STUDIES IN CELL PERMEABILITY

THE UPTAKE OF PYRUVATE BY YEAST*

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INTRODUCTION

A previous study (1) described the permeability properties of yeast cells to added citrate, a substrate which is not metabolized by the intact cell. The present report deals with the uptake of pyruvate by the intact cell. Added pyruvate is rapidly metabolized by yeast in the presence of O_2 (2).

Conway and Downey (3) showed that pyruvic acid freely enters an outer region comprising about 10 per cent of the whole yeast cell. They identified this region with the cell wall. It will be shown below that pyruvate ion also rapidly penetrates this region under all conditions tested. Before pyruvate can be metabolized it must penetrate further through a plasma membrane separating the outer from the inner region of the cell. This readily occurs with undissociated pyruvic acid which is metabolized under both aerobic and anaerobic conditions. Pyruvate ions on the other hand are not metabolized in the absence of oxygen, a finding attributed by Smythe (2) to the lowering of cell permeability in nitrogen. The difficulty of visualizing an influence of O_2 on the physical diffusion from the outer into the inner region of the cell led to the view that the uptake of pyruvate may depend on some active metabolic process. The experiments reported in this paper were carried out to test this hypothesis.

Theoretical and Experimental

In a cell metabolizing pyruvate the intracellular concentration of pyruvate at time t (P_T) will be given by

$$P_t = P_0 + \Delta_{in} - \Delta_{met} \qquad (1)$$

in which P_0 is the concentration at time 0, Δ_{in} is the net amount of pyruvate which has entered the cell during the time interval t, and Δ_{met} is the amount of pyruvate metabolized during this period. All quantities are expressed in micromoles per milliliter of cells. Pyruvate formed from substrates within the cell is neglected.

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Δ_{met} in Equation 1 is temperature-dependent. If Δ_{in} expresses the amount of pyruvate which has entered the cell by physical diffusion independent of any metabolic process it follows that reduction of Δ_{met} by decreasing the temperature should lead to an increase of P_t. At low temperatures at which Δ_{met} can be neglected, P_t at equilibrium should be entirely a function of the extracellular concentration of pyruvate. If on the other hand Δ_{in} represents a process with an energy of activation similar to that represented by Δ_{met}, P_t will be independent of temperature or of the extracellular concentration at 0°C. Further if $P_t = P_0$ when pyruvate is metabolized, it follows that $\Delta_{in} = \Delta_{met}$.

Similarly it is possible to distinguish between the two alternatives of physical diffusion and of energy-requiring uptake of pyruvate by reducing Δ_{met} under anaerobic conditions. If it is assumed that physical diffusion is independent of O_2, P_t should be higher in N_2 than in O_2. Conversely P_t should remain constant if Δ_{in} is linked to Δ_{met}.

Experimentally the values of the terms in Equation 1 were determined as follows. All experiments were carried out with freshly purchased Anheuser-Busch commercial bakers' yeast. This was washed three times and suspended in distilled water immediately before use. The pH of the suspension was adjusted to the desired value by the addition of HCl. Na pyruvate was added at pH 6, the internal pH of the yeast cell (4). Free pyruvic acid, obtained from Na pyruvate by treatment with dowex 50 ion exchange resin, was added at pH 2. As the outer region of the cell has little buffering capacity (4) the pH in this region will fall with addition of HCl to the suspension. At an extracellular pH of 2, pyruvic acid in the outer region will thus be undissociated.

The yeast was shaken under the stated conditions. After incubation the flask contents were rapidly cooled when necessary. Pyruvate was estimated by the method of Friedemann and Haugen (5) as follows:—

(a) Extracellular pyruvate concentration (E) in micromoles per milliliter was determined on the supernatant from the suspension spun at 0°C.

(b) Total pyruvate concentration (T) in micromoles per milliliter was obtained from a portion of the suspension acidified with trichloracetic acid (final concentration 12 per cent w/v).

