ERRATUM

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THE LACK OF PHOSPHOFRUCTOKINASE ACTIVITY IN SEVERAL SPECIES OF RHODOTORULA

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Certain disparities observed in the growth response of Rhodotorula glutinis to various carbohydrate substrates have led to a study of its pathways of carbohydrate metabolism. The substance of this report is concerned with our inability to detect phosphofructokinase activity in several members of this species, and what appears to be diminished PFK levels in other oxidative yeasts.

METHODS

Log phase populations were harvested by centrifugation from cultures incubated at 28 C on a rotary shaker in a neopeptone (0.5%) - glucose (1.0%) medium. The collected cells were washed once in 0.03 M phosphate buffer with added 0.05 M β-mercaptoethanol and transferred to a chilled 50 ml vessel containing 20 g glass beads (0.45-0.50 mm dia.). The cells were disrupted in a Bronwill homogenizer model MSK operating at 4,000 cycles/min for 15 sec. Liquid CO_2 was used to maintain temperatures between 5-10 C during this treatment. After disruption, 10 ml chilled PO_4-βME buffer were added to the vessel and the homogenate decanted. This preparation was then centrifuged at 15,000 x G for 15 min at 4 C and the resulting cell free suspension assayed for enzyme activity.

Phosphofructokinase (E.C. 2.7.1.11) activity was assayed by the procedure of Sols and Salas (1966) using a portion of the crude extract that had

been treated with 20 mM NaF in order to permit the use of ATP in the assay procedure. In those crude extracts demonstrating PFK activity, this method of relieving ATP inhibition was as effective as the use of NH^+ ions or GTP. In those extracts demonstrating no PFK activity, the use of NH^+ ions or GTP in the assay did not result in enzyme activation. Glucose 6-phosphate dehydrogenase (E.C. 1.1.1.49) was assayed by the procedure of Kuby and Noltmann (1966). The unit of activity and the specific activity of each enzyme have been calculated as described in these references.

The oxidation of NADH and the reduction of NADP were determined at room temperature by measuring the change in absorbance at 340 mμ in cuvettes with a 1 cm light path using an Hitachi Perkin-Elmer Model 139 spectrophotometer. The protein concentration of each extract was estimated using the methods of Lowry et al. (1951) and Warburg and Christian (1957).

All of the organic reagents used in these assays were purchased from the Sigma Chemical Company except fructose 6-phosphate (Grade B). This was purchased from Calbiochem and converted from the barium salt by adding stoichiometric amounts of sodium sulfate. All other chemicals used were of reagent grade.

The members of the genera Rhodotorula and Saccharomyces used in this study also were grown in defined media consisting of yeast nitrogen base w/o amino acids (Difco 0119-15) supplemented with D-glucose (1%), D-xylose (1%), or DL-aspartate (0.7%), and the cell free extracts assayed as described above. The numbered cultures used in this investigation were originally obtained through the generosity of Dr. W. B. Cooke, Taft Sanitary Engineering Center, Cincinnati, Ohio.

RESULTS AND DISCUSSION

Because initial attempts to detect a PFK in R. glutinis MU-R were unsuccessful, extracts of S. cerevisiae MU-S were prepared to serve as controls of the reactants and the assay procedure. The concurrent measurement

of dehydrogenase activity was established as a control of the technique used to prepare the extracts. The various mechanisms of inhibition for PFK and the relief of same, recently reviewed by Wood (1966), could be easily reproduced in the extracts of S. cerevisiae. Repeated attempts to relieve an inhibition in R. glutinis, by the same methods, failed. Other species of Rhodotorula were examined in the event that this isolate happened to be a mutant. The data of Table 1 represent averaged activities from the assays performed as described. Activities of extracts from cells grown in defined media with the various carbohydrates were not significantly different and will not be separately reported.

A single report of a PFK deficiency in Rhodotorula affords something less than complete assurance that an unusual glycolytic pathway is operative. However, the absence of previously considered essential enzymes of intermediary carbohydrate metabolism, i.e., aldolase (DeMoss et al., 1951) and phosphohexokinase (Fraenkel and Horecker, 1964) in specific microbial types, and, especially the reported PFK deficiency in skeletal muscle (Tarui et al., 1965), encourage some speculation.

We interpret the data to indicate a specific uniqueness in glycolysis of Rhodotorula. There is no evidence to date that the PFK of this genus is more easily denatured during extract preparation than the PFK of other yeasts, that it is regulated or inhibited by substances that do not similarly react with the PFK of other species, or that the synthesis of this enzyme is influenced by the temperature of culture incubation.

