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Glucose Repression in Yeast Cells. Comparison of the Effect of Galactose with that of Glucose on the Formation of Respiratory Enzymes and Cytochromes

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The effect of glucose and galactose on the formation of respiratory enzymes and cytochromes in yeast cells was compared both in aerobic culture and during adaptation of anaerobically grown cells to oxygen.

(1) In aerobic culture, activities of reduced nicotinamide-adenine dinucleotide (NADH) cytochrome c reductase and malate dehydrogenase and QO_2 of cells were reduced considerably with increasing concentration of glucose in the medium. On the other hand, galactose did not repress NADH cytochrome c reductase significantly and QO_2 of cells was not reduced with increasing concentration of gllactose in the growth medium, although it slightly repressed malate dehydrogenase.

(2) Formation of cytochromes, and NADH cytochrome c reductase and malate dehydrogenase during the adaptation of anaerobic cells to oxygen were inhibited by a high concentration of glucose but not by galactose, and glucose was consumed rapidly but galactose was consumed slowly. On the other hand, when yeast cells grown anaerobically in the presence of galactose were transferred to aerobic culture in the presence of galactose, it was consumed rapidly and the formation of cytochromes, NADH cytochrome c reductase and malate dehydrogenase was inhibited. NADH ferricyanide reductase activity did not changed significantly during the adaptation of anaerobic cells to oxygen.

The yeast, *Saccharomyces cerevisiae*, is a fucultative anaerobic organism, and the development of mitochondrial structure and the formation of respiratory chain cytochromes and respiratory enzymes are under the influence of environmental conditions, catabolite repression and the presence of oxygen.[2] The earlier studies in this laboratory have demonstrated that the synthesis of respiratory enzymes in yeast cells was repressed by hexoses such as glucose, fructose and mannose but not by C2 or C3 compounds such as ethanol, acetate or lactate.[3]

In regards to the effect of galactose on the synthesis of respiratory enzymes in *Saccharomyces cerevisiae*, Strittmatter reported that yeast cells grown on galactose had a higher level of oxidative activities than those grown on glucose.[4] Linnane and Polakis, *et al.* reported that the synthesis of mitochondria was inhibited by glucose but not by galactose.[5,6] These

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authors observed that cytochrome c oxidase was formed anaerobically in yeast cells in the presence of galactose and suggested that the respiratory adaptation of yeast was not an induction by oxygen but a de-repression due to the removal of glucose.[7] On the contrary, Somlo demonstrated that the synthesis of respiratory enzymes did not take place under strictly anaerobic conditions even in the presence of glucose.[8]

In this study, the effect of galactose on the formation of respiratory chain in yeast was compared with those of glucose. The results in this paper indicated that the formation of oxidative enzymes and cytochromes were inhibited by glucose but not by galactose. When yeast cells adapted to galactose were used, galactose was consumed as rapidly as glucose and the repression of respiratory chain by galactose was observed.

Methods and Materials

Organism——*Saccharomyces cerevisiae*, No. 3027, a Bakers' yeast, Faculty of Agriculture, Hokkaido University was used in this study. It was maintained on agar medium containing 1% yeast extract, 1% peptone and 1% glucose and subcultured monthly.

Aerobic Culture——Cells were cultured in a liquid medium containing 1% yeast extract, 1% glucose and 1% peptone on standing for 20 hr at 30°. Harvested cells were washed twice with sterilized water and a portion (1 mg dry weight) of cells was transferred to 200 ml of semi-synthetic medium as described by Ephrussi[9] containing 10 g of yeast extract, 0.7 g of $MgSO_4 7H_2O$, 1.0 g of KH_2PO_4, 0.4 g of $CaCl_2$, 1.2 g of $(NH_4)_2SO_4$ and 0.005 g of $FeCl_3$ per liter. Glucose or galactose was supplemented as indicated. Cells were cultured aerobically in an one liter shaking flask containing 200 ml of medium at 30°. At the end of incubation, cells were rapidly cooled, centrifuged and washed twice with cold distilled water. The cells thus obtained were suspended in cold water.