The hematocrit reading (H) was determined in Wintrobe tubes after spinning for 25 minutes at 800 g. No changes in hematocrit reading were observed during incubation. The suspensions were adjusted to a final hematocrit reading of 60 per cent. The concentration of pyruvate in micromoles per milliliter of packed cells (C) was then calculated as

$$C = \left[T - \frac{E(100 - H)}{100} \right] \times \frac{100}{H} \tag{2}$$

To obtain the true intracellular level of pyruvate C_{in}, C was corrected for the 25 per cent extracellular phase observed in centrifuged yeast by Conway and Downey (3).

$$C_{in} = (C - 0.25E) \times \frac{1.00}{0.75} \tag{3}$$

The ratio R of intracellular to extracellular pyruvate concentration was then given by

$$R = \frac{C_{in}}{E} \tag{4}$$

R will remain constant with changes of temperature and external concentration if pyruvate enters the cell by physical diffusion independent of an active metabolic process.

C_{in} as determined by Equation 3 includes the pyruvate present in both the outer and the inner region of the cell. As pyruvate metabolism takes place only in the inner region, only the pyruvate concentration of that inner region is of

TABLE I

Influence of Different Variables on Permeability of Yeast Cells to Pyruvate Ion

pH 6.0; concentration of pyruvate in micromoles per milliliter of suspension before incubation.

Conditions of incubation	Variable		R
0°C., air, 20 min.	Pyruvate concentration:	0.6	0.17
		3.7	0.08
		7.4	0.10
0°C., O_2, pyruvate 2.6	Time in min.:	0	0.17
		20	0.14
		30	0.08
O_2, 30 min., pyruvate 2.6	Temperature in °C.:	0	0.08
		28	0.14
28°C., 30 min., pyruvate 2.6	O_2 pressure:	O_2	0.14
		Air	0.14
		N_2	0.11

significance for the calculation of the terms in Equation 1. To determine this concentration advantage was taken of the observation that R as calculated from Equation 4 rapidly reached a fairly steady value in good agreement with the value obtained for the volume of the outer region of the cell by Conway and Downey (3). This observation which is discussed in further detail below implies that the outer region of the cell is in rapid equilibrium with the extracellular phase. To obtain P_t, the concentration of pyruvate at time t in the inner region, the yeast after incubation was accordingly quickly washed twice with a large volume of water at 0°C. Any pyruvate remaining in the cell after washing was equated with P_t.

RESULTS

In Table I are shown the values of R for pyruvate ion obtained under a variety of conditions of incubation. In O_2 at 28°C. about 1.5 μM pyruvate were me-

tabolized per ml. of suspension in 30 minutes. No appreciable pyruvate was dissimilated in N_2 or at 0°C.

It is apparent that even in the short time required to mix the suspension and to withdraw samples for analysis R has attained a reasonably steady value of

TABLE II

Permeability of Yeast Cells to Undissociated Pyruvic Acid

Yeast incubated for 20 minutes at 0°C. and pH 2.

Pyruvic acid in μM/ml. of suspension	R after incubation
0.6	0.62
0.7	0.50
3.0	0.50
3.7	0.34
6.0	0.69
7.4	0.41

TABLE III

Influence of Different Variables on Concentration of Pyruvate in Inner Region (P_i)

pH 6.0; concentration of pyruvate in micromoles per milliliter of suspension before incubation.

Conditions of incubation	Variable		P_i
			μM/ml. cells
0°C., air, 30 min.	Pyruvate concentration:	0.5	0.23
		2.2	0.23
		6.2	0.24
25°C., air, pyruvate 6.0	Time in min.:	0	0.30
		10	0.21
		20	0.17
		30	0.25
Air, 30 min., pyruvate 6.0	Temperature in °C.:	0	0.24
		30	0.25
30°C., 30 min., pyruvate 6.0	O_2 pressure:	O_2	0.25
		N_2	0.26

about 0.1 to 0.2. The water for solution in the outer region of the cell was found by Conway and Downey to be 0.10 to 0.11 of the total cell volume or about 15 per cent of the total cell water. As the concentration of pyruvate at equilibrium will be the same in the outer region of the cell and in the extracellular phase, it follows that most of the intracellular pyruvate is present in this outer region. The attainment of this equilibrium is rapid even under conditions under which no pyruvate is metabolized.

Table II shows the R values obtained with undissociated pyruvic acid at pH 2. As the outer region of the cell has little buffering capacity, the total concentration of pyruvic acid at equilibrium at pH 2 will again be the same in the outer region and in the extracellular phase. The R values at pH 2 are therefore much greater than can be accounted for by the pyruvate in the outer region. The results thus demonstrate the penetration of undissociated pyruvic acid into both the outer and the inner regions of the cell. Like the penetration of pyruvate ion into the outer region, the uptake of the undissociated acid takes place under conditions under which no pyruvate is metabolized.

