RESPIRATORY DEFICIENCY IN SACCHAROMYCES CARLSBERGENSIS 4228 CAUSED BY THIAMINE AND ITS PREVENTION BY PYRIDOXINE

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SUMMARY The cells of Saccharomyces carlsbergensis 4228 (ATCC 9080), growing at a limited rate after a long lag period in the presence of thiamine and absence of pyridoxine, exhibited a markedly low respiration rate, despite the fact that the cultivation was carried out under aerobic conditions. The cytochrome oxidase activity of the cells was also negligible. The characteristic spectra of cytochrome pigments were not detected. Addition of pyridoxine to the medium prevented the growth inhibition and eliminated these effects of thiamine.

It is well known that S. carlsbergensis 4228 (ATCC 9080) requires pyridoxine for growth when thiamine is added to a synthetic medium. This phenomenon has been utilized for a microbiological assay of vitamin B_6 (1). Rabinowitz and Snell (2) demonstrated that pyridoxine was required to prevent the growth inhibition caused by thiamine. There are several reports (3-6) dealing with the inhibitory effect of thiamine and the preventing action of pyridoxine on the growth of the yeast. However, unequivocal explanation for the mechanism has not been presented as yet. Chiao and Peterson (3) reported that the above-mentioned effect of thiamine was observed only under aerobic conditions, and that the anaerobic growth of the yeast was rather stimulated by thiamine. This fact led us to investigate the respiratory activity of the yeast cells growing aerobically in the presence of thiamine.

EXPERIMENTAL

S. carlsbergensis 4228 was grown aerobically at 30°C in the medium of Atkin et al. (1) with a slight modification. Thiamine-HCl and pyridoxine-HCl were added to the medium at the final concentrations of 1 μg/ml and 0.02 μg/ml, respectively. The growth of the yeast was measured turbidimetrically at 610 nm and the values were converted to the dry cell weights (mg/ml-culture). The oxygen-uptake by the cells was determined manometrically by using a conventional Warburg apparatus. The absorption spectrum of cell suspension was taken on a Shimadzu recording spectrophotometer 50 L equipped with an opalescent glass plate according to the method of Chance (7). Cell-free extracts were prepared with a cell suspension in 0.05 M potassium phosphate

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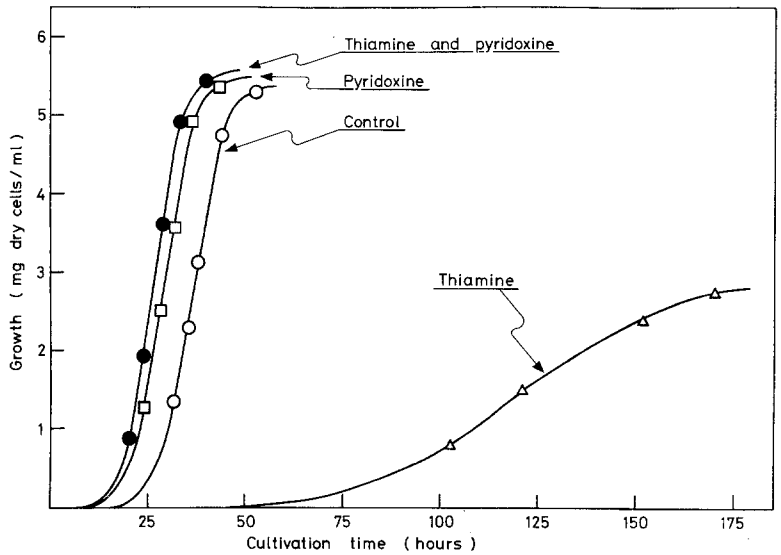

Figure 1. Effects of thiamine and pyridoxine on the growth of S. carlsbergensis.
The cultivation of the yeast and the growth measurement were carried out as described in the text.

buffer (pH 6.8) by using a sonic disintegrator at 20 KC for total 20 minutes at 5 minute-intervals, followed by centrifugation at 1,000 X g for 10 minutes. Cytochrome oxidase activity of the cell-free extracts was assayed by measuring a decrease in the absorbance of reduced cytochrome c at 550 nm. Enzyme unit was defined as μmole of the substrate utilized per minute. Specific activity is expressed as units per mg of protein. Protein determination was done by the method of Lowry et al. (8). The glucose concentration of culture was determined by an anthrone-H_2SO_4 method (9). Cytochrome c was obtained from Sigma Chemical Co., St. Louis, Mo., U. S. A..

RESULTS

Effects of thiamine and pyridoxine on the growth of S. carlsbergensis

Addition of thiamine to the growth medium caused a delay of the beginning of the growth, a decrease in the growth rate, and a lowering of the maximal level of growth under aerobic conditions (Figure 1). The growth inhibition by thiamine was prevented completely by addition of pyridoxine. The lag period of growth was reduced considerably as compared with that of a control experiment in which both of thiamine and pyridoxine were omitted. Addition of pyridoxine alone also caused a reduction of the lag time.

