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Effect of Hexoses on the Levels of Pyruvate Decarboxylase in *Mucor rouxii*

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Pyruvate decarboxylase activity in the dimorphic fungus *Mucor rouxii* increased 25- to 35-fold in yeastlike and mycelial cells grown in the presence of glucose as compared to the activity observed in mycelial cultures grown in the absence of glucose.

The fungus *Mucor rouxii* has been used as a model to study factors that influence morphogenesis because of its ability to grow in either filamentous or yeastlike form. Basically it forms a mycelium when grown in the presence of predominantly aerobic conditions and exhibits yeastlike morphology when grown under conditions that favor fermentative metabolism (12). However, the overall type of metabolism imposed on *Mucor* is itself not sufficient to dictate morphology. Anaerobic conditions can be used to produce either mycelial or yeastlike cells (1), and both types of morphology can also be produced aerobically (5, 8). The only invariable observation is that the formation of yeastlike cells, aerobically or anaerobically, requires the presence of a fermentable hexose (1). To understand the role of fermentable hexoses in the determination of yeastlike morphology, more information is needed on the activity of key enzymes of glucose catabolism under different nutritional conditions which result in specific morphological changes.

One of the studies that address catabolite metabolism in relation to morphogenesis deals with the effects of cyclic AMP on the morphogenesis of *M. rouxii* (7). This study showed that the morphogenetic effect of cyclic AMP could be due to activation of phosphofructokinase, which has its highest activity in cells grown under anaerobic conditions. Friedenthal et al. showed that pyruvate kinase also responds to conditions that affect morphology in *M. rouxii* (3). The presence of glucose favors the production of the type I isozyme, whereas growth under oxidative conditions in the absence of glucose favors the production of the type III isozyme. Depending on the nature and concentration of fermentable carbon sources used for growth of aerobic hyphae, a hybrid form, type II, is also found. *Mucor racemosus*, which catabolizes glucose through both the Embden-Meyerhof-Parnas and pentose phosphate pathways (4), also has multiple forms of pyruvate kinase which respond to growth conditions (9). Type A is induced by the presence of glucose, and type B is formed in the absence of glucose. A particularly strong positive correlation between the level of NAD-dependent glutamate dehydrogenase, which functions as a catabolic enzyme, and hyphal morphology has also been found in *M. racemosus* (10).

Pyruvate decarboxylase (2-oxo-acid carboxylase, EC 4.1.1.1) is a key enzyme in the fermentation of hexoses to ethanol. It catalyzes the conversion of pyruvate to acetaldehyde and CO_2 and is known to be induced by glucose in at least some species of *Saccharomyces* and *Candida* (11). It is possible that the pyruvate decarboxylase could play a role in the morphogenesis of *M. rouxii* through the production of CO_2, which has been shown to influence morphology (2), or by acting in concert with the changes in phosphofructokinase and pyruvate kinase activities described above. We have found that the specific activity of pyruvate decarboxylase from *M. rouxii* was relatively high when cells were grown in the presence of glucose or fructose, whereas greatly reduced levels of activity were found in cells grown in the presence of glycerol, acetate, or peptone as major carbon sources.

The mycelial form of *M. rouxii* (NRRL 1894) was grown aerobically in yeast extract-peptone (YEP) medium, which contained 1% (wt/vol) peptone (Difco) and 0.3% (wt/vol) yeast extract (Difco) adjusted to pH 4.5 with sulfuric acid. Yeastlike cells were grown in YEP medium with 2% (wt/vol) glucose (YEPG) in the presence of a 30% CO_2–70% N_2 atmosphere. All cultures were initiated by the addition of 10^5 sporangiospores per ml followed by incubation at 28°C for 18 h. The cells were harvested by filtration or centrifugation, suspended in 0.01 M potassium phosphate buffer (pH 6.4) with 1.5 mM thiamine pyrophosphate and 25 mM magnesium chloride, and ruptured in an Eaton cell at 10,000 lb/in^2.

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The supernatant fraction from an initial centrifugation at 1,000 × *g* for 10 min was centrifuged at 27,000 × *g* for 10 min. The supernatant fraction from the second centrifugation contained the pyruvate decarboxylase activity. Partial purification of the enzyme used for determination of apparent K_m values was achieved by ammonium sulfate precipitation. The fraction that precipitated between 55 and 75% saturation at 4°C contained the pyruvate decarboxylase activity. An alcohol dehydrogenase-coupled assay that measures NAD^+ formation spectrophotometrically was used to measure enzyme activity (13). Specific activity values are expressed as micromoles of NADH oxidized per minute per milligram of protein. Protein concentrations were determined with a Bio-Rad protein assay kit.