Anaerobic Culture——One liter of the above medium supplemented with 20 mg of ergosterol and 4.4 mg of Tween 80 in an one liter Erlenmeyer flask was layered with liquid paraffin and inoculated with 5 mg of yeast cells, and incubated with gentle stirring with a magnetic stirrer at 30°.

Adaptation of Cells Grown Anaerobically to Oxygen——The cells grown anaerobically were harvested as described above, washed twice with distilled water and suspended in 100 ml of solution containing 0.067 M KH_2PO_4, 0.1% casamino acid and different concentrations of glucose or galactose. Three or four mg (dry weight) per ml of cells in the above medium were incubated aerobically at 30° in a 500 ml shaking flask. Cells were harvested at the indicated times, centrifuged and washed with and suspended in cold water.

Preparation of Cell Free Homogenate——Cells (from 400 to 500 mg dry weight) suspended in 10 ml of solution containing 10 mM Tris HCl buffer, pH 6.5, 0.1 mM EDTA, and 0.65 M solbitol were placed in a Homoblendor, Nihon seiki Seisakusho, Co., together with 10 g of glass beads (0.2 mm diameter) and homogenized at top speed (about 15000 r.p.m.) for 5 min below 2°. After removal of glass beads on standing, unbroken cells and cell debris were removed by centrifugation at $1000 \times g$ for 5 min and the supernatant was used as cell free homogenate.

Analytical Methods——Glucose and galactose were determined by Ashwell's procedure.[9] Protein was determined by a biuret method using bovine serum albumin as a standard.[10]

Assay of Enzyme Activities——Enzyme assay was carried out spectrophotometrically at room temperature by use of Shimazu MPS spectrophotometer. Activity of reduced nicotinamide-adenine dinucleotide (NADH) cytochrome c reductase (NADH-cytochrome c oxidoreductase EC 1.6.99.3) was measured by Green's method.[11] Malate dehydrogenase (L-malate-NAD oxidoreductase EC 1.1.1.37) was measured as described by Ochoa.[12] NADH ferricyanide reductase activity was measured as described by Lindenmayer.[13] Enzyme activitywas expres sed in μmoles of substrate oxidized per minute per mg protein.

Manometric Measurement of Glycolytic and Respiratory Activities of Whole Cells——Respiratory activity was measured manometrically with air as the gas phase at 30°. The incubation medium contained 200 μmoles of potassium phosphate buffer, pH 6.0 and 2–6 mg dry weight of cells in a total volume of 2.8 ml. Center well

7) E.R. Tustanoff and W. Bartley, *Biochem. J.*, **91**, 595 (1964).
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contained 0.2 ml of 10 N KOH. In the side arm, 0.2 ml of 1.0 M ethanol was placed and tipped into the reaction mixture after 7 minutes of preincubation, and the oxygen taken up was determined. Glycolytic activity was measured manometrically under nitrogen as gas phase. After preincubation, 0.2 ml of 0.5 M glucose was tipped into the medium and the evolution of CO_2 was determined.

Difference Spectrum——Reduced minus oxidized difference spectrum of cell suspension was obtained at room temperature with a Shimazu MPS spectrophotometer. The reference cuvette contained 150 μmoles of phosphate buffer pH 7.0, 750 μmoles of hydrogen peroxide and 50 mg (dry weight) of yeast cells in a total volume of 3.0 ml. A sample cuvette contained a few grains of sodium dithionite and 50 mg (dry weight) of yeast cells in 3.0 ml of the above buffer.

Chemicals——NADH was purchased from Sigma Chemicals Company and other chemicals were purest grade available.

Results

Growth and Consumption of Sugars by Yeast Cells Grown on Glucose or Galactose Aerobically

Yeast cells were grown aerobically on varying concentrations of glucose or galactose. As seen in Figure 1, the growth in a medium of glucose started after 6 hr of lag period and virtually ceased after 18 hr of incubation. Glucose disappeared 12 hr after innoculation from the medium containing initially 0.9% glucose. In the case of 10.8% glucose, the sugar remained in the medium after 24 hr of incubation. When yeast cells were cultured with Galactose as carbon source, growth started after 12 hr of lag period. The amount of cells obtained after 24 hr of culture with galactose did not differ significantly from that of cells obtained with glucose, while the consumption of the sugar was slower in the culture of galactose.

