GLYCOLYTIC ENZYMES IN *ZYMOMONAS MOBILIS*

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ABSTRACT

RAPS, SHIRLEY (University of Illinois, Urbana) AND R. D. DEMOSS. Glycolytic enzymes in *Zymomonas mobilis*. *J. Bacteriol.* **84**:115-118. 1962—An enzyme extract of *Zymomonas mobilis* (*Pseudomonas lindneri*) was capable of fermenting glucose-6-phosphate to CO₂ and ethanol. The extract was found to contain phosphohexoisomerase, aldolase, and glyceraldehyde-3-phosphate dehydrogenase, but no demonstrable phosphohexokinase. The lack of isotope-mixing found in earlier studies is, thus, explained on an enzymatic basis.

Degradation of glucose and fructose by *Zymomonas mobilis* (*Pseudomonas lindneri*) results in an alcoholic-type fermentation (Kluyver and Hoppenbrouwers, 1931). Tracer experiments (Gibbs and DeMoss, 1951, 1953) suggest that the organism degrades glucose in a manner similar to the mechanism proposed for *P. saccharophila* by Entner and Doudoroff (1952). Since the results of tracer experiments are incompatible with even the partial operation of the complete Emden-Meyerhof pathway, the absence of one or more of the hexose-phosphate enzymes is predicted. This report deals with experiments designed to inquire as to the presence of the hexose-phosphate enzymes in *Z. mobilis*.

MATERIALS AND METHODS

Z. mobilis (ATCC 10988) was obtained from the American Type Culture Collection. The organism was grown as described previously (Gibbs and DeMoss, 1953).

RESULTS AND DISCUSSION

To ensure the presence in active form of all the enzymes of the fermentative pathway, cell-free extracts (Hugo, 1954) were prepared by several methods (Table 1). The efficacy of the Hughes-press extract dictated its utilization in the determination of phosphohexokinase, phosphohex-

isomerase, aldolase, and glyceraldehyde-3-phosphate (G3P) dehydrogenase.

Phosphohexokinase activity was determined as (i) acid production manometrically by the release of CO₂ from bicarbonate buffer, and (ii) decrease in acid-labile phosphorus. The results of the manometric analyses are presented in Table 2. A similarly prepared extract of *Escherichia coli* was included as a control of the methodology. The slight activity exhibited by the *Z. mobilis* preparation can be ascribed to adenosine triphosphatase or phosphatase action. A significant decrease in acid-labile phosphorus was not observed, a result which leads to the conclusion that phosphohexokinase activity is not present.

By use of crude Hughes-press extracts, diphosphopyridine nucleotide (DPN) reduction was observed in the presence of glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), or fructose-1,6-phosphate (F1,6P) as shown in Fig. 1. Since phosphohexokinase activity could not be demonstrated in these extracts, it is assumed that the DPN reduction is due to G6P-dehydrogenase. Prior incubation of F6P without DPN resulted in an increased rate of DPN reduction. DPN reduction in the presence of F6P indicates the presence of phosphohexoisomerase.

The slow rate of DPN reduction observed with F1,6P as substrate may be due either to the combined action of a phosphatase, phosphohexoisomerase, and G6P-dehydrogenase, or to the action of aldolase and G3P-dehydrogenase. The first alternative is eliminated by the results presented in Fig. 2. If phosphatase action is responsible for DPN reduction, prior incubation of F1,6P with enzyme (for 30 min) should result in the accumulation of G6P with an increased rate of DPN reduction over that observed without prior incubation. The equilibria of the aldolase and triose phosphate isomerase reactions are such as to preclude a significant accumulation of G3P.

Figure 2 suggests that the combined action of aldolase and G3P-dehydrogenase is responsible for the observed DPN reduction. To verify the

presence of aldolase and G3P-dehydrogenase, the effects of iodoacetate (Warburg and Christian, 1939) and of arsenate (Krebs, Rafter, and Junge, 1953) on DPN reduction were determined with F1,6P as substrate. Figure 3 shows that arsenate doubles the rate of DPN reduction, whereas iodoacetate effects an inhibition (44%). These

results infer the presence of aldolase and G3P-dehydrogenase. The upper two curves, indicating the absence of inhibitory action by iodoacetate, may be taken as further evidence against the role of G6P-dehydrogenase in DPN reduction, when F1,6P is the substrate.

