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Why Saccharomyces cerevisiae can oxidize but not decarboxylate external pyruvate

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Abstract

Protoplasts of the yeast Saccharomyces cerevisiae oxidized externally added pyruvate by pyruvate oxidase system but were not able to decarboxylate it anaerobically by pyruvate decarboxylase at pH 6.4 in isotonic solutions. The decarboxylation set in in hypotonic solutions in which the integrity of the plasma membrane was being impaired. Yeast cells incubated with $^{1\text{-}14}C$ pyruvate accumulated radioactivity under conditions allowing oxidation of pyruvate, but virtually no pyruvate was taken up when the oxidation had been arrested by inhibition or mutation. In view of a large difference between K_M for pyruvate of pyruvate decarboxylase (30 mM) and of pyruvate oxidase (0.16 mM), the results may be accounted for by the assumption that transport of pyruvate across the yeast plasma membrane is trans-inhibited by relatively high concentrations of intracellular pyruvate. This arrangement would allow utilization of external pyruvate by the cell energy-transforming machinery and, at the same time, prevent it wastage by futile decarboxylation.

Keywords: Plasma membrane; Pyruvate; Transport; (*S. cerevisiae*)

1. Introduction

It has been known since the 1920s that externally added pyruvate can be oxidized by Saccharomyces cerevisiae aerobically but not decarboxylated anaerobically [1]. It was speculated that this may be due to different locations of pyruvate decarboxylase and of

pyruvate oxidase in the cell, the former being in the center of the cell and the latter close to periphery [1]. As an alternative, Smythe proposed that the incapability of pyruvate decarboxylation could be accounted for by the fact that pyruvate can enter the cells only under aerobic conditions and not anaerobically [2]. This hypothesis was later taken over by Suomalainen et al. [3].

The present paper shows that the accumulation of externally added pyruvate and of its metabolites in yeast cells can be virtually arrested by a respiratory inhibitor or by mutation to respiratory deficiency and that its decarboxylation can set in upon an impairement of the plasma membrane. The analysis provides a rational for the intricacy of pyruvate metabolism in the yeast.

2. Materials and Methods

A haploid strain *S. cerevisiae* MMY 2 (MATa, ura3) was cultured in a medium containing 1% peptone, 1% yeast extract and 2% glucose for 24 h at 30° on a shaker. The cells were harvested, washed twice in water and used immediately for transport measurements. The same strain was also checked in a manometric experiment, but most of the manometric measurements were done with a diploid wild-type strain DT XII, grown and harvested in the same manner. The two strains did not differ in the properties examined in this study. A respiration-deficient (rho-) mutant of strain MMY 2 was isolated after a prolonged culturing of the cells in the presence of ethidium bromide [4]. Protoplasts were prepared by a snail-gut procedure [5]. The weight of the cells was determined after drying at 95°.

To measure the transport of pyruvate, the cells were mixed with a solution containing buffer and labelled pyruvate and incubated with shaking at 30°. Aliquots (100 µl) were taken off at different time intervals, filtered on nitrocellulose filters, washed with buffer, dried and the radioactivity retained by the cells was determined by scintillation counting.

Antimycin A was provided by Dr. A. O. M. Stoppani or purchased from Serva. 2-deoxy-D-glucose was from Serva and $^{1\text{-}14}$C pyruvate (Na salt) from New England Nuclear.

3. Results

In isotonic solutions, yeast protoplasts could oxidize pyruvate aerobically but not decarboxylate it when the oxidation was prevented by inhibition with antimycin A. In slightly hypotonic solutions, in which protoplasts maintained their shape but their plasma membrane may have no longer been intact, the protoplasts acquired the capacity to decarboxylate pyruvate while retaining the oxidation activity (Fig. 1). In strongly hypotonic solutions the decarboxylation became prominent and the oxidation activity was lost. The result indicated that it was the plasma membrane and its transport machinery which made external pyruvate accessible to mitochondrial oxidation but not to pyruvate decarboxylase in intact cells.