Effects of thiamine and pyridoxine on the respiratory activity of S. carlsbergensis

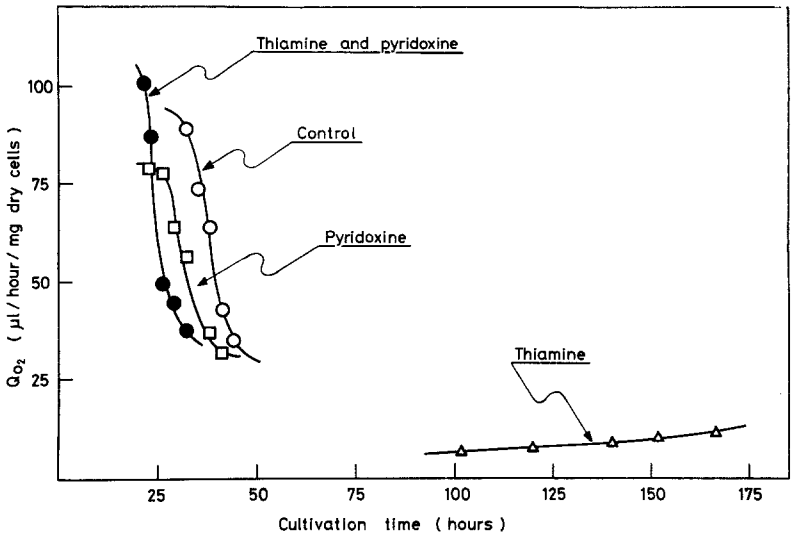

Figure 2. Effects of thiamine and pyridoxine on the respiration rate of intact cells of S. carlsbergensis.
The cells harvested at the indicated time of incubation were washed and suspended in 0.05 M citrate buffer (pH 5.3). The suspension containing 6 mg of cells and the same buffer (167 µmoles) were transferred to a Warburg flask. Glucose (17 µmoles) was added as substrate from a side arm. Oxygen-uptake was measured every 5 minutes for 40-60 minutes. The respiratory activity of the cells was calculated as Q_{O_2} (µl of oxygen uptaken per hour per mg cells).

Figure 2 shows the time-course changes in respiration rate of the cells, the growth curves of which are given in Figure 1. The oxygen-uptake by the cells grown with thiamine was markedly low at any phase of growth. Pyridoxine added to the growth medium, singly or concomitantly with thiamine, gave a normal respiratory activity to the yeast. The low respiration rate of the cells grown with thiamine may not be due to glucose effect, because the residual glucose concentrations of the thiamine-added culture were not higher, in any growth phase, than those of the normal or pyridoxine-added culture.

Effects of thiamine and pyridoxine on the cytochrome c oxidase activity of S. carlsbergensis

The above-mentioned effects of thiamine and pyridoxine on the respiratory activity were confirmed by experiments using cell-free extracts. As shown in Figure 3, the time-course changes in cytochrome c oxidase activity of the cells grown under the above-mentioned conditions were in good agreement with those of respiratory activity depicted in Figure 2.

Effects of thiamine and pyridoxine on the cytochrome pattern of S. carlsbergensis cells

The absorption spectrum of whole cells of S. carlsbergensis grown in the

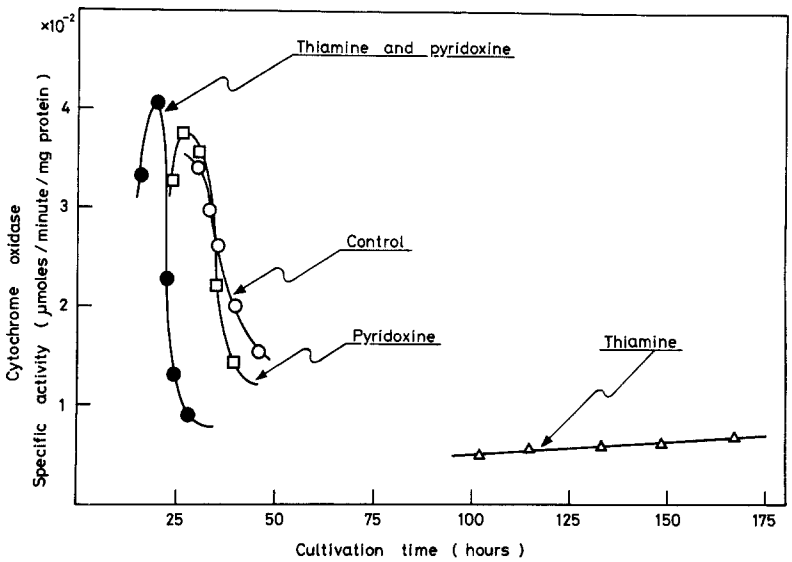

Figure 3. Effects of thiamine and pyridoxine in growth media on the cytochrome oxidase activity of cell-free extracts.
Preparation of cell-free extracts and assay of cytochrome oxidase were carried out as described in the text. The reaction mixture contained 0.15 μmole of cytochrome c reduced with an equimolar amount of ascorbic acid, 200 μmoles of potassium phosphate buffer (pH 6.8) and the enzyme in a final volume of 3 ml.

