A STUDY OF THE ENZYME EQUIPMENT OF THE YEAST RHODOTORULA GRACILIS

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Received 13 May 1969

1. Introduction

The red, lipid forming yeast *Rhodotorula gracilis* lacks the ability to metabolize sugars under anaerobic conditions [1]. Experiments with specifically labelled ^{14}C-D-glucose have shown that the pentose phosphate cycle is the main pathway for glucose catabolism [2]. Brady and Chambliss [3] reported their failure to detect any phosphofructokinase activity in various species of *Rhodotorula,* including the one used in this communication. Our further analyses have supported fully the findings of the mentioned authors, in that we also could not find PKF in our strain of *Rhodotorula.*

In order to obtain a better picture of the enzyme equipment of *R. gracilis* cells, we have estimated the activities of other enzymes involved in glycolysis and in the pentose phosphate cycle.

2. Materials and methods

The collection strain used, *Rhodotorula glutinis* 5/Fres/Harrison, was cultivated as described earlier [4].

2.1. *Enzymes of the glycolytic sequence*

The activities of glycolytic enzyme were determined by conventionally used reactions (see e.g. Bergmeyer [5]), coupled with oxidation or reduction of nicotinamid adeninedinucleotides, in different fractions of a cell-free French press extract. The redox state of the nicotinamid adenine dinucleotides was measured in an Eppendorf fluorometer. The cell-free French press extract was made of a 90% (w/v) suspension of the yeast cells in 50 mM triethanolamine buffer, pH 7.0, containing 20 mM KCl and 5 mM $MgCl_2$ and subjected twice to 80–90 atm pressure. The extract was spun down 20 min at 27000 *g*; the supernatant again for 60 min at 100,000 *g*. The supernatant after the second centrifugation is referred to as the "crude extract". Acetone powders were prepared from the two sediments. The "crude extract" was further fractionated by successive saturation with ammonium sulphate. Distilled water extracts of the obtained sediments and the final supernatant were used for the assays. The assays were carried out at 25° in 50 mM triethanolamine buffer, pH 7.8, containing 20 mM KCl and 5 mM $MgCl_2$.

2.2. *Enzymes of the pentose phosphate cycle*

A 50% suspension of the yeast cells was subjected to 30 min sonification at 7 A (alternatively 30 sec sonification and 60 sec standby intervals, in order to maintain the suspension at 2°C). The extract was then centrifuged 30 min at 60,000 *g*. The supernatant was used for the assays. G6PDH and 6PGDH were determined according to Bücher et al. [6], TK according to Horecker [7], TA according to Tschola and Horecker

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[8], and the system TK + Epi + Iso according to Heber et al. [9].

2.3. *Fructosediphosphate phosphatase and Glycoxylate reductase*

For the above two enzymes, both the fractioned French press extracts and the toluene autolysate [10] were analysed. The enzyme preparations were dialyzed overnight at 0° against distilled water. The dialyzed preparations were analyzed for FDPase and glycoxylate reductase activities according to Racker and Schroeder [11] and Klotzsch and Bergmeyer [12], respectively. The FDPase activity was tested over a pH range of 6.0–9.0.

The pure enzymes and substrates used for the assays were products of Boehringer GmbH, Mannheim, Germany. All the other reagents were commercial products of analytical purity.

3. Results and discussion

Table 1 summarizes the results of assays of glycolytic enzymes. It can be seen that, except for the lack of PFK, all the enzymes of the sequence of glycolytic reactions are present, including the final dehydrogenase, viz. LDH and ADH. These two enzymes displayed, indeed, the least activity. However, both lactate and ethanol stimulate cell respiration with RQ values corresponding to theoretical expectation [2]. With ethanol, the yeast suspension in the main compartment of a Warburg flask even responds to an ethanol tension with a 10% ethanol solution in the side arm. To find out whether the gap in glycolytic reactions between F6P and FDP cannot be overlapped at least in one direction to allow for gluconeogenesis, the fructosediphosphate phosphatase (FDPase) activity was tested. Neither in French press extract nor in toluene autolysate was FDPase detectable; see table 2.

The high aldolase activity may be, as the highly active phosphoglucose isomerase too, involved in the reactions of the pentose phosphate cycle. This question will be considered elsewhere [13,14].

Table 2 shows the activity of glyoxylate reductase. This enzyme is important for enabling the C_2-units formed by the splitting of xylulose-5-phosphate, to enter the tricarboxylic acid cycle via the glyoxylate shunt, as suggested by Betz and Höfer [15].

Table 3 summarizes the obtained activities of the pentose phosphate cycle enzymes. A comparison of the values given in tables 1 and 3 confirms the postulated predominance of the pentose phosphate cycle for sugar catabolism in *R. gracilis*.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft and the Stiftung Volkswagenwerk.

Table 1
Activities of glycolytic enzymes in *Rhodotorula gracilis*.

	PGI	PFK	ALD	TPI	GAPDH	G1PDH	PGK	PGM	ENO	PK	LDH	ADH
"Crude extract"	3400	0	–	–	–	–	–	–	62	–	–	–
40% $(NH_4)_2SO_4$ Sediment	–	0	1500	–	0	0	–	–	14.6	400	2.5	–
50% $(NH_4)_2SO_4$ Sediment	–	0	52	–	0	–	–	–	0	700	0	–
50% $(NH_4)_2SO_4$ Supernat.	–	0	44	–	3*	44	3	–	–	500	0.6	–
Aceton Powder 27,000 *g*	–	0	1500	46	12.9*	0	61.5	2.1	2.1†	–	–	3.5
Aceton Powder 100,000 *g*	–	0	1500	374	5.7	0	10	47	47†	100	0.1	2.5

Values of activity are expressed in mμmoles NADH oxidized at 25° and pH 7.8 per min per mg protein of the individual fraction. –: Activity not tested. 0: No detectable activity. *: Reaction sensitive to 0.5 mM IAA. †: Reaction sensitive to 20 mM NaF. Abbreviations used: PGI: phosphoglucose isomerase; PFK: phosphofructokinase; ALD: aldolase; TPI: triosephosphate isomerase; GAPDH: glyceraldehyd-phosphate dehydrogenase; G1PDH: glycerolphosphate dehydrogenase; PGK: phosphoglycerate kinase; PGM: phosphoglyceromutase; ENO: enolase; PK: pyruvate kinase; LDH: lactate dehydrogenase; ADH: alcohol dehydrogenase.

Table 2
Fructosediphosphate phosphatase (FDPase) and glyoxylate reductase activites in *Rhodotorula gracilis.*

	FDPase	Glyoxylate reductase
Toluene autolysate	0	2.3
French press extract 40% $(NH_4)_2SO_4$ sediment	0	0
French press extract 90% $(NH_4)_2SO_4$ sediment	0	13

Values of activities are expressed in mμmoles/min/mg protein.

Table 3
Activities of pentose phosphate cycle enzymes in *Rhodotorula gracilis.*

	mμmoles NADP/min/mg protein
Glucose-6-phosphate dehydrogenase	10,000
6-phosphogluconate dehydrogenase	2,280
Transketolase	2,300
Transaldolase	6,750
TK + Epi + Iso*	1,650

* Transketolase plus ribulose phosphate epimerase plus ribose phosphate isomerase.

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