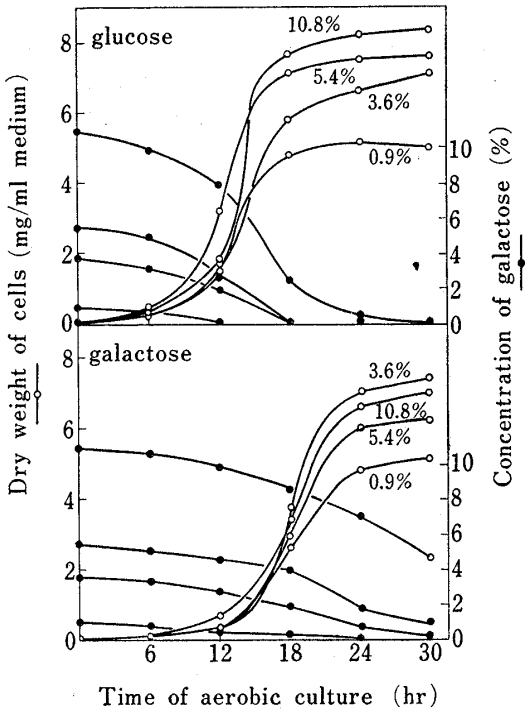

Fig. 1. Aerobic Growth of Yeast Cells in the Presence of Different Concentrations of Glucose or Galactose and Consumption of Sugars

Yeast cells were grown on 0.9, 3.6, 5.4 and 10.8% glucose or galactose at 30 with shaking. Aliquots of cultures were taken up with time, centrifuged, washed twice with distilled water and dry weight of cells and a glucose concentration in the growth medium were determined as described in materials and methods.

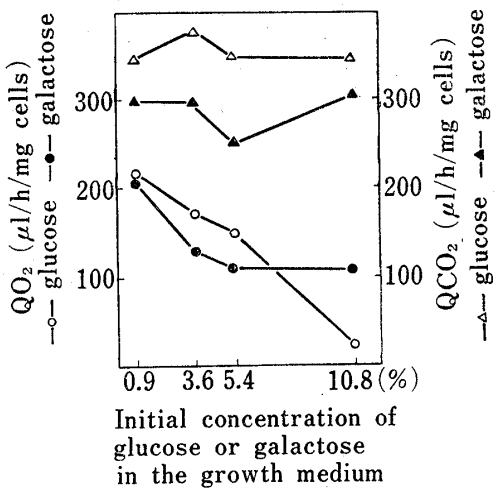

Fig. 2. Glycolytic and Respiratory Activities of Yeast Cells Grown Aerobically on Different Concentrations of Glucose or Galactose

Yeast cells were cultured aerobically at 30 in the presence of different concentrations of glucose or galactose. Cells harvested after 24 hr of cultivation were washed twice with distilled water and suspended in cold water. Glycolytic and respiratory activities were assayed manometrically as described in materials and methods.

○: QO_2 glucose
●: QO_2 galactose
△: QO_2 glucose
▲: QO_2 galactose

After 24 hr of incubation, galactose disappeared from the medium which contained 0.9% galactose at the beginning of culture but remained in the medium containing initially 3.6%, 5.4% and 10.8% galactose.

Glycolytic and Respiratory Activities and Enzyme Activities of Yeast Cells Grown on Different Concentrations of Glucose or Galactose

The glycolytic and respiratory activities of yeast cells grown aerobically for 24 hr on different concentrations of glucose or galactose were shown in Figure 2. In cells grown on glucose, glycolytic activity did not change with increasing concentration of initial glucose in the growth medium. In the case of galactose, the initial concentration of the sugar in the growth medium also did not affect the glycolytic activity. The glycolytic activity of cells grown on glucose was slightly higher than that of cells grown on galactose. On the other hand, the oxidative activity of yeast cells considerably reduced with increasing concentration of glucose. The decrease in the oxidative activity of cells grown with increasing concentration of galactose was within 50%.

Effect of different concentrations of glucose and galactose on the formation of malate dehydrogenase and NADH cytochrome c reductase was shown in Figure 3. With increasing concentration of glucose, activities fo malate dehydrogenase and NADH cytochrome c reductase were remarkably lowered. The activity of malate dehydrogenase was considerably repressed by galactose but the activity of NADH cytochrome c reductase was not lowered significantly.