As shown in Fig. 2, yeast cells accumulated radioactivity from external pyruvate, when incubated with radioactively labelled pyruvate. The rate and capacity of accumulation was lower in cells grown on pyruvate instead of on glucose as a carbon source. Respiration deficient mutants did not take up the labelled pyruvate as also did not glucose-grown wild type cells that had been starved by aeration in buffer without a carbon source for 24 h. The latter observation may indicate that the uptake of pyruvate depended on an internal energy source which had been depleted by starvation. Antimycin A also strongly (80 to 90 per cent) inhibited the accumulation of radioactivity by wild-type cells (not shown).

The accumulated radioactivity could not be washed out of the cells either by buffer or by non-labelled pyruvate and was only slowly dissaprearing when the labelled cells were

incubated with or without unlabelled pyruvate. Also, the cells preincubated for 20 min with non-labelled pyruvate and subsequently incubated with radioactive pyruvate accumulated radioactivity with the same rate as did the non-preincubated cells (Fig. 3). Apparently, the radioactivity accumulated in the cells did not represent free pyruvate but rather its metabolic products, including, perhaps, polysaccharides and proteins. In fact, Fowlkes 6 reported that concentrations of intracellular pyruvate in yeast, incubated with external pyruvate, were maintained constant (about 0.25 μmol/ml cells) independently of concentrations of external pyruvate, of time of incubation and of temperature.

The metabolism must have been preceded by transport of pyruvate across the cell membrane. To assess the properties of the transport system, the accumulation of radioactivity ic cells was measured at the initial linear rate during first three minutes. The rate had a maximum at pH 5.5, being only 62% at pH 3.5 and 14% at pH 8.5. It exhibited the saturation kinetics and the apparent K_M for pyruvate was found to be 0.7 mM (Fig. 4). It was strongly inhibited by glucose and the inhibition was of a mixed type, diminishing V_{max} and increasing K_M. The inhibitor was probably glucose itself and not its metabolite(s) and it exerted the inhibition at the entry step, since non-metabolizable 2-deoxy-D-glucose inhibited in the same manner (not shown).

4. Discussion

The results are consistent with the view that in glucose-grown *S. cerevisiae* there is a pyruvate carrier in the plasma membrane. It must be different from the monocarboxylate transport system described by Cássio et al. [7], since the latter was found only in lactate grown cells and could not be detected in cells grown on glucose. The rate, optimum at pH 5.5, energy requirement and saturation kinetics rule out the possibility that the transport of pyruvate could be accounted for by a free diffusion of non-dissociated pyruvic acid. The rate of pyruvate transport under standard conditions (V_{max} equal to 5.5 $umol.g^{-1}.min^{-1}$) is at least twenty times higher than would be free diffusion of undissociated pyruvic acid. The latter value could be derived from the data of Cássio et al. [7], assuming that free diffusion of pyruvic acid is the same as that of lactic acid.

The unique feature of the pyruvate carrier is that its activity is somehow synchronized with the rate of pyruvate oxidation and it is switched off when pyruvate is not oxidized and would accumulate in free form inside the cell. This feature may be explained by the trans inhibition of the pyruvate carrier by higher concentrations of intracellular pyruvate. Minute steady-state concentrations of pyruvate in the cell should be sufficient to allow oxidation by pyruvate oxidase system, which may have the K_M for pyruvate as low as 0.16 mM [8] and, if slightly raised, to be large enough to exert the strong trans-inhibition of the carrier, but still too low to be markedly accessible to pyruvate decarboxylase whose K_M for pyruvate is 30 mM [9]. A rough estimate of the constantly maintained intracellular concentration of pyruvate, 0.25 mM, derived from Fowlkes' measurement [6] is consistent with this assumption. Without the trans-inhibition, pyruvate would enter the cell in an unrestricted manner and be wasted by decarboxylation which would furnish no free energy. Thus, the peculiar transport system would have a reasonable teleonomic explanation. The trans-inhibition of transport would not be unique to this system; it was previously invoked to account for the peculiarities of amino acid uptake in yeast [10, 11, 12]. Incidentally, the Kluyver effect in yeast, the inability to ferment certain disaccharides despite the fact that they can be utilized by oxidation [13, 14], may be accounted for by a similar arrangement and could be approached by the simple procedure used in this study.

