REGULATION OF PYRUVATE DECARBOXYLASE (E.C.4.1.1.1) SYNTHESIS BY COENZYME INDUCTION IN SACCHAROMYCES CEREVISIAE.

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Aerobic glycolysis increases rapidly after the addition of ammonia to yeast cells which are oxidizing glucose. This increase in glycolytic rate is caused by ammonia dependent ATP-consuming synthetic reactions and the subsequent increase of the levels of orthophosphate and ADP, which are rate-limiting for glycolysis (Holzer,1953; Holzer and Witt, 1958; Witt and Holzer,1964). This rapid regulation of glycolysis depends on the activation or inhibition of glycolytic enzymes by metabolites or coenzymes.

In this paper we present results for a slower glycolytic control mechanism involving the regulation of the synthesis of a rate-limiting enzyme.

Incubation of starved yeast cells with glucose and a nitrogen source led to a 2 - 4 fold increase of the pyruvate decarboxylase (PD) activity and the thiamine content within 3 hours. The activity of PD could also be increased by addition of thiamine to the medium. Therefore we assume that the PD activity may be regulated by the thiamine content of the cells. This assumption was supported by the observation that in thiamine-deficient mutants of Saccharomyces cerevisiae the rate of PD synthesis depended on the thiamine content of the growth medium.

MATERIALS AND METHODS

Chemicals: Thiamine·HCl and thiamine pyrophosphate were purchased from E.Merck AG, α-hydroxythiamine·HCl and pyrithiamine·HBr from the Sigma Chemical Company, DL-p-fluorophenylalanine and 5-fluorouracil from Calbiochem and proflavine from Nutritional Biochemicals Corp.

Organisms: The yeasts used were commercially available fresh

bakers' yeast prepared by the Hefefabrik Sinner AG (Karlsruhe) or the Hefefabrik Weingarten GmbH (Württemberg).

Thiamine-deficient mutants of Saccharomyces cerevisiae were obtained from Prof. F.Lingens (Tübingen) and were grown on minimal medium as described by Lingens and Oltmanns (1964).

Incubation of yeast cells: The yeast cells were washed and starved as described earlier (Witt and Holzer,1964). 1 g of yeast cells (wet weight) was incubated at 30° C in 250 ml medium which consisted of (per 1 liter): 1.0 g KH_2PO_4, 0.5 g NaCl, 0.6 g $MgCl_2 \cdot 6\ H_2O$, 0.6 g $CaCl_2 \cdot 2\ H_2O$, 12.0 mg $Na_2SO_4 \cdot 10\ H_2O$, 10 mg $FeCl_3$, 2 mg ß-alanine, 1 mg L-leucine, 5 mg m-inositol, 40 µg pyridoxin hydrochloride , 6 µg calcium pantothenate, 0.2 µg biotin and 0.05 M citrate buffer pH 6.0.

Preparation of yeast extract: The harvested and washed cells were ground with three times their weight of alumina powder, Alcoa A 305, extracted with three volumes of 0.1 M citrate buffer pH 6.0 and centrifuged for 30 min at 34,000 g.

Measurement of enzyme activities: Glucose-6-phosphate dehydrogenase (E.C.1.1.1.49) was estimated according to Kornberg and Horecker (1955). The pyruvate decarboxylase (E.C.4.1.1.1) assay was a modification of the method of Holzer et al.(1956).The reaction mixture consisted of: 0.09 M citrate buffer pH 6.0, $2.1 \cdot 10^{-3}$M thiamine pyrophosphate, $2.1 \cdot 10^{-2}$M $MgSO_4$, $0.2 \cdot 10^{-3}$M NADH, $5.4 \cdot 10^{-3}$M sodium pyruvate and 0.6 mg alcohol dehydrogenase.

Determination of thiamine: Thiamine was estimated as described by Strohecker and Henning (1963), except that thiamine phosphates were hydrolysed with acid phosphatase and thiamine was isolated on an amberlite CG 50 (H^+) column.

RESULTS AND DISCUSSION

After the incubation of starved yeast cells with glucose and a nitrogen source for 3 hours the specific activity of PD was four times the value without nitrogen (Table I). NH_4Cl or $(NH_4)_2SO_4$ were somewhat more effective in this respect than the amino acids studied (aspartate, methionine, lysine, glutamate). The activities of other glycolytic enzymes, for example glucose-6-phosphate dehydrogenase (Table I), were nearly unchanged. The activity of this enzyme was used as a control in the following experiments.

In addition to the increase of the PD activity an increase of the thiamine content of the cells was found (Table I).

A similar interrelationship between PD activity and thiamine content was observed by Suomalainen and Oura (1959). They reported that the PD activity and thiamine content both decrease as yeast cells pass from anaerobic to strictly aerobic conditions.

Table I

Enzyme activites and thiamine content in yeast cells after incubation for 3 hours with different nitrogen sources.