Difference of Contents of Enzymes and Cytochromes between Aerobically and Anaerobically Grown Cells

Cells were cultured aerobically or anaerobically on glucose as seen in Figure 1 and 4, and the enzyme activities of the cell free extract were compared (Table I). Activities of malate dehydrogenase and NADH cytochrome c reductase of aerobic cells were about 67 fold and 80 fold higher than those of anaerobic cells, respectively, while NADH ferricyanide reducing activity of aerobic cells was only 1.7 fold higher than that of anaerobic cells. In aerobic

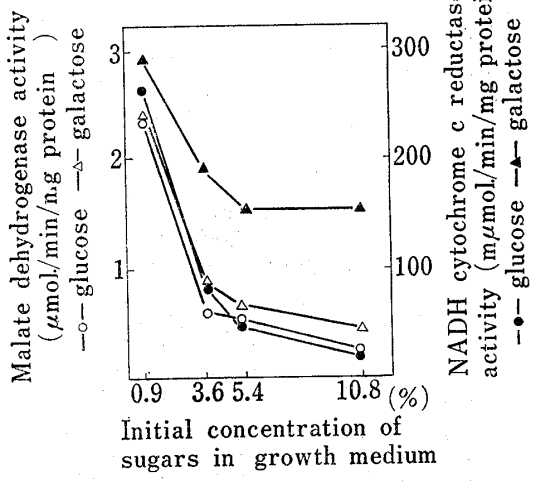

Fig. 3. Activities of Malate Dehydrogenase and NADH Cytochrome c Reductase of Yeast Cells Grown on Different Concentrations of Glucose or Galactose

Cells were cultured for 24 hr and the harvested cells were washed twice with distilled water. Preparation of cell free extract and measurement of malate dehydrogenase and NADH cytochrome c reductase activities were performed as described in materials and methods.

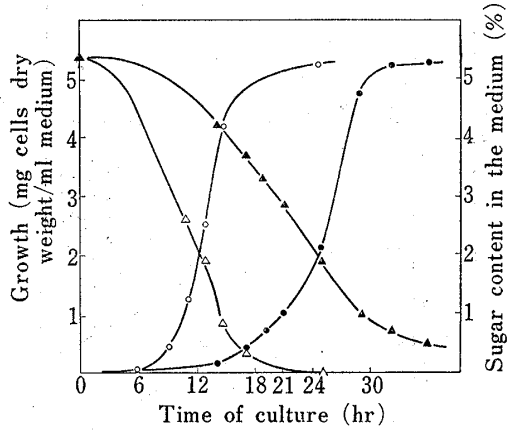

Fig. 4. Anaerobic Growth of Yeast Cells in the Presence of Glucose or Galactose

Experimental conditions were described in materials and methods. In the case of galactose, cells cultured on agar slant of 1% galactose were transferred to the preculture medium of galactose and incubated for 20 hr at 30°. The cells were inoculated to the experimental medium containing 5.4% galactose and cultured anaerobically at 30°.

growth
—○—: glucose
—●—: galactose
sugar concentration
—△—: glucose
—▲—: galactose

cells, typical absorption spectra of cytochrome a, b, and c were observed, while in cells grown anaerobically, absorptions of these cytochromes could not be detected but an absorption peak at 556 mμ was observed.

Effect of Glucose or Galactose on the Formation of Respiratory Enzymes and Cytochromes during the Adaptation of Anaerobically Grown Cells to Oxygen

To know the difference between glucose and galactose on the formation of respiratory systems,

TABLE I. Comparison of Enzyme Activities of Yeast Cells Grown on Glucose Aerobically and Anaerobically

	Aerobic cells	Anaerobic cells
	mμmoles/min/mg protein	
Malate dehydrogenase	2020	30
NADH cytochrome c reductase	320	4
NADH ferricyanide reducing activity	413	238

Cells were cultured aerobically on 0.9% glucose or anaerobically on 5.4% glucose for 24 hr, respectively. Preparation of cell free extract and assays of enzyme activities were described in materials and methods.

