Glucose-induced Crypticity Toward Succinate Metabolism in Saccharomyces lactis

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Saccharomyces lactis grown on glucose adapted very slowly to growth on succinate. This initial inability of glucose-grown cells to grow on succinate was paralleled by their inability to oxidize succinate. The possibility that repression by glucose of respiratory chain components was responsible for these observations was examined. Glucose-grown cells were able to respire glucose, ethyl alcohol, and lactate and were able to initiate growth on ethyl alcohol as rapidly as succinate-grown cells. Respiratory enzyme levels were essentially the same in cells grown on succinate or on glucose. Spectroscopic analysis revealed that glucose-grown cells possessed a full complement of cytochrome bands. Since by these criteria glucose-grown S. lactis appears to possess a competent respiratory system, the penetration of succinate-2,3-14C into succinate- and glucose-grown cells was examined directly. Glucose-grown cells exhibited a strong permeability barrier to succinate. Comparison of glucose oxidation by S. lactis and by S. cerevisiae suggests that the crypticity to succinate does not depend upon a strong Crabtree effect in S. lactis.

The ability of a variety of yeasts to utilize tricarboxylic acid cycle intermediates was observed by Barnett and Kornberg (3) to depend on the conditions of growth. They suggested that the cellular component modified by the growth conditions was the permeability system for acids of the tricarboxylic acid cycle.

In their studies of the hybrid yeast Saccharomyces fragilis × S. dobzhanskii, MacQuillan and Halvorson (18) found that growth of these cells on glucose markedly inhibited their ability to oxidize succinate and other tricarboxylic acid cycle intermediates. The glucose-grown cells contained succinate dehydrogenase activity, although at a reduced level. The recovery of succinate-oxidizing activity by cells exposed to glucose was susceptible to inactivation by ultraviolet light. It was postulated that the glucose-induced crypticity of this yeast to succinate metabolism was due to a permeability barrier between external succinate and the succinate dehydrogenase in the cell.

The influence of glucose on the formation of the mitochondrial respiratory system in S. cerevisiae is well known. Growth in the presence of high concentrations of glucose leads to repression of the formation of cytochromes (10, 22) and of other respiratory enzymes (20, 21). The succinate dehydrogenase activity of the hybrid yeast was assayed by measuring the reduction of ferricyanide which is relatively nonspecific in its interaction with the respiratory chain (11, 12). It was possible, therefore, that in the hybrid yeast a repression of the respiratory system, e.g., cytochromes, by glucose was also involved in the crypticity towards succinate.

Herman and Halvorson (13) have reported that the ability of S. lactis to oxidize succinate is inhibited by exposure to glucose and that the overall respiratory properties of this yeast are similar to those of the hybrid yeast. It seemed of interest to examine the glucose-induced inhibition of succinate oxidation by these yeasts in more detail, and to determine how closely related are the effects of glucose on the respiratory system of S. cerevisiae and on succinate oxidation by S. lactis.

MATERIALS AND METHODS

Yeast strains. The experiments in this report were performed with a haploid, heterothallic strain of S. lactis Y123 obtained from H. O. Halvorson, University of Wisconsin, and a haploid, heterothallic strain of S. cerevisiae X2180-1A obtained from R. K. Mortimer, University of California. Some growth studies were also carried out with the following strains of yeast: S. lactis Y14, S. fragilis Y610, S. dobzhanskii Y1974 [species which Van der Walt (24) assigned to the genus Kluyveromyces], and the hybrid S. fragilis Y610 × S. dobzhanskii Y1974, all of which were obtained from H. O. Halvorson.
Growth conditions. Cells were grown aerobically in a rotatory shaker bath at 30°C in 250-ml flasks containing 100 ml of a synthetic medium at pH 5.4 (23) with the appropriate carbon source. Exponential-phase cells and stationary-phase cells were harvested from cultures grown to an optical density (OD) at 600 nm of about 0.4 and 1.3, respectively. Lag-phase cells were obtained from cultures first inoculated to an OD of 0.1 with stationary-phase cells, allowed to grow for 4 hr, and then harvested. For growth transition studies, cells were transferred either after washing at 30°C in basal medium (synthetic medium with no carbon source) or without washing, as stated in the results.