The evidence obtained with crude extracts of *Z. mobilis* is consistent with the conclusion that the organism contains phosphohexoisomerase, aldolase, and G3P-dehydrogenase. All attempts to demonstrate the presence of phosphohexokinase were negative. It should be emphasized that the unsuccessful attempts to demonstrate

TABLE 1. Carbon dioxide evolution by *Zymomonas mobilis**

Vessel	Method	Substrate	Q _{CO₂}
1	Whole cells	Glucose	51.8
2	Sonic extract	G6P	15.7
3	Hughes press	G6P	62.3
4	Alumina grinding	G6P	0

*CO₂ evolution was measured manometrically at 30 C in a nitrogen atmosphere. Each vessel contained 150 μ moles of potassium phosphate (pH 7.0). Vessel 1 contained glucose, 5 μ moles, and protein (whole fresh cells), 6.3 mg. Vessels 2, 3, and 4 contained MgSO₄, 10 μ moles; cocarboxylase, 0.1 μ mole; reduced glutathione, 5 μ moles; FeSO₄, 10⁻³ μ moles; DPN, 0.9 μ mole; glucose-6-phosphate, 10 μ moles; and protein (cell-free extract), 5 mg. Total volume in each vessel was 3.0 ml. The Q_{CO₂} values (μ liters CO₂ per hr per mg protein) are corrected for endogenous CO₂ evolution.

TABLE 2. Phosphohexokinase determination*

Organism	CO ₂ released per 5 min (μ moles)		
	Complete	Without F6P	Without ATP
<i>E. coli</i>	4.22	1.58	0.96
<i>Z. mobilis</i>	0.97	0.81	0.58

* Phosphohexokinase activity in Hughes-press extracts was determined manometrically at 30 C under a 5% CO₂-95% N₂ atmosphere. Each vessel contained NaHCO₃, 31.5 μ moles (pH 7.3); protein (cell-free extract), 5 mg; MgSO₄, 10 μ moles; ATP, 20 μ moles; and F6P, 10 μ moles; in a total volume of 3.0 ml.

FIG. 1. Relative rates of DPN reduction with G6P, F6P, and F1,6P as substrates. Each cuvette contained potassium phosphate (pH 7.5), 150 μ moles; MgSO₄, 20 μ moles; 5 μ moles of either G6P, F6P, or F1,6P; protein (cell-free extract from Hughes press), 0.5 mg; and DPN, 0.9 μ mole; in a total volume of 3.0 ml. DPN reduction was measured spectrophotometrically at 340 m μ and 25 C.

FIG. 2. DPN reduction with F1,6P as substrate. DPN reduction was measured spectrophotometrically at 340 mμ. Each cuvette contained potassium phosphate (pH 7.5), 150 μmoles; MgSO₄, 20 μmoles; F1,6P, 5 μmoles; protein (cell-free extract from Hughes press), 0.5 mg; with DPN, 0.9 μmole, added either at zero time or after 30 min of incubation. The total volume was 3.0 ml, and the temperature was 25 C.

FIG. 3. Aldolase and G3P dehydrogenase. The effects of iodoacetate and arsenate on DPN reduction with G6P or F1,6P as substrate. DPN reduction was measured spectrophotometrically at 340 mμ. Each cuvette contained potassium phosphate (pH 7.5), 150 μmoles; protein (Hughes-press extract), 0.5 mg; DPN, 0.9 μmole; 5 μmoles of either G6P or F1,6P; and iodoacetate, 3 μmoles, or arsenate, 6 μmoles, when indicated; in a total volume of 3 ml at 25 C.

phosphohexokinase were performed with a cell-free extract which catalyzed the conversion of G6P to CO₂ and ethanol. If phosphohexokinase is actually absent in whole cells, as seems probable from these results, the lack of isotope-mixing in fermentations with specifically labeled glucose is clearly explainable on an enzymatic basis. The conclusions are completely in accord with the work of Ribbons and Dawes (1961), which demonstrated the presence of pyridine nucleotide-linked dehydrogenases in extracts of the same organism.

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