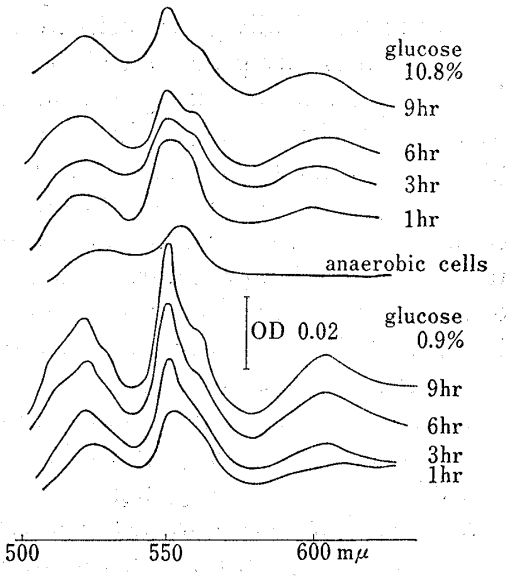

Fig. 5. Effect of Glucose on the Formation of Cytochromes Induced by Oxygen in Yeast Cells Grown on Glucose Anaerobically

Yeast cells grown anaerobically for 15 hr on glucose were harvested, washed and incubated aerobically at 30° as described in legend of Figure 4. Aliquots of cells were harvested at the indicated times and washed, and reduced minus oxidized difference spectra were obtained as described in materials and methods.

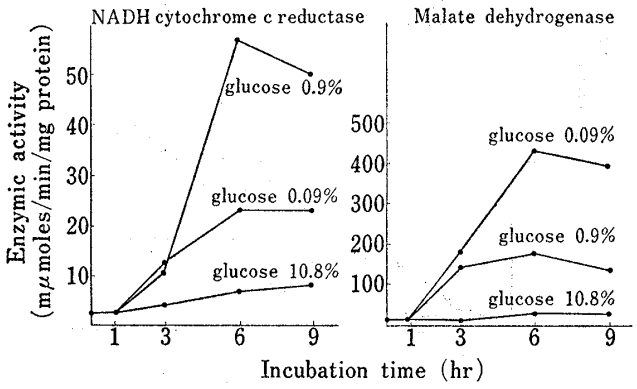

Fig. 6. Effect of Glucose on the Formation of NADH Cytochrome c Reductase and Malate Dehydrogenase Induced by Oxygen in Yeast Cells Grown on Glucose Anaerobically

Yeast cells grown on glucose anaerobically were washed and incubated aerobically with shaking as described in legend of Figure 4. Alliquots of cells were harvested at the indicated times and the activities of the enzymes in cell free extract were measured as described in materials and methods.

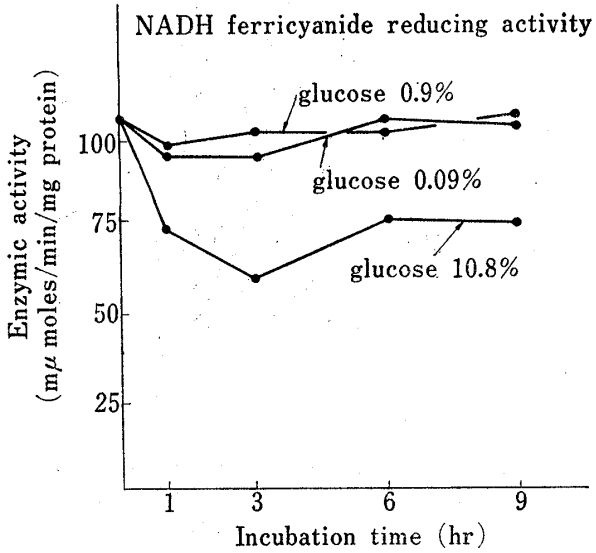

Fig. 7. Changes of NADH Ferricyanide Reductase Activity During the Adaptation to Oxygen of Anaerobically Grown Cells in the Presence of Glucose

Yeast cells grown on glucose anaerobically were washed and incubated aerobically with shaking as described in legend of Figure 4. Aliquots of cells were harvested at the indicated times and the activities of the enzyme in cell free extract were measured.