Manometric procedures. The preparation of cells and manometric conditions have been previously described (18).

Protein determinations. The protein content of cell extracts was determined by the procedure of Lowry et al. (15), with bovine crystalline albumin as a standard. Amounts of protein in washed yeast cells were determined by suspending cells in 1.0 M NaOH and heating the suspensions in a boiling-water bath for 10 min. The suspensions were then cooled and centrifuged, and the protein content of the supernatant fluid was assayed by the above procedure. Bovine crystalline albumin, similarly treated, was employed as a standard.

Succinate uptake studies. Exponential-phase cells were harvested and prepared as for manometric studies. Reaction mixtures contained 0.067 M phosphate buffer (pH 5.4), 7.5 ml; cell suspension, 4 ml; water, 1.5 ml; and succinate-2,3-C14, 35.8 µmole/ml (specific activity, 0.024 µc/µmole), 2 ml. Mixtures were shaken at 15 and at 30°C, and 1.0-ml samples were removed to 3.0 ml of ice-cold phosphate buffer containing 0.01 M sodium azide. Cells were then filtered (0.45 µm, HA; Millipore Corp., Bedford, Mass.) and washed three times with 2.0-ml amounts of ice-cold phosphate-azide buffer (pH 5.4). The filters and cells were then boiled in 4.0 ml of distilled water for 15 min, cooled, and centrifuged. Portions (1 ml) of the supernatant fluid were then evaporated to dryness in aluminum planchets and counted with a Nuclear-Chicago (model 418) beta scintillation crystal. No corrections for self-absorption were made. Succinate accumulation, in micromoles per milligram of protein, was calculated from the hot-water-extractable radioactivity and from protein determinations on the whole cells. Corrections for adsorption of succinate to cells were made by subtracting radioactivity obtained with samples from reaction mixtures, incubated at 0°C, in which phosphate-azide buffer was added before succinate.

Cytochrome measurements. The absorption spectrum of cells grown on solidified medium was determined from examination of liquid nitrogen-frozen cell pastes (1 mm in thickness) employing a microspectroscope (Beck, London).

Enzyme assays. Cells grown in the synthetic medium with succinate or glucose as the carbon and energy source were washed once in 0.067 M phosphate buffer (pH 6.8), and then suspended at a concentration of about 2 X 10^9 cells per ml in 0.01 M tris(hydroxy-
methyl)aminomethane buffer (pH 8.0) containing 0.5 M sucrose and 5 X 10^4 M ethylenediaminetetra-acetate (19). The frozen cell suspensions were passed twice (8) through a BPI X-Press (Biochemical Processes Inc., New York, N.Y.). The extract was centrifuged for 5 min at 3,600 X g in the cold, and the supernatant fluid was retained for enzyme determinations. Assays for succinate dehydrogenase (1), cytochrome c oxidase (EC 1.9.3.1; 17), reduced nicotinamide adenine dinucleotide (NADH) oxidase, NADH-cytochrome c reductase (EC 1.6.2.1), and succinate-cytochrome c reductase (EC 1.3.99.1; 16) were performed at 28°C by use of a Beckman DB recording spectrophotometer. Specific activities are reported as micromoles of substrate converted per minute per milligram of protein.

RESULTS

When cells of S. lactis Y123, previously grown in synthetic medium containing either succinate or glucose as the carbon source, were washed and transferred, each to succinate medium and to glucose medium, marked differences in the initial growth responses were observed (Fig. 1). Glucose-grown cells did not grow for many hours after transfer to succinate. Similar but not identical growth responses during these transitions were shown by S. lactis Y114, S. fragilis Y610, S. dobzhanskii Y1974, and the hybrid S. fragilis Y610 X S. dobzhanskii Y1974. However, the lag

![Fig. 1. Growth response of S. lactis Y123 when washed succinate-grown cells (mid-exponential phase) were transferred to succinate (○) and to glucose (■), and when washed glucose-grown cells (mid-exponential phase) were transferred to glucose (△) and to succinate (▲). Initial concentration of succinate or glucose in the medium was 0.2%.](http://jb.asm.org/)

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on transfer from glucose to succinate was most pronounced for *S. lactis* Y123.