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References

1 Barron, E. S. G., Ardao, M. I. and Hearon, M. (1950) J. Gen. Physiol. 34, 211-228.
2 Smythe, C. V. (1938) J. Biol. Chem. 125, 635-651.
3 Suomalainen, H., Konttinen, K. and Oura, E. (1969) Arch. Mikrobiol. 64, 32-42.
4 Nagley, P. and Linnane, A. W. (1970) Biochem. Biophys. Res. Commun. 39, 989-996.
5 Kováč, L., Bednárová, H. and Greksák, M. (1968) Biochim. Biophys. Acta 153, 32-42.
6 Fowlkes, E. C. (1955) J. Gen. Physiol. 38, 425-432.
7 Cássio, F., Leao, C. and van Uden, N. (1987) Appl. Environ. Microbiol. 53, 509-513.
8 Harada, T. and Hirabayashi, T. (1982) Methods in Enzymol. 89, 420-423.
9 Singer, T. P. (1955) Methods in Enzymol. 1, 460-464.
10 Crabeel, M. and Grenson, M. (1970) Eur. J. Biochem. 14, 197-204.
11 Morrison, C. E. and Lichstein, H. C. (1976) J. Bacteriol. 125, 864-871.
12 Indge, K., Seaston, A. and Eddy, A. A. (1977) J. Gen. Microbiol. 99, 243-255.
13 Weusthuis, R. A., Visser, W., Pronk, J. T., Scheffers W. A. and van Dijken, J. P. (1994) Microbiology 140, 703-715.
14 Kaliterna, J., Weusthuis, R. A., Castrillo, J. I., van Dijken, J. P. and Pronk, J. T. (1995) Microbiol. 141, 1567-1574.

Legends to Figures

Fig. 1. Oxidation and decarboxylation of pyruvate by yeast protoplasts. The main compartment of Warburg flasks contained Tris/maleate buffer (pH 6.4), Na pyruvate and different concentrations of sorbitol. When decarboxylation was measured it also contained 20 µg of antimycin A. When oxidation was measured the central well contained 200 µl of 2 M NaOH. Protoplasts of strain DT XII ($4xl0^8$ in 200 µl of l M sorbitol) were tipped from the side-arm at 40 min. 30°. Final concentrations: 36 mM Tris/maleate, 50 mM Na pyruvate and sorbitol 1 M (in 1), 0.75 M (in 2), 0.5 M (in 3) and 0.25 M (in 4).

Fig. 2. Time-course of incorporation of radioactivity into cells. The incubation mixture contained 2 mM $^{1\text{-}14C}$ pyruvate (Na salt), 20 mM K phtalate buffer (pH 4.5) and the following cells: 1, MMY 2, wild-type, 10.1 mg/ml; 2, MMY 2, wild-type, grown on Na pyruvate instead of glucose for 43 h, l4.5 mg/ml; 3, MMY 2, wild-type, starved by bubbling air through the cell suspension in water for 24 h, 10.1 mg/ml; 4, MMY 2, rho- mutant, 10.8 mg/ml. The radioactivity in the cells was calculated with respect to the radioactivity of the labelled pyruvate.

Fig. 3. Radioactivity in cells after different treatments. 1, cells MMY 2, wild-type (10.3 mg/ml) were incubated for 20 min with 2 mM unlabelled pyruvate (Na salt) in 20 mM K phtalate (pH 4.5), then spinned for 2 min in an eppendorf centrifuge, washed with 1 ml 20 mM buffer and resuspended in the buffer containing 2 mM $^{1\text{-}14C}$ pyruvate. The incorporation of radioactivity into cells was followed. 2 and 3, the cells were incubated for 20 min with 2 mM $^{1\text{-}14C}$ pyruvate in 20 mM K phtalate buffer (pH 4.5), then spinned and washed as above and resuspended in the buffer containing no pyruvate (2) or 2 mM unlabelled Na pyruvate (3). Radioactivity of the cells was determined in different time intervals and was calculated with respect to the radioactivity of the labelled pyruvate.

Fig. 4. Lineweaver-Burk plot of kinetic data. MMY 2 cells, wild-type (7.4 mg/ml) were incubated with different concentrations of labelled Na pyruvate (containing a constant amount of $^{1\text{-}14C}$ pyruvate) in 20 mM K phtalate buffer (pH 4.5) and in the absence (1) or presence of glucose 2 mM (2) and 8 mM (3). Aliquots were sampled at 0, 1.5 and 3 min. v - the rate in μmol.min-1.g-1; s - concentrations of pyruvate in mM.