosphate. Succinate transport across the plasma membrane of *S. cerevisiae* is not coupled with proton transport, but sodium ions are necessary. The plasma membrane of *S. cerevisiae* is established to have a carrier catalyzing the transport of dicarboxylates (succinate and possibly L-malate and malonate).

DOI: 10.1134/S0006297906100142

Key words: succinate, L-malate, malonate, O-palmitoyl-L-malate, competitive inhibition, *Saccharomyces cerevisiae* plasma membrane dicarboxylate transporter, protonophores FCCP and SF, sodium symport

Until the present work, the plasma membrane of *Saccharomyces cerevisiae* was thought to lack a protein-mediated system of C_4-dicarboxylate transport [1-4]. At pH 3.0, only L-malate transport mediated by diffusion of its uncharged form was shown [3]. Based on this finding, French researchers [4] succeeded in expression in the yeast *S. cerevisiae* cells of the gene *MAE1* encoding the dicarboxylate transporter in *Schizosaccharomyces pombe* [5] and demonstrated an increase in the rate of exogenous malate transport, which was sensitive to protonophores [4]. We have earlier shown [6] that after a prolonged preincubation of *S. cerevisiae* Y-503 cells at 0°C and pH 5.5 (under these conditions the diffusion of undissociated dicarboxylate is unlikely or even impossible) succinate significantly stimulates malonate-sensitive respiration (malonate inhibits mitochondrial succinate dehydrogenase). It was suggested that the *S. cerevisiae* plasmalemma should contain a dicarboxylate transporter.

We have earlier found that higher aliphatic derivatives of C_4-dicarboxylates are effective inhibitors of the liver mitochondrial dicarboxylate transporter [7] and O-palmitoyl-L-malate does not penetrate across the plasma membrane of *S. cerevisiae* at pH 5.5 [8]. Under these conditions, it was expected to influence proteins only of this membrane. Studies on isolated cells are preferable because in this case possible changes in the dicarboxylate transporter properties associated with its reconstruction into liposomes can be prevented, as shown for the tricarboxylate [9] and adenylate [10] mitochondrial transporters. Using the endogenous system of succinate oxidation by mitochondria as a coupled system to determine the rate of the substrate entrance into the cell allows us to work without radiolabeled substrates. Such substrates in intact cells can lead to artifacts associated with the substrate adsorption on the cell surface and its retention in the periplasm [11], as well as metabolic transformations of the labeled substrate, which considerably hampers the analysis [12].

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The known dicarboxylate transporters of the plasma membrane of yeasts, including *Schizosaccharomyces pombe* [13], *Candida utilis* [14], *Kluyveromyces marxianus* [15], and *Pachysolen tannophilus* [16], transfer the substrate in symport with protons. As a rule, the transporters, in addition to L-malate and succinate, can also transport other dicarboxylates; thus, the *Sch. pombe* transporter can transfer oxaloacetate, malonate, and maleate [5], and the *K. marxianus* transporter can transfer D-malate, fumarate, and oxaloacetate [15]. Sodium-dependent symporters of dicarboxylates have been studied in the plasmalemma of higher eukaryotes [17-19] but are unknown for yeasts. In the plasma membrane of *S. cerevisiae* a Na^+/H^+ antiporter and a Na^+-dependent symporter of phosphate ions are present [20, 21]; therefore, under certain conditions (alkaline pH values, presence of NaCl in the medium) a Na^+ gradient can be created on this membrane. The substrate specificity and transport mechanism for the hypothetical *S. cerevisiae* transporter are unknown.

MATERIALS AND METHODS

The *S. cerevisiae* strain Y-503 (from the collection of the State Institute for Genetics and Selection of Industrial Microorganisms (GNII Genetika), Moscow, Russia) obtained by the Caspian Institute of Biological Resources, Dagestan Research Center, Russian Academy of Sciences [22], was used. The strain was shown to belong to the *S. cerevisiae* taxon by the UP-PCR (universal primer polymerase chain reaction) approach in the group of S. Bulat (Laboratory of Eukaryote Genetics, Department of Molecular and Radiation Biophysics, St. Petersburg Institute of Nuclear Physics, Russian Academy of Sciences). In some cases, the *S. cerevisiae* strain S288c obtained from L. Mallet (Institute of Genetics and Microbiology, University of Paris) was used. The yeast was grown for 10 h under conditions favorable for proliferation of mitochondria. The cells were pretreated and respiration was measured as described in [6]. In some experiments, cells grown on a YNB (yeast nitrogen base) synthetic medium were used. Mitochondria were isolated by a modification of a published method [23] using lyticase (Sigma, USA) to prepare spheroplasts, and the mitochondria were precipitated at 5000*g* for 20 min. The mitochondrial protein was determined by a modification of Bradford's method [24]. Cell respiration was measured at 30°C in 50 mM potassium phosphate buffer (pH 5.5 and in some cases 6.5) and in special cases in sodium phosphate buffer (pH 5.5). Mitochondrial respiration was measured in medium containing 0.6 M mannitol, 1 mM EDTA-Na, 10 mM KH_2PO_4, and 10 mM MES (pH 6.5). The oxygen level was determined amperometrically [6, 25] under stationary conditions. All activities (including those of different substrate transporters) measured using "oxidase" coupled systems are presented in nmol O_2 per min.

Influences of effectors on oxidation of pyruvate were studied when its rate was limited by the plasma membrane transporter (after 2-6 h of aerobic preincubation of the cells at 0°C and substrate concentration from 5 to 24 mM). The activity of "pyruvate oxidase" was 162.2 ± 8.1 nmol/min per mg dry weight and the K_m value was 7.62 ± 2.5 mM (the mean of five independent measurements). This value is close to the K_m value for pyruvate of the monocarboxylate H^+-symporter of the *S. cerevisiae* plasma membrane (5.8-4.1 mM [26]), but significantly differs from the K_m value of the monocarboxylate transporter in *S. cerevisiae* mitochondria (0.8 mM) [27].