Several reports (9, 22) have described the inability of respiratory deficient yeasts to grow on substrates which can be respired but not fermented. If the long lag shown by *S. lactis* on transfer from glucose to succinate was due to the repression of some component(s) of the respiratory chain, then cells transferred from growth on glucose to other respiratory carbon and energy sources might exhibit a similar lag. Figure 2 illustrates the pattern of growth responses when glucose-grown cells and succinate-grown cells were washed and transferred to synthetic media containing ethyl alcohol, acetate, lactate, and glycerol as carbon sources. Cells grown on glucose showed two kinds of growth response. They adapted very slowly to acetate and to glycerol just as to succinate. They exhibited a much shorter lag period on lactate and on ethyl alcohol and initiated exponential growth at a time when similar cells on acetate, glycerol, or succinate were unable to grow. It seems clear from these results that while glucose-grown cells adapt to respiratory substrates less readily than do succinate-grown cells, the period of adaptation depends on the nature of the substrate rather than on some generalized respiratory property of the cells.

The more rapid adaptation of succinate-grown cells to growth on these respiratory carbon sources was reflected in their higher rates of oxygen uptake on respiratory substrates (Table 1). Only glucose was oxidized by succinate-grown cells at a rate which was lower than that of glucose-grown cells. The rate of glucose oxidation by succinate-grown cells was not increased by washing and resuspending the cells in basal medium rather than in phosphate buffer. Oxidation of respiratory substrates by glucose-grown cells roughly corresponded to their ability to grow on these compounds. The high rates of oxidation of glucose and of ethyl alcohol by glucose-grown cells, however, suggest that the respiratory system was highly active and that the reduced ability of glucose-grown cells both to oxidize and to grow on some respiratory carbon sources was the result of poor access to the respiratory system rather than to an overall impairment of the system.

The progressive changes in succinate and glucose respiration which developed after transfer of *S. lactis* from succinate to glucose medium were examined (Fig. 3). Initially, these cells exhibited a low rate of glucose oxidation which was consistent with the growth lag observed. The subsequent increase in the rate of glucose respiration during the growth lag suggests that glucose utilization may be, to a large extent, inducible.

In contrast to these results for the oxidation of glucose by these cells, the rate of succinate oxidation was high at first but decreased within 5 hr to very low levels. This loss of ability to oxidize succinate may be attributable to one of the following reasons: (i) a progressive decrease in the concentration of succinate carried over when the cells were transferred, implying perhaps that succinate is necessary for the preservation of the succinate-utilizing system; (ii) the deleterious

![FIG. 2. Growth of *S. lactis Y123* when mid-exponential-phase cells grown on succinate or on glucose were washed and transferred to media containing the following carbon sources at a concentration of 0.2%: lactate, ●; ethyl alcohol, ○; acetate, ▲; and glycerol, △.](image)

**Table 1. Respiration of various substrates by *S. lactis Y123* grown on glucose or on succinate**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Carbon source$^a$ for growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td>Glucose</td>
<td>353$^b$</td>
</tr>
<tr>
<td>Succinate</td>
<td>5</td>
</tr>
<tr>
<td>Malate</td>
<td>8</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>261</td>
</tr>
<tr>
<td>Lactate</td>
<td>165</td>
</tr>
<tr>
<td>Acetate</td>
<td>32</td>
</tr>
<tr>
<td>Glycerol</td>
<td>10</td>
</tr>
</tbody>
</table>

$^a$ Glucose or succinate (0.2%) in a synthetic medium.

