SOME DIFFERENCES IN PYRUVATE KINASE REGULATION IN SELECTED YEASTS

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Summary. The pyruvate kinase of Rhodotorula glutinis is insensitive to activation by fructose 1-6 diphosphate. The same enzyme of two other oxidative yeasts, Endomycopsis javenensis and Torulopsis famata is similarly unresponsive. Three fermentative species examined displayed significant FDP-activation of PK, and the extent of activation was influenced by cultural substrate. The PK of R. glutinis is moderately sensitive to ATP inhibition and insensitive to possible feed-back control by citrate and several fatty acids.

The regulation of glycolysis in Saccharomyces is keyed to the formation of fructose 1-6 diphosphate (FDP). The activity of the enzyme catalyzing its formation, phosphofructokinase (PFK), is modulated by both energy charge and citrate levels (1, 2). Further, FDP serves as a positive modifier of the allosteric pyruvate kinase (PK) in Saccharomyces, and can also relieve the inhibition of this PK established by ATP (3). The members of the genus Rhodotorula evidence no PFK activity (4). However, the availability of FDP is postulated because of the presence of aldolase and triose phosphate isomerase (5). This report is of the general insensitivity of the PK of R. glutinis, and other oxidative yeasts showing diminished PFK activities, to a control mechanism characteristic of those yeast species displaying a fermentative mode of glucose dissimilation.

MATERIALS AND METHODS

Log phase populations were harvested by cold centrifugation from

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cultures incubated at 28 C on a rotary shaker in either neopeptone (0.5%) - glucose (1.0%) medium or defined yeast nitrogen base w/o amino acids (Difco 0119-15) - glucose (1.0%) medium. The techniques used for the preparation of cell extracts, omitting B-mercaptoethanol from the buffer in order to inactivate aldolase, were as previously described (4). Pyruvate kinase (ATP: pyruvate phosphotransferase, EC 2.7.140) activity was determined using the compound optical assay of Bücher and Pfleiderer (6). Specific activities are recorded as micromoles of substrate per minute per milligram of extract protein. The cultures used are from the departmental collection.

RESULTS

The data of Fig. 1 show the apparent inability of FDP to serve as a positive modifier of the PK of R. glutinis, and the degree of sensitivity of this enzyme to inhibition by ATP. Further, it was not possible to relieve

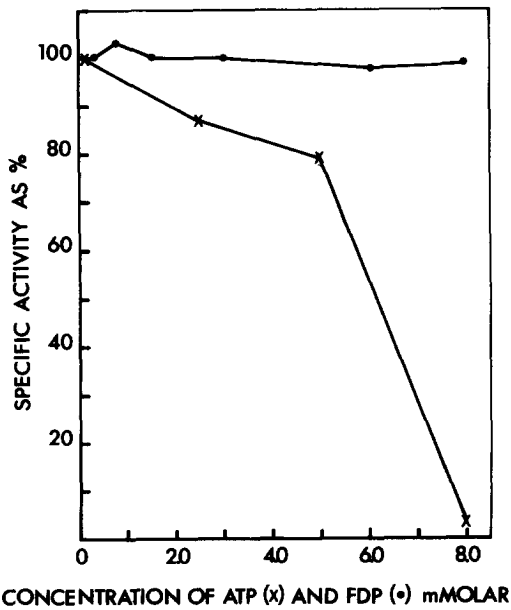

Fig. 1. The effect of ATP and fructose 1-6 diphosphate on the pyruvate kinase activity of Rhodotorula glutinis. Extracts were prepared from cultures grown on defined medium as described in Methods. Specific activity calculated as μmoles/min/mg extract protein. Assay reactants as reported in Table 1.

Table 1. The influence of fructose 1-6 diphosphate on the pyruvate kinase activity of several yeasts grown in defined and complex media[1]

Yeast	Specific Activities[2] Defined		Complex	
	FDP-none	FDP-added	FDP-none	FDP-added
(Oxidative)				
Rhodotorula glutinis L-1041	.184	.184	.230	.226
Endomycopsis javenensis L-287	.283	.307	.402	.424
Torulopsis famata L-475	.591	.669	.957	1.196
(Fermentative)				
Saccharomyces cerevisiae 64-456	.034	.322	.358	.596
Candida pulcherrima L-1292	.081	.342	.233	.484
Torulopsis stellata 64-286	.004	.470	.288	.513

1. Preparation of extracts, media used, and calculation of specific activities as described in Methods.

2. Assay Reactants (millimolar): KCL, 75.0; $MgSO_4$, 8.0; NADH, 0.15; phosphoenolpyruvate, 0.78; ADP, 0.23; lactic acid dehydrogenase, 37 mg/L; FDP, 0.33; Tris buffer, 50; extract, .05-.20 ml; final volume, 3.0 ml.