the formation of respiratory enzymes and cytochromes induced by the aerobic incubation of anaerobically grown cells were followed in the presence of glucose or galactose. Yeast cells anaerobically grown on glucose were harvested at 15 hr of growth, washed, suspended in the medium containing various concentrations of glucose or galactose and incubated aerobically with shaking. In the medium containing 10.8% glucose initially, the content of glucose was lowered to 3.2% during 9 hr of incubation and in the media of 0.9% and 0.09% glucose, glucose disappeared during 1 hr of incubation. Changes of reduced minus oxidized difference spectrum and the activities of malate dehydrogenase and NADH cytochrome c reductase were followed during the adaptation to oxygen. As seen in Figure 5, with aeration of anaerobic cells in the presence of 0.9% glucose, cytochrome a, b, and c were formed with time, while the formation of cytochromes were markedly depressed in the presence of a high concentration of glucose. Activity of NADH cytochrome c reductase was increased 22 times and 9 times during 6 hr of aeration in the presence of 0.9% and 0.09% glucose, respectively. In the presence of 10.8% glucose, the formation of the enzymes was repressed remarkably. Activity of malate dehydrogenase also increased during the adaptation to oxygen in the presence of low glucose while the activity did not increased in the presence of a high concentration of glucose (Fig. 6). On the other hand, NADH ferricyanide reductase activity did not change significantly by the adaptation of anaerobically grown cells to oxygen (Fig. 7).

The effects of galactose on the synthesis of cytochromes, NADH cytochrome c reductase and malate dehydrogenase were examined during the adaptation of cells grown on glucose anaerobically to the oxygen. Sugar consumption during aerobic incubation was much slower

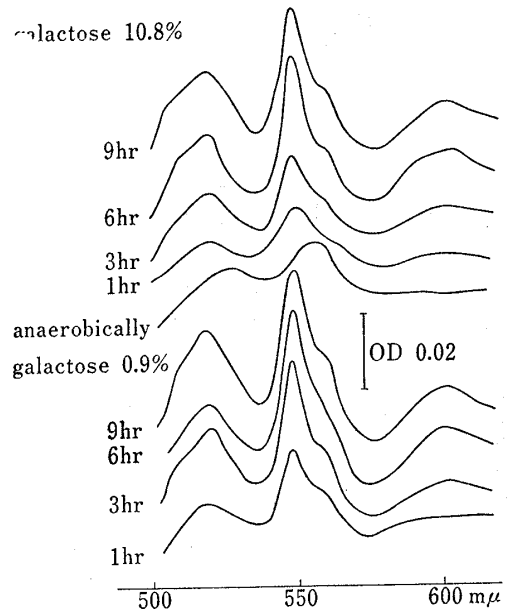

Fig. 8. Effect of Galactose on the Formation of Cytochromes Induced by Oxygen in Yeast Cells Grown on Glucose Anaerobically

Yeast cells grown on glucose anaerobically were harvested, washed and incubated aerobically at 30° as described in legend of Figure 8. Aliquots of cells were harvested at the indicated times and washed, and reduced minus oxidized difference spectra of cells were obtained as described in materials and methods.

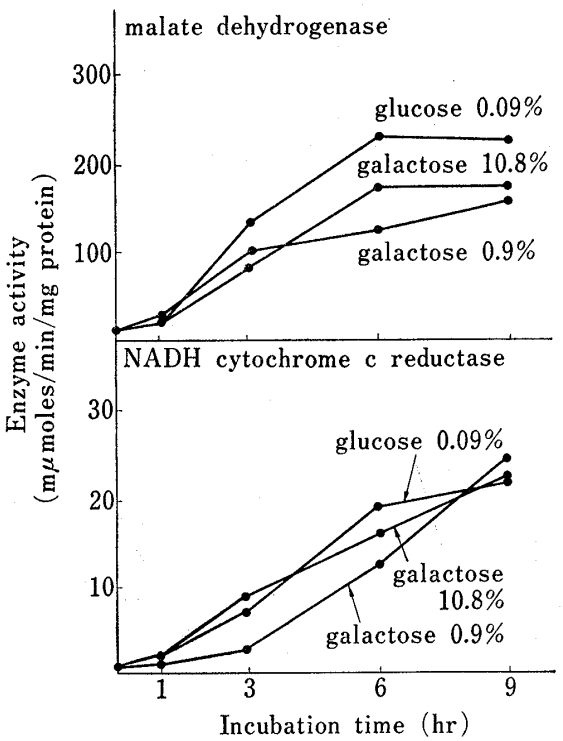

Fig. 9. Effect of Galactose on the Formation of Malate Dehydrogenase and NADH Cytochrome c Reductase Induced by Oxygen in Yeast Cells Grown on Glucose Anaerobically