$^b$ Expressed as microliters of O$_2$ X hour$^{-1}$ X milligram$^{-1}$ of protein.
effects of glucose utilization; or (iii) a combination of (i) and (ii).

It seems unlikely that the loss of succinate-oxidizing ability is caused solely by the depletion of succinate in the cells. Higher concentrations of succinate (0.2%) added to the glucose medium did not reduce the rate at which the cells lost the ability to oxidize succinate. Moreover, washed, succinate-grown cells might have been expected to lose a succinate-dependent, succinate-oxidizing ability more rapidly than cells transferred in the presence of succinate. In fact, when washed cells were transferred to glucose medium they exhibited a longer growth lag (Fig. 1), and the ability to oxidize succinate decreased more slowly (Fig. 3). It is apparent, therefore, that something more than the mere absence of succinate contributes to the progressive restriction of succinate oxidation by these cells. Glucose itself or glucose metabolism might be this contributing factor. The inhibitory effects of glucose on succinate oxidation might be accomplished in a number of ways. For example, glucose or its metabolism might exert control over the function or the formation of that part of the respiratory chain which specifically deals with succinate oxidation. Alternatively, the inhibition may be directed towards the succinate permeability system of the cell.

The repression of cytochrome formation which results from the growth of *S. cerevisiae* on glucose is manifest in the "Crabtree effect," wherein high rates of glucose fermentation lead to a reduced respiratory ability (5, 6, 10). A useful measure of the Crabtree effect can be obtained from the ratio of glucose fermented to glucose respired by a culture (6). Accordingly, the fermentative capacity of *S. lactis* was compared by this means with that of *S. cerevisiae* (Table 2). For the latter yeast, a very strong Crabtree effect (ratio >1) was observed. A puzzling but reproducible feature of glucose inhibition with this strain was the relatively low rate of glucose fermentation by cells grown on 2% glucose. Nevertheless, the Crabtree ratio was high. Glucose-grown *S. lactis*, however, showed no evidence of a Crab-

![Fig. 3. Changes in the ability of *S. lactis* Y123 to oxidize succinate (○), or glucose (●), when mid-exponential-phase cells were transferred, unwashed, from succinate medium to glucose medium. Growth in glucose medium (×). Succinate oxidation when cells were washed before transfer to glucose medium (△).](image)

**Table 2. Influence of glucose on the production of a Crabtree effect in yeast**

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>Respiration (O2 uptake)</th>
<th>Aerobic fermentation (CO2 evolution)</th>
<th>Glucose utilized (μmoles)</th>
<th>Ratio of glucose fermented to glucose respired</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. cerevisiae</em> X2180-1A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2% Glucose</td>
<td>0.22</td>
<td>1.65</td>
<td>0.04</td>
<td>0.83</td>
</tr>
<tr>
<td>2% Glucose</td>
<td>0.85</td>
<td>15.9</td>
<td>0.14</td>
<td>7.95</td>
</tr>
<tr>
<td><em>S. lactis</em> Y123</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2% Glucose</td>
<td>4.2</td>
<td>0.4</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Exponential</td>
<td>13.92</td>
<td>1.52</td>
<td>2.32</td>
<td>0.76</td>
</tr>
<tr>
<td>Stationary</td>
<td>3.8</td>
<td>0.89</td>
<td>0.63</td>
<td>0.45</td>
</tr>
<tr>
<td>2% Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exponential</td>
<td>14.3</td>
<td>0.9</td>
<td>2.38</td>
<td>0.45</td>
</tr>
<tr>
<td>5% Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exponential</td>
<td>11.2</td>
<td>0.6</td>
<td>1.97</td>
<td>0.3</td>
</tr>
<tr>
<td>0.2% Succinate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag</td>
<td>2.9</td>
<td>0</td>
<td>0.48</td>
<td>0</td>
</tr>
<tr>
<td>Exponential</td>
<td>3.1</td>
<td>0</td>
<td>0.52</td>
<td>0</td>
</tr>
<tr>
<td>Stationary</td>
<td>2.1</td>
<td>0</td>
<td>0.35</td>
<td>0</td>
</tr>
</tbody>
</table>

* Expressed as micromoles × hour⁻¹ × milligram⁻¹ of protein.
tree effect (ratio <1) at any stage of growth over a wide range of glucose concentrations (Table 2). Glucose respired always greatly exceeded that aerobically fermented. Aerobic fermentation of glucose was not observed with succinate-grown cells.

