IMPLICATION OF THE DIRECT PHOSPHORYLATION OF PYRUVATE IN CELLULOSE SYNTHESIS BY ACETOBACTER XYLINUM

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Washed glucose-grown A. xylinum cells readily oxidize intermediates of the citrate cycle, but form no cellulose from them. Such synthesis occurs, however, if the cells have been grown on citrate-cycle intermediates. In earlier studies (Benziman and Burger-Rachamimov, 1962), aimed at elucidating the chemical basis for the acquisition of the cellulose-synthesizing ability, it was observed that exogenous CO_2 failed to serve as a carbon source for cellulose synthesized by A. xylinum cells in the presence of pyruvate as the sole carbon source. In addition it was found (Benziman and Heller, 1964) that extracts of succinate grown cells are unable to carboxylate pyruvate or phosphoenolpyruvate (PEP). Thus the net synthesis of PEP from pyruvate as a primary step leading to cellulose is unlikely to be achieved via the generally accepted mechanisms involving carboxylation of pyruvate to oxaloacetate followed by phosphorylative decarboxylation to yield PEP (Utter, 1963). The present communication presents evidence that succinate-grown cells of A. xylinum, contain an enzyme system which effects a direct net synthesis of PEP from pyruvate. Pyruvate is phosphorylated by ATP with the splitting of an additional pyrophosphate bond to yield PEP, AMP and Pi. Such activity was absent in glucose-grown cells.

Materials and Methods. Succinate-grown cells of A. xylinum were grown and harvested as previously described (Benziman and Burger-Rachamimov, 1962). Glucose-grown cells were obtained according to Schramm et al (1957). Cell suspensions in 50 mM Tris-HCl buffer (pH 7.4) were disrupted

by treating them in a French pressure cell under 15000 lb/in^2. The extract was centrifuged for 15 min at 18000 xg and the precipitate was discarded. The supernatant fluid was further centrifuged at 100000 x g for 1 hr in a Spinco ultracentrifuge. The high speed supernatant fraction was fractionated with solid ammonium sulfate at 4°. The material which precipitated between 40 and 50% saturation was dissolved in 50 mM tris-1mM Mg^{++} - 5mM EDTA buffer pH 7.4. This fraction was used in the experiments described. It rapidly lost activity when stored frozen or in ice, but retained activity when kept at room temperature in the presence of penicillin (100 units/ml) and streptomycin (100 γ/ml). Products and reactants of the reaction were estimated by measurement of changes in optical density at 340 mμ by using the following assay systems: for ATP; hexokinase, glucose, glucose-6 phosphate dehydrogenase and NADP; for pyruvate: lactic dehydrogenase and $NADH_2$; for PEP as for pyruvate followed by ADP and pyruvic kinase; for ADP as for pyruvate followed by pyruvic kinase and PEP and for AMP as for ADP followed by adenylate kinase. Böeringers analytical grade enzyme preparations were used for these determinations. Inorganic phosphate was estimated by the method of Fiske and SubbaRow (1925).

Results. The ammonium sulfate fraction isolated from extracts of succinate grown-cells of A. xylinum catalyzed the removal of pyruvate when incubated with this substrate and ATP. It contained inorganic pyrophosphatase, traces of pyruvate decarboxylase (Neeman and Benziman, 1962) and traces of ATPase. It was free from pyruvate kinase and adenylate kinase and did not show any pyruvate carboxylase (Utter and Keech, 1963) or PEP carboxykinase (Utter and Kurahashi, 1954) activities. The products formed by the partially purified enzyme when incubated with pyruvate ATP and Mg^{++} were PEP, AMP and Pi. The results of a quantitative determination of the amounts of products fromed and of reactants removed from the system is given in Table 1. PEP synthesis was completely dependent on enzyme, pyruvate, ATP and Mg^{++}. Substitution of ATP by GTP, CTP, ITP or TTP did not result in any PEP formation. The rate of PEP formation was linear with time and proportional to the amount of enzyme added.

Extracts similarily prepared from glucose-grown cells formed no PEP under similar conditions. Such extracts showed as much pyruvate kinase activity as did extracts of succinate-grown cells.

Table 1

Stoichiometry of PEP synthesis from pyruvate

Component measured	Changes (μmoles) in Complete System	ATP omitted	Pyruvate omitted	Net change
Pyruvate	-0.88	-0.18	0	-0.70
ATP	-1.02	0	-0.28	-0.74
PEP	+0.72	0	0	+0.72
AMP	+0.75	0	0	+0.75
Pi	+1.13	0	+0.30	+0.83
ADP	+0.27	0	+0.27	0

The complete system (1 ml) contained the following in μmoles: Tris-HCl buffer pH 7.4, 100; sodium pyruvate, 3.5; ATP, 4; $MgCl_2$, 10; and enzyme, 1.1 mg protein (ammonium sulfate fraction). Incubaed in air for 30 min at 30°. Reaction terminated by 1 ml 0.6 M $HClO_4$, followed by centrifugation. The protein-free supernatant was neutralized by 2.5 ml 0.15 M K_2CO_3. Determination of components was carried out as described in Materials and Methods.

Discussion. The results presented clearly indicate that succinate-grown cells of A. xylinum contain an enzyme system which catalyzes the net synthesis of PEP from pyruvate and ATP with the concomitant formation of AMP and Pi. The stoichiometry of the reaction (Table 1) is compatible with the reaction of PEP synthase recently suggested by Cooper and Kornberg (1965) for mutants of E. Coli, namely: Pyruvate + ATP $\xrightarrow{Mg^{++}}$ PEP + AMP + Pi.

Activity was dependent on the presence of magnesium ions and ATP. Other nucleotide triphosphates could not be substituted for ATP. The activity of PEP synthase could be separated from the pyruvate kinase activity.

Since other pathways for the formation of PEP from pyruvate are not found in A. xylinum (Benziman and Heller, 1964), it is concluded that the physiological role of PEP synthase in succinate-grown cells is to catalyze the formation of PEP from pyruvate, thus enabling them to synthesize cellulose which is essential for growth in a static liquid medium (Schramm and Hestrin, 1954). This is consistent with the results of our earlier studies (Benziman and Burger-Rachamimov, 1962) which have indicated that the anhydroglucose carbon

chain of cellulose arises from pyruvate in Acetobacter via PEP. Further support for this role of PEP synthase comes from the finding that glucose-grown cells which cannot form cellulose from pyruvate are devoid of PEP synthase activity.

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