Yeast cells grown on glucose anaerobically were washed and incubated aerobically with shaking in the presence of galactose as described in legend of Figure 8. Aliquots of cells were harvested at the indicated times and the activities of the enzymes in cell free extract were measured as described in materials and methods.

in the case of galactose than in the case of glucose. The medium initially supplemented with 0.9% galactose still contained 0.12% galactose after 9 hr of incubation. Figure 8 shows the effect of different concentrations of galactose on the synthesis of cytochromes during the adaptation of these cells to oxygen. Formation of cytochromes was induced by O_2 adaptation in the presence of galactose and inhibition of the formation by a high concentration of galactose was not observed. These results were consistent with those by Linnane.[5] The effects of galactose on the synthesis of malate dehydrogenase and NADH cytochrome c reductase during adaptation to oxygen are presented in Figure 9. Formation of malate dehydrogenase and NADH cytochrome c reductase were not inhibited in the presence of a high concentration of galactose.

Then, the effects of galactose on the synthesis of cytochromes and enzymes were examined with cells grown on galactose anaerobically. Cells cultured on agar slant containing galactose were transferred to a liquid medium of galactose and cultured for 20 hr at 30° on standing. The cells were transferred to the experimental medium of galactose and cultured anaerobically at 30° with gentle stirring as described above. Cells harvested at 25 hr were transferred to the medium of galactose and incubated aerobically. The cells thus obtained, consumed galactose more rapidly during the adaptation to oxygen than those grown on glucose. The galactose concentration in the medium containing initially 0.9% galactose was lowered to 0.14% after one hr of incubation.

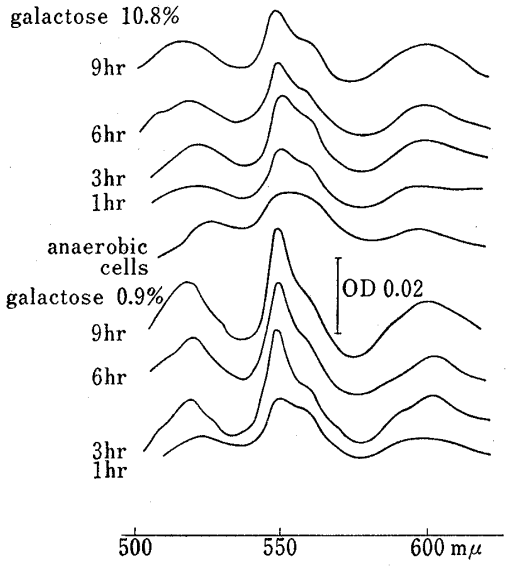

Fig. 10. Effect of Galactose on the Formation of Cytochromes Induced by Oxygen in Yeast Cells Grown on Galactose Anaerobically

Yeast cells grown on galactose anaerobically were harvested, washed and incubated aerobically at 30° as described in legend of Figure 12. Aliquots of cells were harvested at the indicated times and washed and reduced minus oxidized difference spectra were obtained as described in materials and methods.