In spite of the absence of a measurable Crabtree effect in glucose-grown S. lactis, it was still possible that the succinate-oxidizing system was very susceptible to repression by glucose. This possibility was tested by comparing the specific activities of several components of the respiratory chain in cells grown on succinate and on glucose. The results (Table 3) show that glucose-grown cells possess a respiratory enzyme complement which is essentially the same as that of succinate-grown cells. The slight drop in succinate dehydrogenase activity in glucose-grown cells should not account for the loss of succinate oxidation observed within 4 hr of transfer to glucose (Fig. 3).

When the permeability to 14C-labeled succinate was tested in succinate- and glucose-grown cells (Fig. 4), the former were found to accumulate radioactive materials in their hot-water soluble pool, whereas the latter cells showed no evidence of such accumulated material. At 30°C, succinate accumulation by succinate-grown cells was linear for about 15 min, a period which approximately coincides with an initial lag which has been observed for succinate oxidation by these cells. Uptake at 15°C by succinate-grown cells was slower but was linear for a longer time. Taken together, these observations suggest that the growth of S. lactis on glucose severely reduces its permeability to succinate.

As further evidence that the impairment of succinate utilization by glucose-grown S. lactis is mainly due to a glucose effect exerted specific-

**Table 3. Specific activities* of respiratory enzyme systems from S. lactis Y12**

<table>
<thead>
<tr>
<th>Determination</th>
<th>0.2% Succinate</th>
<th>0.2% Glucose</th>
<th>2.0% Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH oxidase</td>
<td>28.0</td>
<td>27.1</td>
<td>27.8</td>
</tr>
<tr>
<td>Ferrocyanochrome c oxidase</td>
<td>18.7</td>
<td>18.9</td>
<td>15.1</td>
</tr>
<tr>
<td>NADH-cytochrome c reductase</td>
<td>4.4</td>
<td>9.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Succinate-cytochrome c reductase</td>
<td>2.4</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Succinic dehydrogenase</td>
<td>0.019</td>
<td>0.015</td>
<td>0.010</td>
</tr>
</tbody>
</table>

* Micromoles of substrate converted per minute per milligram of protein.
  
Exponential-phase cells.

FIG. 4. Succinate uptake at 15°C (●) and 30°C (○) by succinate-grown cells and at 30°C (▲) by glucose-grown S. lactis Y123. Radioactive counts (14C) accumulated are expressed as micromoles of succinate per milligram of cell protein.

...on succinate utilization, rather than on the generalized respiratory system, the following observations are offered.

(i) Counts of mitochondria stained with Janus Green (2) in viable succinate- and glucose-grown S. lactis cells did not differ significantly.

(ii) Counts of the average number of mitochondrial profiles per section in electron microscope photographs of succinate- and glucose-grown cells were almost identical.

(iii) Spectroscopic analysis of S. lactis grown for 24 hr on 0.2, 2, and 5% glucose showed no detectable differences with respect to quality or quantity of the cytochrome content. Succinate-grown cells, however, contained slightly higher amounts of all of the main cytochrome bands. In contrast, when S. cerevisiae grown at the same glucose concentrations was examined, marked decreases in cytochromes c and a were observed at the higher glucose levels.

**DISCUSSION**

Two features of the control of succinate metabolism, produced apparently in response to glucose in S. lactis, which are evident from this study are (i) that crypticity is the result of a relatively specific regulatory effect, probably exerted on the succinate permeability system of the cell, and (ii) that the production of crypticity is not dependent on the presence of a Crabtree effect, i.e., on excess aerobic fermentation as compared to respiration of glucose.