For our experiments, we needed reagents free of even small admixtures of acetate because we have shown it to have a high affinity for "acetate oxidase" of the cells (K_m = 35 ± 15 μM).

Since the observed parameters of the inhibition by O-palmitoyl-L-malate (I_{50}) depended on the concentration of mitochondria or cells (B), the true value of I_{50} was determined by extrapolation to zero concentration of the organelles in I_{50}–(B) coordinates, as proposed by Harvey [28]. The linearity of the dependence allowed us to use the method described in [7].

L-Malic acid and lyticase were from Sigma; D(+)-glucose monohydrate and mannitol from Merck (Germany); BSA from Calbiochem (USA); Tris and MES from Serva (Germany); yeast extract and YNB medium from Difco Laboratories (USA); protonophores SF-4867 (3,5-di-tret-butyl-4-oxybenzylidenemalononitrile) from Sumimoto Chem. Co. (Japan) and FCCP (carbonyl cyanide 4-trifluoromethoxyphenylhydrazone) from Aldrich (USA); 2-thenoyltrifluoroacetone and NaOH from Fluka (Switzerland), and domestic preparations of KH_2PO_4 and KOH (of special purity), succinic and maleic acids, EDTA, sodium pyruvate, and sodium succinate (recrystallized) were used. O-Palmitoyl-L-malate and 2-undecyl malonate were synthesized in our laboratory [7]. Water-insoluble reagents were dissolved in dimethyl sulfoxide.

RESULTS

We first chose conditions suitable for measurement of the transport of dicarboxylic acids (succinate and malonate) across the yeast cell plasmalemma in the case of virtual absence of diffusion of protonated, i.e., uncharged dicarboxylates (pH 5.5 and higher). We have earlier shown that the *S. cerevisiae* cells grown at a low concentration of glucose, i.e., in the absence of glucose repression [29], displayed pronounced "endogenous respiration". This became stable after about 15 min of incubation at 30°C [6]. The respiration was insensitive to malonate and thenoyltrifluoroacetone which are specific

inhibitors of mitochondrial succinate dehydrogenase (data not presented). Even after 8-10 h of aerobic preincubation at 0°C, the rates of acetate and pyruvate oxidation were significantly higher than the rate of endogenous respiration (Fig. 1a). However, the latter could not be neglected on measuring the respiration in the presence of succinate. Therefore, the action of all effectors under study on the endogenous respiration was tested and its value (v_0) was subtracted from the total rate of oxidation in the presence of succinate. The resulting rate was subsequently considered as the rate of succinate oxidation. After the aerobic preincubation at 0°C, the respiration rate was virtually unchanged during the period between the 9 and 26 h (Fig. 1b); therefore, just these conditions were used in our experiments.

To measure the rate of succinate transport in intact cells, we used the endogenous coupled system of succinate oxidation (further called "succinate oxidase"), which consisted of a supposed plasma membrane dicarboxylate transporter, mitochondrial dicarboxylate transporter, succinate dehydrogenase, and "ubiquinol oxidase" (Fig. 2). Antimycin A (an inhibitor of mitochondrial "ubiquinol oxidase") was earlier shown to completely suppress the oxidation of succinate [6]. Therefore, the mitochondrial "succinate oxidase" was the only oxidation pathway of this substrate in the cell under conditions of our experiments. The significantly higher rates of acetate and pyruvate oxidation compared to the rate of succinate oxidation (Fig. 1a) suggested that the final link of "succinate oxidase", which was in common for the oxidation systems of all three substrates (Fig. 2), should not limit the succinate oxidation. The respiration rate within the period between the 9 and 26 h was virtually unchanged (Fig. 1b). Note that under these conditions the rates of succinate oxidation were comparable with those for strains Y-503 and S288c.

It was impossible to fully suppress the activity of mitochondrial NADH dehydrogenases in yeast because of poor solubility of the corresponding inhibitor (flavone). Hence, in our experiments the oxidation of pyruvate, glucose, and acetate by the cells was associated (via the Krebs cycle) with functioning of succinate dehydrogenase (Fig. 2). This was supported by the inhibitory effect of thenoyltrifluoroacetone (a specific inhibitor of succinate dehydrogenase [30]) on the oxidation of each of the above-mentioned substrates (data not presented). Thus, thenoyltrifluoroacetone inhibited the oxidation of acetate (the dependence on the substrate concentration was hyperbolic) with I_{50} of about 0.2 mM (Fig. 3). The inhibition of the succinate oxidation was a sigmoid-