An interesting possibility stems from a prediction made by Hommes (1966) as a result of his comprehensive study of changing ratios in the glycolytic enzymes of Candida parapsillosis. He postulated several operons controlling the synthesis of these enzymes, and found that PFK was distinguished by being controlled by an apparently separate and independent operon leading to the likelihood of a variety of mutations at this site. If such a mutation is responsible for the PFK deficiency observed in Rhodotorula, it may partly

Table 1

Specific Activities of Phosphofructokinase and Glucose 6-Phosphate Dehydrogenase in Rhodotorula Species and Saccharomyces cerevisiae

Organism[1]	Specific Activities	
	PFK[2]	G-6-P-DH[3,4]
R. glutinis MU-R	< 0.001	0.466
R. glutinis L-1041	< 0.001	0.464
R. minuta 1511	< 0.001	0.450
R mucilaginosa L-238	< 0.001	0.450
R. pilimaniae 64-13	< 0.001	0.482
S. cerevisiae MU-S	0.178	0.260

1 Cell-free extracts prepared and specific activities calculated as described in Methods.

2 Assay reactants (millimolar): F-6, 1.0; ATP, 0.13; phosphate buffer, pH 6.5, 30.0; β-mercaptoethanol, 5.0; $MgCl_2$, 5.0; NADH, 0.17; aldolase, 0.325 unit/ml; glycerophosphate dehydrogenase-triose-phosphate isomerase (mixed crystals, 10 mg/ml), 0.033 mg/ml; 0.2 ml extract; 3.0 ml total vol.

3 Assay reactants: 2.5 ml of 0.1 M glycylglycine buffer, pH 8.4; 0.2 ml of 0.15 M $MgSO_4$; 0.1 ml of 0.03 M G-6-P; 0.1 ml of 0.01 M NADP; 0.1 ml extract.

4 These data most probably include the activity of 6-phosphogluconic dehydrogenase because of the use of cell free extracts.

explain why this genus is completely dependent upon pathways of terminal oxidation for survival while possessing glycolytic and gluconeogenic pathways nearly as versatile as other yeasts (Ruiz-Amil et al., 1965). In such a mutant the phosphorylated triose could possibly be supplied only by the monophosphate shunt (a) rather than by the conventional glycolytic pathway (b).

(a) 3 glucose + 3 ATP → 2 glucose-6-P + 3 CO_2 + 1 PGA

(b) 1 glucose + 2 ATP → 2 PGA

If (a) is the only source of the 3-phosphoglyceraldehyde (PGA) which, in turn, is subjected to the subsequent ATP-generating intramolecular oxidations

leading to the formation of pyruvic acid, the resulting ATP deficit could be relieved only by terminal oxidation. There does not appear to be any evidence to ascribe an ATP-generating role to the considerable quantities of reduced NADP produced in the first reaction (Horecker, 1962). The highly reduced lipoidal materials characteristic of this genus may serve as a necessary adjunct to sustain this type of metabolism.

The data of Table 2 clearly indicate that a PFK deficiency is not characteristic of the other oxidative types tested. Less clear, because of limited sampling, is the indication of a shift from greater to lesser PFK activity between the fermentative and oxidative types. We have used the G-6-P-DH/PFK ratio to give some approximation of the interplay between the mono- and diphosphate shunts in cultures grown under identical conditions. H. anomala, an apt description, while possessing higher PFK activity than the

Table 2

Specific Activities of Phosphofructokinase and Glucose 6-Phosphate Dehydrogenase in Several Fermentative and Oxidative Yeasts[1]

Organism	Specific Activities		Ratio
	PFK	G-6-P-DH	G-6-P-DH/PFK
(fermentative)			
Saccharomyces cerevisiae MU-S	0.178	.260	1.46
Torulopsis glabrata 64-461	0.060	.371	6.18
Torulopsis stellata 64-286	0.064	.271	4.23
Hansenula anomala L-301	0.074	.892	12.05
(oxidative)			
Torulopsis famata L-475	0.033	.375	11.36
Torulopsis candida 63-895	0.045	.538	11.96
Endomycopsis javenensis L-287	0.042	1.136	27.05
Rhodotorula glutinis MU-R	< 0.001	0.466	466.00

1 Cell free extracts, calculations and assay reactants as described in Table 1.

oxidative types, possesses considerably more dehydrogenase activity than the other fermentative types tested.

We are continuing to modify the cultural conditions and techniques of extract preparation in order to detect PFK activity in Rhodotorula, and are collecting a significant number of PFK activities from the other yeasts to permit a rigorous comparative analysis.

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