ATP inhibition by the addition of FDP to the reaction. This enzyme proved to be similarly unresponsive to other phosphorylated hexoses that participate in the metabolism of this species; fructose 6-phosphate, glucose 6-phosphate, and 6-phosphogluconate.

Data suggesting that the phenomenon of FDP stimulation of PK activity in yeasts may be correlated to their mode of glucose dissimilation are presented in Table 1. The PK activity of those species evidencing no fermentative capacity is not significantly influenced by FDP. The amount of enzyme produced in complex medium is increased with no substantial change in FDP-sensitivity. The PK activity of the fermentative species, when cultured in minimal medium, is markedly stimulated by FDP. This activation is also

noted when the species are grown in the more enriched medium, but to a lesser extent.

Pyruvate kinase of R. glutinis was further reacted with metabolic intermediates that have been demonstrated to exert a specific inhibition of PK activity in other systems; citrate in Saccharomyces (3) and free fatty acids in liver (7). The citrate was prepared as the sodium salt and both the potassium and sodium salts of hexanoic, octanoic, and decanoic acid were tested. The assay conditions were as reported above. Final concentrations of these intermediates ranged from 0.5 to 5.0 mM. In no instance was there any diminution of activity compared to that of the control. In this test, 3.3 mM sodium citrate was sufficient to reduce the specific activity of pyruvate kinase from S. cerevisiae by one-half.

DISCUSSION

The contrast in sensitivity to FDP displayed by the pyruvate kinase of the two types of yeasts is similar to that of the M and L types in rat tissues described by Tanaka et al. (8). PK activity in the oxidative yeasts is insensitive to FDP as is the M type. This lack of response in R. glutinis has also been observed by Gancedo et al. (9). The moderate sensitivity to ATP displayed by the PK of R. glutinis is also similar to that evidenced by the M type. The pronounced activation of PK by added FDP, noted in the fermentative species, is the same as recorded for the L type. Malcovati and Kornberg (10) report of two types of pyruvate kinases present in Escherichia coli K12. Pyruvate kinase I, inducible and activated by FDP, is comparable to type L. Pyruvate kinase II, comparable to type M, is not activated by FDP and appears to be constitutive. The data of this report suggest that the presence or absence of an allosterically controlled pyruvate kinase in yeast may be associated with the mode of glucose catabolism.

The dampening of glycolytic activity by feedback control of the PK of R. glutinis could not be demonstrated with citrate or certain fatty acids.

The energy charge of the cell, to date, appears to be the only control mechanism of significance.

REFERENCES

1. Vinuela, E., Salas, M. L., and Sols, A., Biochem. Biophys. Res. Comm., 12, 140 (1963).
2. Salas, M. L., Vinuela, E., Salas, M., and Sols, A., Biochem. Biophys. Res. Comm., 19, 371 (1965).
3. Hess, B., Haeckel, R., and Brand, K., Biochem. Biophys. Res. Comm., 24, 824 (1966).
4. Brady, R., and Chambliss, G., Biochem. Biophys. Res. Comm., 29, 898 (1967).
5. Brady, R., Bacteriol. Proc., 134 (1969).
6. Bücher, T., and Pfleiderer, G., Methods in Enzymology, 1, 435 (1955).
7. Weber, G., Convery, H.J.H., Lea, M. A., and Stamm, N. B., Science, 154, 1357 (1966).
8. Tanaka, T., Sue, F., and Morimura, H., Biochem. Biophys. Res. Comm., 29, 446 (1967).
9. Gancedo, J., Gancedo, C., and Sols, A., Biochem. J., 102, 23c (1967).
10. Malcovati, M., and Kornberg, H., Biochim. Biophys. Acta., 178, 420 (1969).