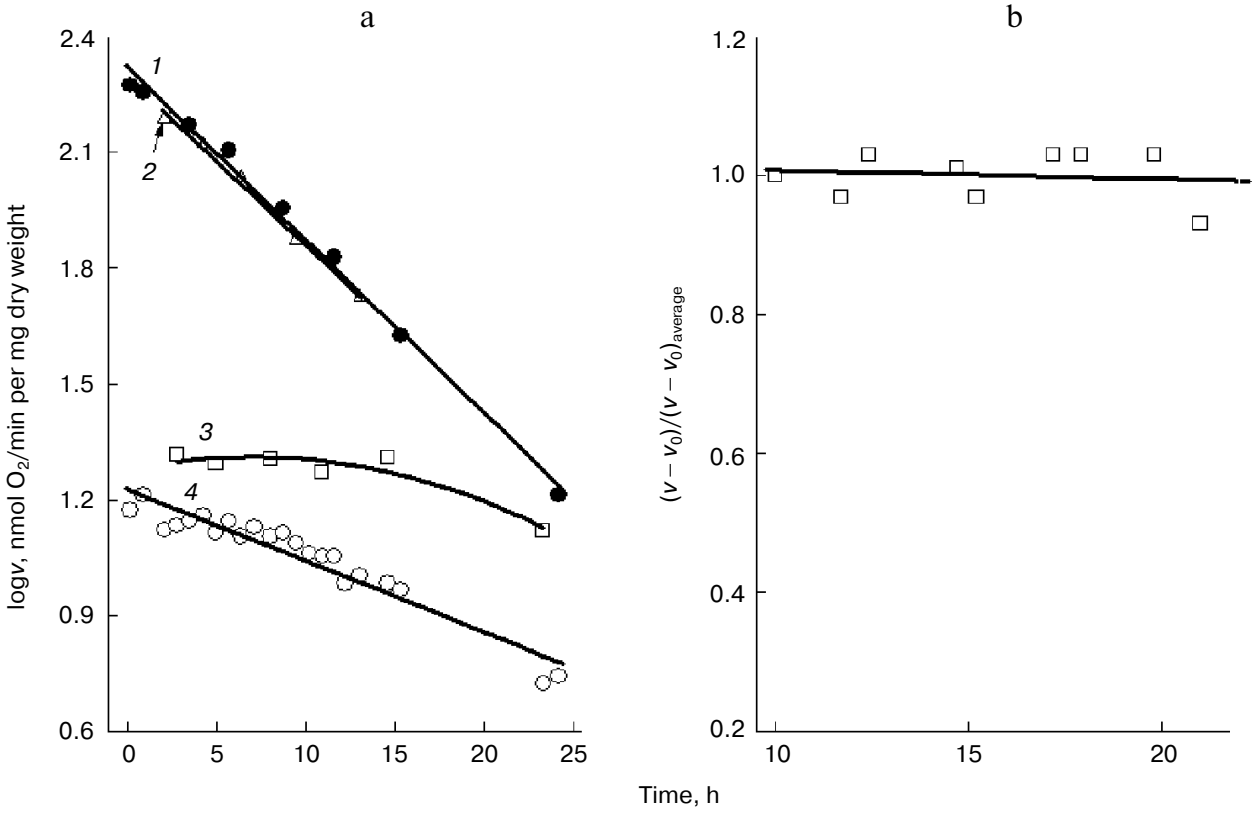

Fig. 1. a) Dependence of oxidation rate by *S. cerevisiae* cells (5 mg wet weight per ml) of 24 mM sodium pyruvate (*1*), 20 mM potassium acetate (*2*), 20 mM sodium succinate (*3*), and in the absence (*4*) of exogenous substrates (v_0) at 30°C on the time of aerobic preincubation at 0°C. b) The relative rate of oxidation of 20 mM sodium succinate during aerobic preincubation at 0°C.

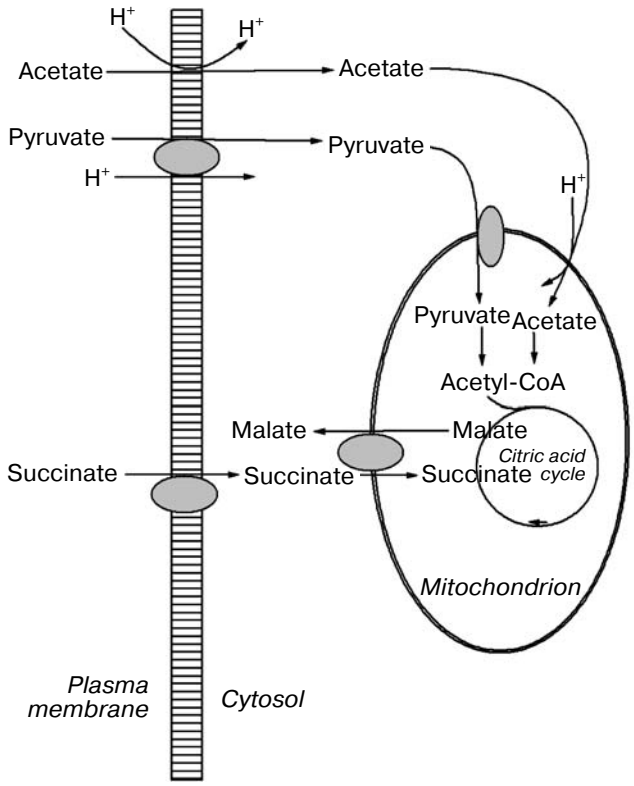

Fig. 2. Endogenous coupled systems used for measurement of the rates of dicarboxylate transport in *S. cerevisiae* cells (scheme).

shaped curve with I_{50} of about 0.8 mM. This indicated that the common for these two processes part of the coupled system of succinate dehydrogenase + "ubiquinol oxidase" failed to limit the oxidation of succinate. Thus, the succinate transport across the plasmalemma is likely to be a limiting link for the rate of succinate oxidation by the cells (Fig. 2).

The oxidation of succinate (24 mM) by the yeast cells, as discriminated from the oxidation of pyruvate and acetate, was nearly completely inhibited by O-palmitoyl-L-malate (Fig. 4).