Pyruvate decarboxylase from mycelial cells grown aerobically in the absence of a carbon source other than that provided by YEP had a specific activity of 0.05. The addition of 2% (vol/vol) glycerol or 2% (wt/vol) sodium acetate to the YEP medium resulted in an increase in specific activity to 0.3, and mycelial morphology was maintained. The specific activity increased to 1.3 in mycelial cells when 2% (wt/vol) glucose or fructose was added to the YEP medium. The enzyme from yeastlike cells grown anaerobically in the presence of 2% glucose had a specific activity of 1.7, a 35-fold increase over the activity from mycelial cells grown without glucose. Thus pyruvate decarboxylase activity increased when cells were grown on a glucose medium regardless of whether aerobic or anaerobic conditions were present, and substantial increase occurred in the absence of morphological changes.

The time-dependent increase in cell mass and pyruvate decarboxylase specific activity of cells grown aerobically in the presence or absence of 2% (wt/vol) glucose is shown in Fig. 1. The amount of growth obtained in the presence of glucose was about 30% greater than that obtained in the absence of glucose, but this increase in cell mass did not account for the 25-fold increase in specific activity in the presence of glucose.

Lineweaver-Burk double-reciprocal plots were used to determine apparent K_m values for pyruvic acid. The value for the pyruvate decarboxylase from mycelial cells grown aerobically in YEPG medium was 2.4 mM (±0.11), and that for the enzyme from yeastlike cells grown anaerobically in YEPG medium was 2.9 mM (±0.14). The value for the enzyme from mycelial cells grown in an aerobic YEP medium was 1.5 mM (±0.34). Although further work is needed to establish the significance of the observed differences between the apparent K_m value for the enzyme from cells grown with glucose and cells grown without glucose, a similar difference has been reported for the isozymes of pyruvate kinase in *M. rouxii* (3).

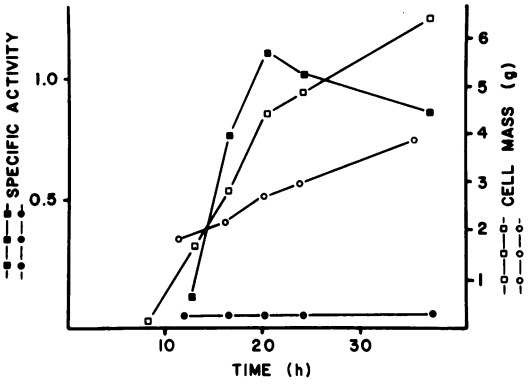

FIG. 1. *M. rouxii pyruvate decarboxylase activity and mycelial cell mass as a function of growth time.* (■) *Specific activity of pyruvate decarboxylase from culture grown with 2% (wt/vol) glucose;* (□) *cell mass per 500 ml of culture medium for cells grown with glucose;* (●) *specific activity of pyruvate decarboxylase from culture grown in the absence of glucose;* (○) *cell mass per 500 ml of culture medium for cells grown without glucose.*

Some of the previous work on the effects of glucose on morphology and levels of catabolic enzymes is summarized and compared with our findings in Table 1. These results are similar to ours on pyruvate decarboxylase in that changes in activity levels, in our case a 25-fold increase, in the presence of absence of glucose under aerobic conditions did not accompany changes in morphology. In the case of phosphofructokinase, pyruvate kinase, and NAD^+-dependent glutamate dehydrogenase, there are substantial additional changes in activity levels when the cultures are grown anaerobically with glucose to give yeastlike morphology. Our study showed that the pyruvate decarboxylase activity from yeast cells grown anaerobically in the presence of glucose does not exhibit a substantial increase over that observed in aerobic mycelial cultures grown with glucose. However, the overall trend established by the work cited in Table 1 is that there is a significant change in the activity level of key catabolic enzymes in response to the presence of glucose, but that these changes alone are not sufficient to cause changes in morphology. Conversely, carbon flow through the major catabolic pathways can remain relatively constant while changes in morphology occur, provided glucose is present (4). Thus, although changes in activity levels of key enzymes in response to the availability of fermentable hexoses may not be sufficient to cause changes in morphology, it is possible that the combined

TABLE 1. *Effect of glucose on levels of catabolic enzymes and morphology in Mucor*

Cell type	Enzyme activity				
	Pyruvate kinase[a]		Phosphofructokinase[b]	Glutamate dehydrogenase[c]	Pyruvate decarboxylase[c]
	1 min	5 min			
Mycelium					
−Glucose	1,056	2	1.7	4.3	0.05
+Glucose	2,930	10	8.04	0.53	1.3
Yeast					
(+Glucose)	4,380	30	63.6	0.03	1.7

[a] Nanomoles of pyruvate formed per milligram of protein in time shown (3, 9).

[b] Micromoles ($\times 10^3$) of fructose 1,6-diphosphate produced per minute per milligram of protein (7).

[c] Micromoles of NADH oxidized per minute per milligram of protein (10).

effects of these changes, in addition to changes in levels of other effectors such as cyclic AMP (6) and end products such as CO_2 (2), may be required for morphological changes to occur.

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