The steady state concentration of pyruvate P_i in the inner region under a variety of conditions is shown in Table III. The fact that P_i remains low under various conditions of incubation agrees with the conclusion drawn from the results presented in Table I that most of the intracellular pyruvate is present in the outer region of the cell.

It is apparent that P_i is independent of external concentration, of temperature, and of O_2 tension. The variation of the absolute concentration with time is also small.

DISCUSSION

Tables I and II clearly show that physical diffusion can explain the entrance of pyruvate ion into the outer region of the cell as well as the uptake of undissociated pyruvic acid by the outer and inner region. Indeed R is independent of temperature and extracellular concentration over a wide range. The entrance of pyruvate ions into the inner region on the other hand clearly occurs only under conditions under which pyruvate is metabolized. From the fact that P_i does not vary greatly from P_0 it follows that Δ_{met} equals Δ_{in}. This suggests the possibility that the system concerned in the uptake of pyruvate may be closely related to the system concerned in its further metabolism. But the requirement for metabolic energy during the uptake of pyruvate ion does not necessarily imply the existence of a transport mechanism involving a metabolic transformation of the molecule. It would be sufficient to assume that the metabolic energy derived from an oxidative reaction is utilized in the maintenance of the plasma membrane in a state in which pyruvate ions could freely diffuse in but not out of the cell. Any pyruvate entering the cell would be metabolized. In such a case P_i would either represent bound pyruvate not available for metabolism, or it would be a measure of the affinity of the pyruvate-metabolizing system for its substrate.

Another possibility considered was that the energy requirement for pyruvate absorption arose out of the need to maintain electroneutrality within and without the cell by uptake of cation or exchange of anion. It was observed, however, in a separate experiment that yeast, which is freely permeable to propionic acid but unable to metabolize it, remains impermeable to Na propionate even under conditions under which Na acetate is dissimilated. This

suggests that the energy-requiring limiting process in anion uptake is neither the transport of cation nor the non-specific facilitation of physical diffusion. It is conceivable that the required energy is utilized in the production from endogenous substrate of anions like succinate or bicarbonate which could then exchange for the external anions. The intracellular concentration of the latter would be kept low either by their metabolism or by the existence of a Donnan equilibrium. Such an explanation does not easily account for the observation that as in the case of low concentrations of citrate (1) repeated freezing of the cell abolishes the permeability barrier to pyruvate (1 μM/ml.). The cold-treated cells appear to be morphologically intact. In spite of the continued existence of a Donnan equilibrium across the cell membrane pyruvate now penetrates the cell at 0°C.

There is no evidence that inorganic phosphate is involved in the active uptake of pyruvate. No phosphate was added to the washed yeast suspensions in the present experiments.

Many substances, both electrolytes and non-electrolytes, are known to be taken up actively by various cells. Pyruvate concentration by subcellular particles was described by Bartley and Davies (6). Whether the mechanism involved in pyruvate uptake by yeast is the same as that involved in other examples of active uptake cannot be determined at the moment.

SUMMARY

The uptake of pyruvate by yeast was studied under a variety of conditions of temperature, extracellular concentration, O_2 pressure, and pH. It was shown that physical diffusion adequately explains the permeation of undissociated pyruvic acid into the outer and inner regions of the cell. The entrance of pyruvate ion into the outer region appears to take place by the same process. The passage of pyruvate ion across the membrane separating the outer from the inner region was found to occur only under conditions under which pyruvate is metabolized. The mechanism of the active process involved in the uptake of pyruvate is not known.

BIBLIOGRAPHY

1. Foulkes, E. C., J. Bact., 1954, 68, 505.
2. Smythe, C. V., J. Biol. Chem., 1938, 125, 635.
3. Conway, E. J., and Downey, M., Biochem. J., 1950, 47, 347.
4. Conway, E. J., and Downey, M., Biochem. J., 1950, 47, 355.
5. Friedemann, T. E., and Haugen, G. E., J. Biol. Chem., 1943, 147, 415.
6. Bartley, W., and Davies, R. E., Biochem. J., 1954, 57, 37.