The linear dependence of the O-palmitoyl-L-malate-caused inhibition (Fig. 5a) in the Dixon plots suggests that the action on the limiting link of the endogenous coupled system and the inhibitor binding with it should occur at the same point. It has been said above that O-palmitoyl-L-malate does not penetrate across the plasma membrane under the experimental conditions [8]; therefore, the dicarboxylate transporter of this membrane is the limiting link of "succinate oxidase" of the cells. The K_m value for succinate of "succinate oxidase" of yeast cells was found to be 4.4 ± 1.3 mM (the mean of three independent measurements), which was nearly fivefold higher than the K_m value (0.85 ± 0.173 mM) (the mean of three independent measurements) for succinate of mitochondrial "succinate oxidase" of these yeast cells. And the pH values of the incubation medium were chosen close to pH values of the cytoplasm and mitochondria of

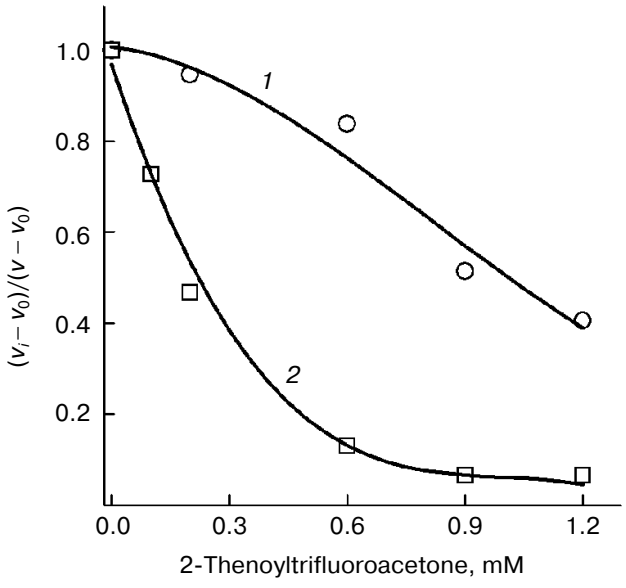

Fig. 3. Dependence of the oxidation rate by *S. cerevisiae* cells (2 mg wet weight per ml) of 20 mM sodium succinate (*1*) and 20 mM potassium acetate (*2*) on the thenoyltrifluoroacetone concentration after aerobic preincubation of the cells for 12 h at 0°C.

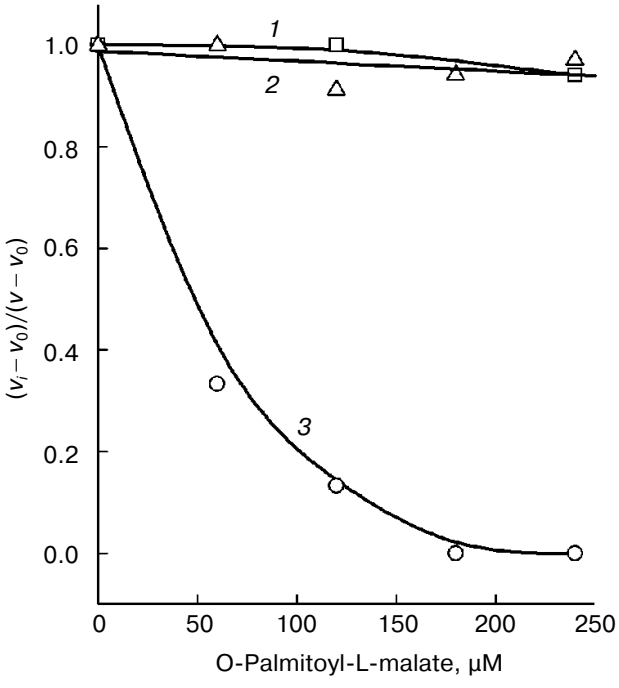

Fig. 4. Dependence on O-palmitoyl-L-malate concentration of the oxidation rate by *S. cerevisiae* cells (5 mg wet weight per ml) of 24 mM sodium pyruvate (*1*) and 10 mM glucose (*2*) after aerobic preincubation of the cells for 2 h at 0°C and of 10 mM sodium succinate (*3*) after aerobic preincubation of the cells for 14 h at 0°C.

S. cerevisiae cells (6.5 and 7.0, respectively [31]). The increase in pH value from 5.5 to 6.5 resulted in a virtually twofold increase in the affinity for the substrate of cellular "succinate oxidase" (8.5 ± 2.1 and 4.4 ± 1.3 mM, respectively). It seemed that it is just the succinate dianion bound to the active site of dicarboxylate transporters of the plasmalemma and mitochondrial inner membrane because its fraction at pH 5.5 and 6.5 was 40 and 85%, respectively [32]. Values of I_{50} of O-palmitoyl-L-malate extrapolated to zero concentration of the cells (dependence not presented) allowed us to calculate the K_i value to be 6.6 ± 1.3 μM (the mean of two independent determinations).

The maximal rate of L-malate oxidation by the cells in the presence in the medium of either potassium or sodium only at pH 5.5 was no more than 13% of the respiration in the presence of succinate and gradually decreased 6-8-fold by the 18th hour of aerobic preincubation at 0°C (in either medium). This allowed us to investigate the suppression of succinate oxidation by L-malate (Fig. 5b), neglecting its contribution to the respiration rate. This inhibition also was linear in the Dixon plots. The K_i value for L-malate was 17.5 ± 1.1 mM (from two independent determinations using data of Fig. 5b), which was incomparable to the K_i value for O-palmitoyl-L-malate equal to 6.6 ± 1.3 μM. Consequently, the acylated substituent significantly increased the affinity of the substrate derivative for the transporter. The increase in the succinate concentration lowered the I_{50} value for L-malate (Fig. 5b). Thus, the two substrates seemed to compete for a common binding site.

2-Undecyl malonate, similarly to O-palmitoyl-L-malate, had no effect on the endogenous respiration of the cells but inhibited the oxidation of succinate. The inhibition was linear in the Dixon plots with K_i = 7.8 ± 1.2 μM (from two independent determinations). It seems that the active site of the plasmalemma dicarboxylate transporter binds not only L-malate but also malonate. Both O-palmitoyl-L-malate and 2-undecyl malonate increased the K_m value of the yeast cell "succinate oxidase" without changing the maximal rate of the reaction (in the absence of the inhibitor K_m = 8.2 mM and V_{max} = 12.4 nmol/min per mg dry weight) (Fig. 6a). The competition of these inhibitors suggested their interaction with the same point of the substrate binding in the active site of the transporter.