presence or absence of thiamine under aerobic conditions is presented in Figure 4. The cells grown with thiamine clearly shows a complete loss of the characteristic absorption peaks of cytochromes. This effect of thiamine was also prevented by the concomitant addition of pyridoxine to the medium.

DISCUSSION

It is well known that the respiratory activities of yeasts are very sensitive to environmental conditions. The main factors which influence the respiratory activities are the concentrations of oxygen and glucose in the medium. Cells growing on glucose of high concentrations exhibit markedly decreased Q_{O_2} values even when the cultivation is conducted under maximal aeration. However, there may be no report dealing with such respiratory depression caused by physiologically active substances other than glucose. The present communication clearly indicated that thiamine added exogenously to the growth medium resulted in a remarkable reduction of the respiratory activity of S. carlsbergensis 4228 under aerobic conditions. From the data obtained here, it would be supposed that an inhibition of biosynthesis of heme or cytochromes, or a stimulation of their degradation occurred in the presence of thiamine. The prevention of this effect of thiamine by simultaneous addition of pyridoxine suggests a close relationship of these phenomena to the well-known inhibitory

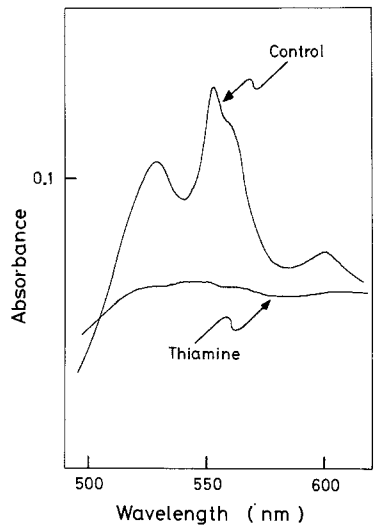

Figure 4. Reduced minus oxidized difference spectra of suspensions of the cells grown in the presence or absence of thiamine. The suspensions contained 50 mg of cells (dry weight) per ml, and were prepared as described in the text. The sample cuvette was reduced with few crystals of hydrosulfite, and the reference cuvette was oxidized with 0.03 % H_2O_2.

effect of thiamine on the growth of S. carlsbergensis 4228 and its reversal by pyridoxine. In the presence of thiamine added exogenously, the yeast may be forced to gain energy via glycolysis, due to the lack of respiratory activity even under aerobic conditions. Although the mechanism of this effect remains still unsolved, a clue to this phenomenon would be offered by the fact that the vitamin B_6 content of the cells grown with thiamine alone was significantly low, especially at the early growth phase as reported by Kawasaki and Yamada (10) and as observed by the present authors (unpublished data). This fact, together with the protecting effect of pyridoxine, would suggest that the decrease in the vitamin B_6 content might be a primary result derived from the single addition of thiamine. The loss of respiratory activity may be resulted from the low vitamin B_6 level in the cells grown with thiamine alone. The requirement of pyridoxal phosphate for the synthesis of heme was first observed by nutritional experiments (11, 12), and now established at the step of δ-amino levulinate synthesis (13-17). The vitamin B_6 deficiency may cause a lowering of the enzyme activity and consequently a reduction of heme synthesis. Accumulation of some specific porphyrin-relating substances as reported in a respiratory deficient mutant of S. cerevisiae (18) or appearance of abnormal hemoproteins was not observed in our experiments, as judged from the absorption spectra of cell suspensions. However, more extensive studies

would be necessary to establish a definite interrelationship between the vitamin B_6 deficiency and the disappearance of cytochrome pigments in the cells of S. carlsbergensis grown with thiamine alone. Furthermore, it should not be precluded that the vitamin B_6 deficiency possibly has a significant influence on the biosynthesis of the protein moieties of cytochromes.

In subsequent papers, we will report a reduction in the levels of unsaturated fatty acids and the absence of ergosterol in the yeast cells grown in the presence of thiamine and absence of pyridoxine. These findings would be correlated with the results of the present communication, since these lipids substances are known to be formed in endoplasmic reticulum by the reactions depending upon cytochromes (19, 20).

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