With regard to the first feature, the focusing
of this glucose effect on a specific component of the succinate-oxidizing system seems evident from the following summarized findings. (i) Glucose-grown cells can grow on ethyl alcohol and on lactate with relatively little difficulty, exhibiting a lag which is considerably shorter than that shown on transfer to succinate. (ii) Ethyl alcohol and lactate are readily oxidized by glucose-grown cells, although lactate oxidation initially proceeds at one-half the rate exhibited by succinate-grown cells. Furthermore, glucose-grown cells can oxidize glucose such that respiration greatly exceeds aerobic fermentation. These observations suggest that a fully operative respiratory system exists. (iii) No major differences in respiratory enzyme levels between succinate- and glucose-grown cells have been found. (iv) Although succinate-grown cells have slightly higher amounts of the cytochromes, high levels of all major cytochrome bands have been observed spectroscopically in glucose-grown cells. Concentrations of glucose which lead to strong repression of cytochromes a and c in S. cerevisiae do not effectively diminish the amounts of cytochromes in S. lactis.

These properties of glucose-grown S. lactis cells leave little doubt that they possess an active and efficient respiratory system, despite the fact (Table 1) that they oxidize some respiratory substrates at rates lower than do succinate-grown cells. The inability of glucose-grown cells to oxidize succinate, therefore, results from a relatively specific effect on the succinate-oxidizing system. The very striking difference in the ability of succinate- and glucose-grown cells to accumulate radioactivity in the extractable pool in the presence of exogenous 14C-succinate points very strongly to a marked cell permeability barrier in the glucose-grown cells. These results clearly suggest that a major site of action of the glucose-induced crypticity to succinate metabolism in S. lactis is on the succinate entry process.

Concerning the second feature of the control of succinate metabolism, there is no a priori reason to expect that a control mechanism, which presumably results from glucose metabolism, must depend on a high rate of glucose fermentation rather than respiration. However, repression of respiratory chain components in a fermentative yeast, S. cerevisiae, is believed to result from fermentation of glucose, and expression of this inhibitory phenomenon gives rise to the Crabtree effect. It is of interest, therefore, that in a respiratory yeast, S. lactis, the control exerted by glucose on the succinate permeability system, which is functionally related to mitochondrial enzymes, is not dependent on a strong Crabtree effect. The lack of a Crabtree effect in S. lactis suggests that either control by glucose fermentation is simply ineffective or some countermeasure exerting opposite controls exists. Perhaps S. lactis exhibits a strong Pasteur effect. Bulder (4) reported that the rate of aerobic fermentation by S. lactis increases in the presence of acriflavine, which inhibits respiratory enzyme synthesis. Similar findings for S. fragilis, which also exhibits a negative Crabtree effect, were reported by De-Deken (7). These reports are consistent with the possibility that lowering the rate of respiration relieves a Pasteur effect control.

The low rate of glucose oxidation by succinate-grown S. lactis might suggest an inhibitory control. Kulka et al. (14) have reported that succinate blocks the transfer of electrons from nicotinamide adenine dinucleotide (NAD)-linked substrates to the cytochromes by monopolizing the respiratory chain in animal tissues. If such a mechanism were operative in producing the low rates of glucose oxidation by succinate-grown S. lactis by virtue of endogenous succinate, one would expect, first, that it would only be a transient inhibition and, second, that the oxidation of other NAD-linked substrates would similarly be inhibited. However, malate and ethyl alcohol (Table 1) were readily oxidized by succinate-grown cells. For the moment, the low rate of glucose oxidation by succinate-grown S. lactis cannot be explained. These same cells do not ferment glucose, an observation which reinforces my suspicion that respiratory metabolism controls glucose utilization. Whether such control is also operative at the level of cell permeability or at an intracellular metabolic location might be deserving of further study.

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LITERATURE CITED