As differentiated from the mitochondrial dicarboxylate transporter, which was inhibited even in the presence

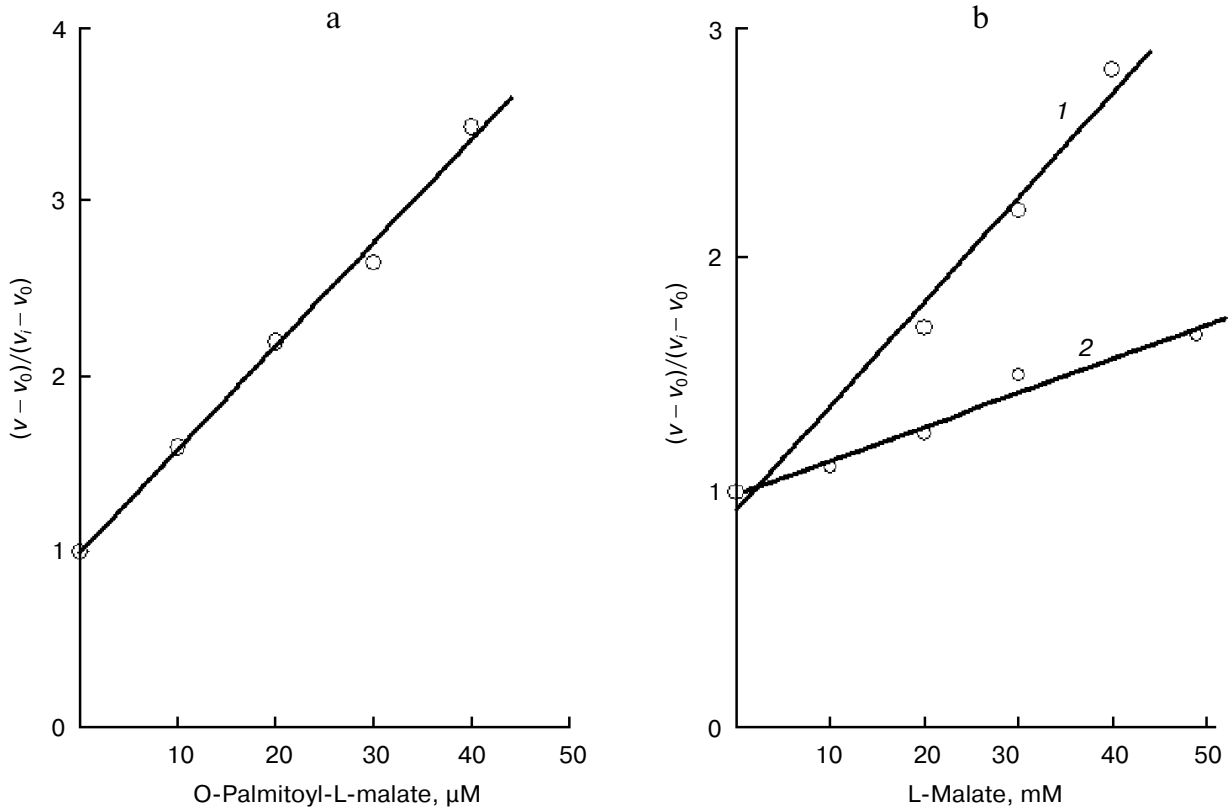

Fig. 5. Dependence of the oxidation rate of 8 mM succinate on the concentration of O-palmitoyl-L-malate (a) and dependence of the oxidation rate of 8 mM (*1*) and 20 mM (*2*) sodium succinate on the concentration of potassium L-malate (b) in the Dixon plots. The *S. cerevisiae* cells (10 mg wet weight per ml) were preincubated aerobically at 0°C for 18 h.

