RECIPROCAL EFFECTS OF CARBON SOURCES ON THE LEVELS OF AN AMP-SENSITIVE FRUCTOSE-1,6-DIPHOSPHATASE AND PHOSPHOFRUCTOKINASE IN YEAST*

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Growth from non-sugar precursors requires glyconeogenesis. This cannot be accomplished by a simple reversal of the glycolytic chain (Krebs and Kornberg, 1957). The physiologically irreversible phosphofructokinase (PFK) reaction is by-passed in animal tissues by a specific fructose-1,6-diphosphatase (FDPase), subject to regulation of its synthesis (Weber et al., 1965) and activity (Taketa and Pogell, 1963) and with a very great affinity for its substrate (Salas et al., 1964).

It is reported here that the FDPase activity previously observed in a yeast grown without sugar (Heredia and Yen, 1963) involves an inducible, specific FDPase with kinetic properties similar to those of the enzyme in animal tissues. Moreover, induction of FDPase in yeast tends to be accompanied by a decrease in the level of the antagonistic PFK.

METHODS

Sac. cerevisiae, strains PM-1 and 1724-14A were grown in a synthetic medium as described by Olson et al. (1949) except that sodium citrate was replaced by sodium chloride (0.25 g/l), with glucose (2%), a mixture of glycerol and lactate (1% each) or ethanol

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(2%) as carbon and energy source, shaking in air, at 30°. The yeast was harvested and washed by centrifugation, ground with alumina, and extracted with 5 vols. of 10 mM $MgCl_2$.

FDPase activity was studied spectrophotometrically or fluorometrically as described by Salas et al. (1964). Hydrolysis of other phosphoric esters was measured by estimation of inorganic P liberated, also at pH 7. Total PFK activity (Viñuela et al., 1964) was measured spectrophotometrically with 0.4 mM F6P and 0.05 mM ATP in 0.025 M phosphate buffer, pH 6.5. Glucose phosphate isomerase was measured spectrophotometrically with glucose-6-P dehydrogenase. Protein in the extracts was estimated by ultraviolet absorption.

RESULTS

Extracts of S. cerevisiae grown without sugar have FDPase activity. That this activity involves a specific FDPase is indicated by the following facts: 1) specific hydrolysis of the 1-P of FDP, as indicated by the fact that over 90% of the substrate added could be recovered as F6P by the coupled enzyme system, and 2) the

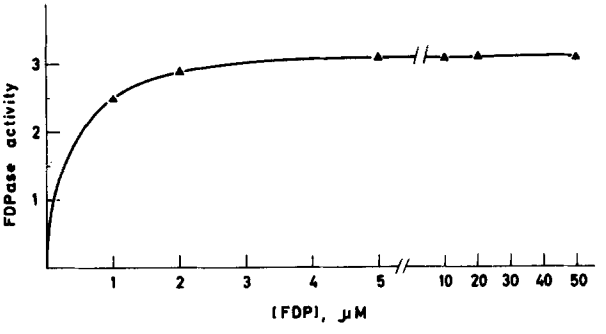

Fig. 1. Effect of the substrate concentration on the activity of yeast FDPase

The FDPase activity of a dialyzed extract of lactate-glycerol grown yeast (PM-1) was assayed fluorometrically as indicated in Methods with FDP concentrations as indicated in the figure. Initial rates (corresponding to less than 20% utilization in the case of the lowest initial substrate concentration) are plotted as fluorometer units per minute.