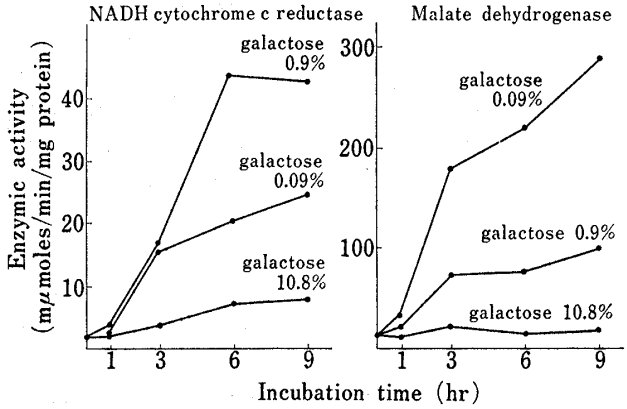

Fig. 11. Effect of Galactose on the Formation of Malate Dehydrogenase and Cytochrome c Reductase Induced by Oxygen in Yeast Cells Grown on Galactose Anaerobically

Yeast cells grown on galactose anaerobically were washed and incubated aerobically with shaking as described in legend of Figure 12. Aliquots of cells were harvested at the indicated times and the activities of the enzymes in cell free extract were measured as described in materials and methods.

Figure 10 showed the formation of cytochromes during the adaptation of the cells to oxygen in the presence of galactose. Synthesis of cytochromes were obviously inhibited by a high concentration of galactose in this case. As seen in Figure 11, the formation of malate dehydrogenase and NADH cytochrome c reductase was evidently inhibited by a high concentration of galactose in cells grown on galactose anaerobically.

Discussion

In this paper it was observed that the oxidative activity of intact cells decreased with increasing concentration of glucose in growth medium, while the activity did not decrease significantly when galactose was used as carbon source (Fig. 2). From the experiment of the adaptation of anaerobic cells to oxygen in the presence of sugars, it was found that the formation of cytochromes, malate dehydrogenase and NADH cytochrome c reductase in yeast cells was inhibited by a high concentration of glucose but not by galactose (Fig. 4, 5, 7, 8). Glucose was consumed rapidly during the adaptation while galactose was consumed slowly. Strittmatter reported that the oxidative activity of yeast cells markedly decreased when the initial concentration of glucose in the growth medium increased, while the high initial concentration of galactose caused a relatively slight decrease of oxidative activity.[2j] Linnane and Polakis also observed that the formation of cytochromes and respiratory enzymes and mitochondrial structure were repressed by glucose but not by galactose.[2k,14] These studies were in agreement with the results in this experiment.

When yeast cells grown anaerobically in the presence of galactose were used for adaptation to oxygen, galactose in the medium was consumed rapidly similarly to glucose, and the formation of cytochromes was inhibited by increasing concentration of galactose (Fig. 10). The similar relationship between repression and sugar consumption was observed in NADH cytochrome c reductase and malate dehydrogenase (Fig. 11). From these results, it was demonstrated that glucose repression is a phenomenon attributed to the catabolism of sugar and therefore catabolite repression was induced by galactose when galactose was catabolized as quickly as glucose. NADH ferricyanide reductase activity did not change significantly during the adaptation to oxygen. NADH ferricyanide reductase activity in anaerobic yeast cells was reported previously by Lindenmayer but the physiological function is not known clearly.[13]

It is interesting that the activity of malate dehydrogenase but not NADH cytochrome c reductase reduced significantly with increasing concentration of galactose (Fig. 2). Rijn and Wijk reported that the cytoplasmic malate dehydrogenase activity in yeast cells was inactivated and that the mitochondrial malate dehydrogenase activity was repressed by glucose in yeast cells.[15]

With respect to the mechanism of glucose repression of β-galactosidase in *E. coli*, Magasanik reported that glucose inhibited the induction of the enzyme but did not affect the production of enzyme.[16] Goldenbaum and Dobrogosz observed that catabolite repression by glucose of β-galactosidase synthesis in *E. coli*, was reversed to a great extent by the addition of cyclic adenosine monophosphoric acid (AMP).[17] Pastan and Perlman also postulated that the repression of enzyme synthesis by glucose might be due to the lowering of the concentration of cyclic AMP by this compound.[18] It has been previously shown that the respiratory activity of yeast cells grown on a high concentration of glucose increased considerably by incubation of the cells for 3 hr in the presence of glucose and the increase of enzyme activity due to the release from glucose repression was inhibited by high concentration of glucose, fructose or mannose, or inhibitors of protein synthesis such as D,L-ethionine and cycloheximide.[3] The precise mechanism of glucose repression in yeast respiratory systems remains for further studies to be done.

Acknowledgements The authors thank professor Makoto Ishimoto for his helpful advice.

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