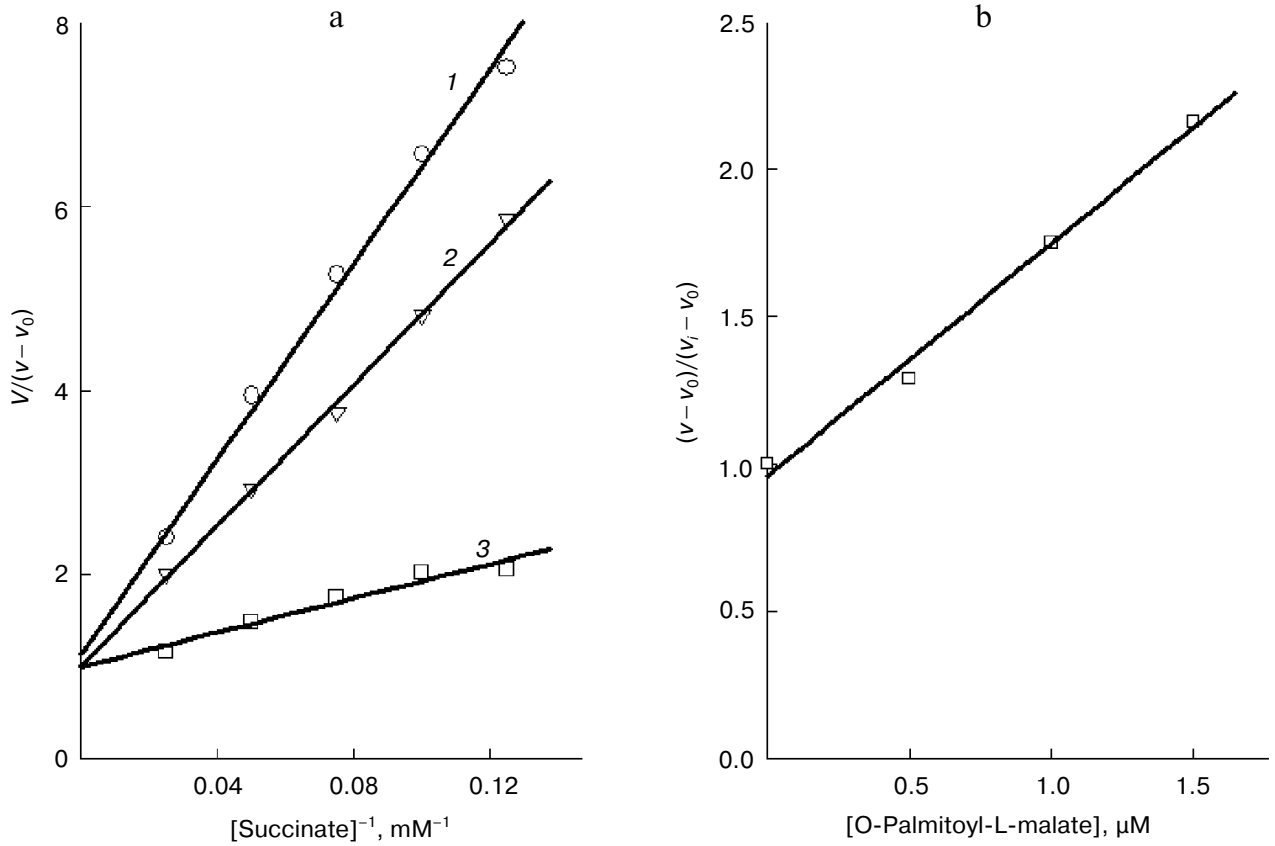

Fig. 6. a) Dependence of oxidation rate on sodium succinate concentration in Lineweaver–Burk coordinates in the presence of 50 μM O-palmitoyl-L-malate (*1*), 50 μM 2-undecyl malonate (*2*), and without the inhibitor (*3*). The *S. cerevisiae* cells (10 mg wet weight per ml) were preincubated at 0°C for 18 h. b) The dependence of the oxidation rate of 4 mM sodium succinate by mitochondria of *S. cerevisiae* (0.1 mg protein per ml) on the concentration of O-palmitoyl-L-malate in the Dixon plots in the presence of 1 mM sodium pyruvate and 0.025 μM SF.

of 10 mM phosphate [7], the K_m value and the maximal rate of cellular "succinate oxidase" of the cells were not influenced by change in phosphate buffer concentration from 10 to 50 mM (K_m = 7.8 mM and V_{max} = 12.6 nmol/min per mg dry weight and K_m = 8.2 mM and V_{max} = 12.4 nmol/min per mg dry weight, respectively).

It is highly probable that at pH 6.5 O-palmitoyl-L-malate does not penetrate across the inner membrane of *S. cerevisiae* mitochondria. Therefore, the linear dependence of the O-palmitoyl-L-malate-caused inhibition of the succinate oxidation by *S. cerevisiae* mitochondria in the Dixon plots on the inhibitor concentration (Fig. 6b) suggests that the dicarboxylate transporter of the inner mitochondrial membrane should be a limiting link of the mitochondrial "succinate oxidase". The I_{50} values for O-palmitoyl-L-malate extrapolated to zero concentration of mitochondria allowed us to calculate the value of K_i = 0.24 ± 0.10 μM (from two determinations). This value was lower than the K_i value for the plasma membrane transporter (6.6 ± 1.3 μM), and this did not allow us to selectively inhibit the succinate transport across the plasmalemma at acidic pH. Thus, the inhibitor effectively decelerated the yeast growth at pH 4.5 (possibly due to penetration by diffusion across the plasmalemma to mitochondria and inhibition of the mitochondrial inner membrane dicarboxylate antiporter) but not at pH 5.5 (data not presented). To determine the mechanism of succinate transport across the plasmalemma, effects of protonophores on the oxidation of succinate and, for comparison, of glucose and pyruvate were studied. The protonophore FCCP increased the rate of glucose oxidation by *S. cerevisiae* cells (Fig. 7a). It seemed that the transport of glucose into the cell did not limit its utilization, because the protonophore-induced deenergization of the mitochondrial inner membrane in the yeast cells [33] increased the rate of oxidation by the mitochondria of glucose metabolites in the cytoplasm after glucose had been energy independently transported across the plasmalemma [34]. The value of I_{50} for the inhibition by FCCP of pyruvate oxidation was about fivefold lower than the A_{50} value of the uncoupler-caused activation of glucose oxidation (0.21 and 1.0 μM, respectively, Fig. 7a). A similar ratio of A_{50}/I_{50} (4.1) was obtained for the protonophore SF-6847 of different chemical structure.