Nitrogen source	Enzyme activities (μmoles/min/mg protein)		μg thiamine per g yeast (dry weight) [water content]
	Pyruvate decarboxylase	Glucose-6-phosphate dehydrogenase	
none	0.24	0.10	18.3 [75%]
0.01 M NH_4Cl	1.05	0.17	28.2 [81%]
0.01 M aspartate	0.89	0.13	28.6 [82%]
0.01 M methionine	0.86	0.13	37.4 [86%]

To test whether there was a correlation between the increase of the PD activity and the increase of the thiamine content, we studied the influence of thiamine on the PD activity in yeast cells. It was found, that the PD activity increased by 50% during the incubation of yeast cells with glucose and thiamine and by 300% when the cells were incubated with glucose, thiamine and a nitrogen source (Table II).

These results suggest that the increase of the PD activity during the incubation of the yeast cells with the various nitrogen sources may be caused by the increase of the thiamine content.

Incubation of the yeast cells with oxythiamine also caused a considerable increase in the PD activity. Incubation with pyrithiamine had no influence on the enzyme activity. Addition of thiamine, oxythiamine or pyrithiamine to the assay system for PD activity showed that there was no effect of thiamine or its derivatives on the PD reaction in vitro.

The increase of the PD activity caused by NH_4Cl, thiamine or oxythiamine was inhibited by the addition of proflavine to the incubation medium. Besides inhibiting DNA synthesis proflavine strongly

Table II

Enzyme activities in yeast cells after incubation for 3 hours with NH_4Cl, thiamine or oxythiamine.

Addition to media	Enzyme activities (μmoles/min/mg protein)	
	Pyruvate decarboxylase	Glucose-6-phosphate dehydrogenase
None	0.46	0.15
0.01 M NH_4Cl	0.90	0.18
$2.3 \cdot 10^{-3}$ M thiamine	0.68	0.18
$2.7 \cdot 10^{-3}$ M oxythiamine	1.30	0.17
0.01 M NH_4Cl + $2.3 \cdot 10^{-3}$ M thiamine	1.30	0.22
0.01 M NH_4Cl + $2.7 \cdot 10^{-3}$ M oxythiamine	1.70	0.19

Table III

Influence of inhibitors on the increase of the pyruvate decarboxylase activity during incubation for 3 hours with NH_4Cl.

Addition to media	Pyruvate decarboxylase activity (% of the value with NH_4Cl)
0.01 M NH_4Cl	100
None	35
0.01 M NH_4Cl + $1.2 \cdot 10^{-3}$ M proflavine	45
0.01 M NH_4Cl + $3.3 \cdot 10^{-3}$ M DL-p-fluorc-phenylalanine	57
0.01 M NH_4Cl + $3.1 \cdot 10^{-3}$ M 5-fluorouracil	40

inhibits glycolysis of yeast cells (Witt and Müller, 1966). Therefore the effect of proflavine on the increase of the enzyme activity can be caused by an inhibition of the DNA-directed RNA synthesis as well as by decreased energy production. It was found that the rise of the PD

activity could also be prevented by the addition of p-fluorophenylalanine or 5-fluorouracil to the incubation medium (Table III).

These results indicate that the reason for the higher activity of PD after incubation with a nitrogen source or thiamine was a stimulation of the de novo synthesis of the enzyme and not an activation of preexisting enzyme molecules. The stimulation of the enzyme synthesis may be caused by induction or derepression.

The dependence of the PD synthesis on thiamine could also be demonstrated for thiamine-deficient mutants of S.cerev. . After the cultivation of these mutants with increasing amounts of thiamine there was a corresponding rise of the PD activity (Table IV). The activity of the glucose-6-phosphate dehydrogenase was unchanged under these different culture conditions.

Table IV

Enzyme activites in a thiamine-deficient mutant of S.cerev. after growth in media with different thiamine concentrations.

Thiamine in the media (g/ml)	Enzyme activities after 40 hours of growth (μmoles/min/mg protein)	
	Pyruvate decarboxylase	Glucose-6-phosphate dehydrogenase
$0.4 \cdot 10^{-9}$	0.09	0.38
$0.8 \cdot 10^{-9}$	0.14	0.37
$2.0 \cdot 10^{-9}$	0.36	0.35
$4.0 \cdot 10^{-9}$	1.40	0.42

The observed rise of the specific activity of PD during the incubation of yeast cells with a nitrogen source and the corresponding increase of the thiamine content as well as the elevation of the enzyme level by incubation with thiamine or oxythiamine supports the following assumption: Thiamine besides its function as a coenzyme of the PD reaction regulates the synthesis of the protein moiety of the PD by induction or derepression. This regulation by "coenzyme induction" is a very valuable mechanism, because the cell is not able to use an apoenzyme without the cofactor.

There exist other examples which support the hypothesis that cofactors of an enzyme in addition to regulating the activity of the existing enzyme, may influence in vivo the amount of the protein

moiety of the same enzyme. Greengard and Gordon (1963) showed a pyridoxin-induced rise of rat liver tyrosine transaminase level *in vivo*. Hammel and Bessman (1966) demonstrated the dependence of the synthesis of hemoglobin from the synthesis of heme.

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