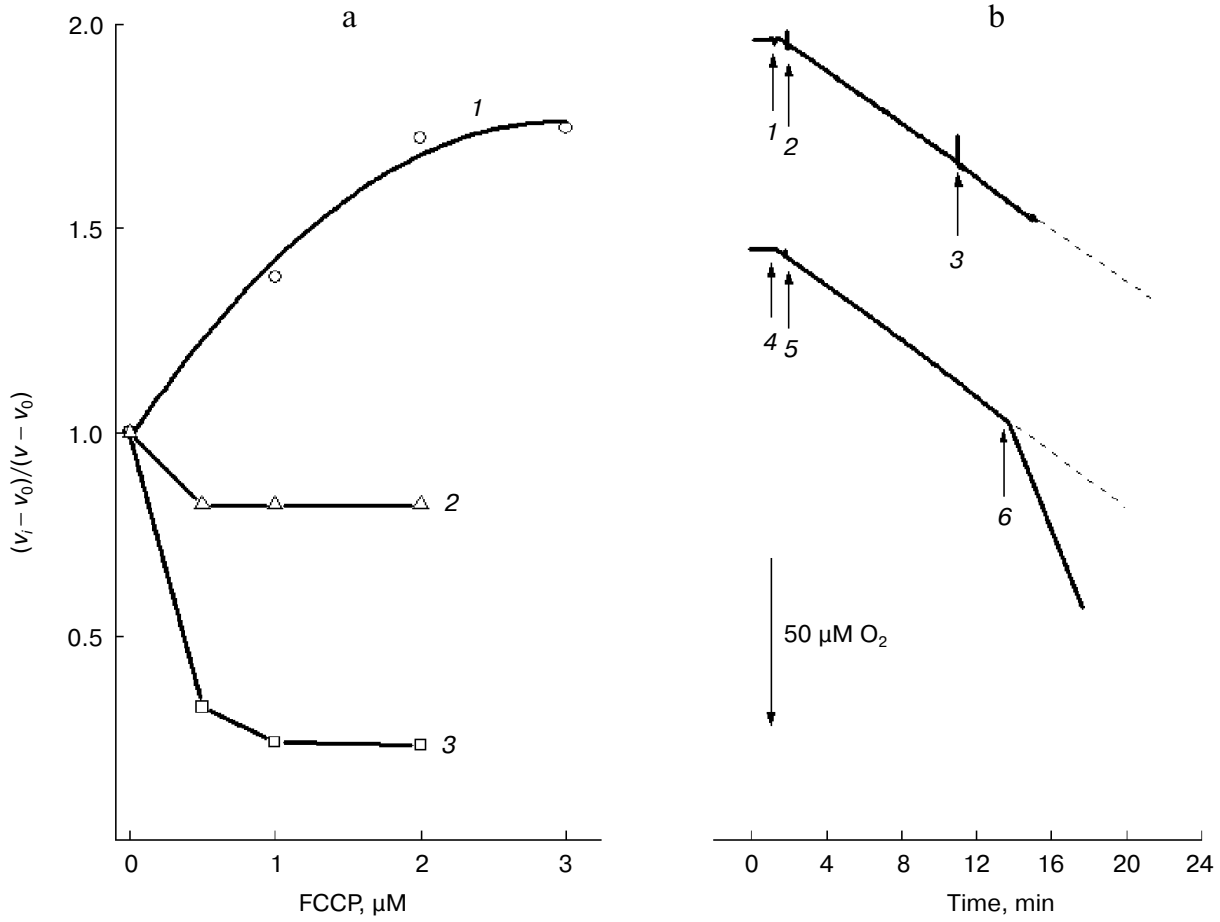

Fig. 7. a) Dependence of the oxidation rates of 10 mM glucose (*1*), 20 mM sodium succinate (*2*), and 20 mM sodium pyruvate (*3*) on the concentration of FCCP. The *S. cerevisiae* cells (10 mg wet weight per ml) were preincubated aerobically at 0°C for 10-14 h. The substrate was added after the protonophore addition. In the presence of FCCP, sodium succinate, sodium pyruvate, and glucose the rates became stable 3, 4, 10, and 2 min, respectively, after the addition of the effector (time dependences not presented). b) Rates of succinate oxidation in monocationic media. Above: 50 mM potassium phosphate buffer (pH 5.5). Additions: *S. cerevisiae* cells (5 mg dry weight per ml) grown in YNB and preincubated aerobically at 0°C for 14 h (*1*), 1 μM FCCP (*2*), 20 mM potassium succinate (*3*). Below: 50 mM sodium phosphate buffer (pH 5.5). Additions: the *S. cerevisiae* cells (5 mg dry weight per ml) grown in YNB and preincubated aerobically at 0°C for 14 h (*4*), 1 μM FCCP (*5*), 20 mM sodium succinate (*6*).

These results are consistent with the direct evaluation of the ratio between the protonophore FCCP concentration required for deenergization of different membranes during the respiration of *S. cerevisiae* cells [33]. It seemed that low concentrations of the protonophore suppressed pyruvate transport into the cytoplasm across the plasmalemma that occurs in symport with protons [35]. Low concentrations of the protonophore caused nearly no suppression of the oxidation of succinate and its high concentrations did not activate it (Fig. 7a). It seemed that, as distinguished from pyruvate, this dicarboxylate was transported across the plasma membrane not in symport with protons. The rates of succinate oxidation by cells preincubated at 0°C for 14 h in phosphate buffer containing either potassium or sodium ions only are sharply different (Fig. 7b). The respiration of succinate could be recorded only in the case of preincubation in sodium phosphate buffer. Because the increase in the concentration of potassium phosphate buffer (and, correspondingly, of potassium) did not change the values of K_m and V_{max} of the cellular "succinate oxidase", the result shown in Fig. 7a cannot be explained by the inhibitory effect of potassium on the transport. Succinate seems to be transported across the plasmalemma in symport with Na^+. To prevent the exit of sodium ions from the cells, the incubation medium was supplemented with the protonophore via the corresponding plasmalemma antiporter [20].

DISCUSSION

It has been shown that the respiration of *S. cerevisiae* cells in the presence of succinate is associated with exis-

tence of a dicarboxylate transporter in the plasma membrane. This is supported by the following data.

1. The cells oxidize succinate (pK_a values are 4.21 and 5.72 [32]) at pH 6.5 in the absence of its undissociated form.

2. The oxidation rate dependence on the succinate concentration is a curve with saturation (Fig. 6a). The K_m values for succinate of the plasmalemma and mitochondrial transporters are different (4.4 ± 1.3 and 0.85 ± 0.173 mM, respectively). As discriminated from the latter [36, 37], phosphate anion is not bound with the plasmalemma dicarboxylate transporter and seems to be not transported by it.

3. O-Palmitoyl-L-malate does not penetrate into the cell [8], competes with succinate, and effectively inhibits the transport (K_i = 6.6 ± 1.3 μM) (Fig. 6a).

The increase in the rate of succinate oxidation after aerobic preincubation of the cells at 0°C (Fig. 1a) cannot be explained by appearance of cells with damaged plasma membrane. O-Palmitoyl-L-malate inhibited oxidation of succinate by mitochondria nearly 30-fold stronger than its oxidation by intact cells (the K_i values were 0.24 ± 0.10 and 6.6 ± 1.3 μM, respectively).

4. "Succinate oxidase" manifested itself in the Na^+-containing medium (Fig. 7b). The dicarboxylate transport was not observed in the monopotassium medium.

The insensitivity of the *S. cerevisiae* plasmalemma to uncouplers of the dicarboxylate transporter (Fig. 7a) is a unique property compared to the proton symporters of dicarboxylates of other yeast genera [5, 13, 15, 16, 38]. The transport of succinate cannot be mediated by proton symporters of *S. cerevisiae*, which transfer into the cell a substrate with the succinate-like tetracarbon structure, such as ureidosuccinate [39].

It seems that the *S. cerevisiae* plasma membrane transporter functions in symport with sodium ion, similarly to the dicarboxylate transporter of rabbit kidney plasmalemma [19]. In the absence of sodium, no succinate oxidation is detected (Fig. 7b). But the affinity for sodium ions is as low as that of transporters of higher eukaryotes [19].

The *S. cerevisiae* mutants with the "damaged" Krebs cycle have been shown to eject succinate and malate from the cell into the growth medium [40]. Because the cytoplasm pH is 7.0 [18], this "excretion" seems to be mediated by a transporter and not diffusion of undissociated dicarboxylic acids across the plasmalemma. The transporter seems to act as a "safety valve" protecting the cell against the dicarboxylate excess in the cytoplasm [6]. This can be also an explanation of the relatively poor affinity for L-malate and succinate (17.5 ± 1.1 and 7.3 ± 1.1 mM, respectively), the L-malate accumulation in the growth medium [6], and the relatively low activity of the transporter. Among the yeast genera [5, 13, 15, 16, 38], only the *Sch. pombe* transporter has the low activity close to that of the *S. cerevisiae* transporter (6.0 ± 0.1 [13] and 13.8 ± 0.4 nmol/min per mg dry weight, respectively) and similar affinities for succinate at pH 5.5 (K_m values are 7.3 ± 1.1 and 4.5 ± 0.5 mM, respectively [13]). However, the *Sch. pombe* transporter binds the monoanionic form of substrate (L-malate).

For *Candida sphaerica*, V_{max} of the plasmalemma dicarboxylate transporter by succinate is 240 nmol/min per mg dry weight [38]. At pH 5.5, the plasmalemma transporters of other yeast genera (capable of building up an additional biomass in the presence of succinate) have also a considerably higher affinity for this substrate. Thus, the K_m values for succinate transport measured directly with radiolabeled substrates are 0.064 mM for *P. tannophilus* [16], 0.031 mM for *K. marxianus* [38], and 0.124 mM for *Candida utilis* [14]. The ejection of malate by the cells grown in the presence of glucose at pH 5.5 [6] indicates that the dicarboxylate transporter of *S. cerevisiae* can function and is constitutive, as differentiated from the inducible transporter of *P. tannophilus* [16] or *C. utilis* [14], which are susceptible to glucose repression.

Suppose that the plasmalemma monocarboxylate transporter could carry monoionic succinate into the cell. Then this transporter would be responsible for the lower rate of the succinate oxidation compared with that of pyruvate (13.8 ± 0.4 and 162.2 ± 8.1 nmol/min per mg dry weight, respectively). However, the oxidation of pyruvate (Fig. 4) was insensitive to O-palmitoyl-L-malate and suppressed by the protonophore (Fig. 7a).

Due to existence of the succinate transporter in the S288c strain, which has been used for interpretation of the *S. cerevisiae* genome [41], it is fundamentally possible to identify the corresponding gene. However, data of inhibitory analysis allow us to study the substrate specificity of the transporter. Because the inhibition of succinate oxidation by O-palmitoyl-L-malate in the Dixon plots displayed a linear dependence (with no breaks), it was suggested that the plasmalemma should have the only transporter of succinate. L-Malate activated cell respiration after 2 h of aerobic preincubation at 0°C. This could not be associated with diffusion of undissociated L-malate, because even O-palmitoyl-L-malate (a hydrophobic derivative of L-malate) did not penetrate into *S. cerevisiae* cells at pH 5.5 [8]. However, L-malate competitively inhibited the transport of succinate, and the dependence of succinate oxidation inhibition in the Dixon plots was linear (with no breaks) (Fig. 5b). It seems that L-malate and succinate are transported by the same transporter. Because the alkylated derivative of malonate, similarly to the acylated derivative of malate (Fig. 6a), competitively inhibited the transport of succinate, malonate was also supposed to penetrate into the cell by means of the only plasmalemma dicarboxylate transporter. Low concentrations of oxaloacetate and malonate completely suppressed the oxidation of succinate by the cells [6]. These substances effectively inhibit succinate dehydrogenase, and this masks the expected suppression of the transport.

The acylated substituent substantially increased the affinity of O-palmitoyl-L-malate for the transporter (K_i = 6.6 ± 1.3 μM) compared to that of L-malate (K_i = 17.5 ± 1.1 mM). It seems that a large lipophilic area exists closely to the substrate-binding point in the active site of the transporter. The use of effective inhibitors with such structure [7] was very informative for studies on the dicarboxylate transport mechanisms.

The authors are grateful to Sh. A. Abramov for presenting the baker's yeast strain Y-503, S. Bulat for verification of this strain as belonging to the *S. cerevisiae* taxon, and S. V. Benevolenskii and D. G. Kozlov for establishing the strain Y-503 ploidy.

This work was supported by the Russian Foundation for Basic Research (project No. 04-04-49670).

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