THE SYNTHESIS OF PHOSPHOENOLPYRUVATE FROM PYRUVATE AND ATP BY EXTRACTS OF PHOTOSYNTHETIC BACTERIA

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In 1964, Bachofen et al. found a ferredoxin-dependent synthesis of pyruvate from acetyl coenzyme A and CO_2 (Eq. 1) in cell-free extracts of the anaerobic bacterium, Clostridium pasteurianum.

(1) Acetyl-CoA + CO_2 + Ferredoxin_{red} $\longrightarrow$ Pyruvate + CoA + Ferredoxin_{ox}

Buchanan et al. (1964) showed the presence of this system, tentatively named pyruvate synthase, in extracts of the photosynthetic bacterium, Chromatium. Evans and Buchanan (1965) found pyruvate synthase in extracts of another photosynthetic bacterium, Chlorobium thiosulfatophilum, and purified the enzyme about 30-fold. Pyruvate synthase has also been found recently in other anaerobic, non-photosynthetic bacteria: in Clostridium kluyveri by Andrews and Morris (1965) and Stern (1965); and in Clostridium acidi-urici by Raeburn and Rabinowitz (1965). Pyruvate synthase from C. acidi-urici has been partially purified (Raeburn and Rabinowitz, 1965).

The metabolic use of newly synthesized pyruvate would in a large degree depend on its conversion to phosphoenolpyruvate (PEP). PEP is known to be a key metabolic intermediate in photosynthetic bacteria, particularly in the synthesis of amino acids (Losada et al., 1960; Fuller et al., 1961) and sugars (Evans, 1965). The generally accepted mechanism for the synthesis of PEP from pyruvate in photosynthetic bacteria, as in other cells (Utter and Kurahashi, 1954; Utter and Keech, 1963) is the carboxylation of pyruvate to oxaloacetate followed by a decarboxylation of the oxaloacetate in the presence of ATP and ITP, respectively.

This communication presents evidence for a direct synthesis of PEP from pyruvate and ATP by enzyme fractions isolated from the three photosynthetic bacteria, Chlorobium thiosulfatophilum, Chromatium and Rhodospirillum rubrum. The synthesis of PEP by these preparations depends on PEP synthase, which Cooper and Kornberg (1965) have recently isolated and partially purified from mutants of Escherichia coli. Cooper and Kornberg showed that, in the presence of $MgCl_2$, PEP synthase utilizes the energy of two rather than of one of the energy-rich phosphate bonds of ATP.

$$(2)\quad \text{Pyruvate} + \text{ATP} \xrightarrow{MgCl_2} \text{Phosphoenolpyruvate} + \text{AMP} + P_i$$

Methods

C. thiosulfatophilum (Evans and Buchanan, 1965) and Chromatium, strain D (Arnon et al., 1963), were grown with carbon dioxide as the sole carbon source and thiosulfate as the reductant; R. rubrum was grown anaerobically in the light on malate (Gest et al., 1950). Cell-free extracts were prepared by sonic oscillation and the soluble enzyme fraction was isolated by centrifugation in a preparative ultracentrifuge as described previously (Evans and Buchanan, 1965). Solid ammonium sulfate was added to the soluble enzyme fraction to a final concentration of 80% saturation at 4° with continuous stirring. The suspension was then centrifuged for 10 minutes at 39,000 x g, and the supernatant fluid was discarded. The precipitate was dissolved in 0.1 M potassium phosphate buffer, pH 7.4, equivalent to one-half the volume of the original soluble enzyme fraction and was used without further treatment.

Results and Discussion

Table 1 shows that the ammonium sulfate fractions isolated from Chromatium, C. thiosulfatophilum and R. rubrum catalyzed the synthesis of PEP when assayed according to the method of Cooper and Kornberg (1965). PEP synthesis was strictly dependent on pyruvate, ATP and $MgCl_2$. In all cases, the reaction rate was linear with time for at least 30 minutes and

Table 1

REQUIREMENTS FOR THE SYNTHESIS OF PHOSPHOENOLPYRUVATE

Treatment	μmoles PEP formed		
	C. thiosulfatophilum	Chromatium	R. rubrum
Complete	0.37	0.17	0.49
Pyruvate omitted	0.10	0.05	0.01
ATP omitted	0.01	0.00	0.07
$MgCl_2$ omitted	0.05	0.00	0.00

The complete system contained ammonium sulfate fractions of C. thiosulfatophilum (0.34 mg protein), Chromatium (1.3 mg protein), R. rubrum (0.88 mg protein), as indicated, and the following in μmoles: Tris-HCl buffer, pH 7.4, 100; sodium pyruvate, 4; ATP, 4; and $MgCl_2$, 10. Final volume, 1.2 ml. The reaction was carried out in air at 25° for 30 minutes in test tubes and was stopped by the addition of 0.5 ml 3 N perchloric acid. The mixture was neutralized with 0.5 ml 3 N potassium carbonate and phosphoenolpyruvate was quantitatively estimated with crystalline lactic dehydrogenase (EC 1.1.1.27) and pyruvate kinase (EC 2.7.1.40) from rabbit muscle as described by Czok and Eckert (1963).

was proportional to the protein concentration at the levels used.

The enzyme fractions of C. thiosulfatophilum, which had PEP synthase activity, showed no pyruvate kinase (E.C. 2.7.1.40) or PEP carboxykinase (E.C. 4.1.1.32) activity. These preparations, however, did contain adenylate kinase (E.C. 2.7.4.3), which prevented an accurate estimation of the AMP formed. For this reason, a determination of the stoichiometry of the PEP synthase reaction, catalyzed by these preparations, could not be made.

We conclude that the photosynthetic bacteria, C. thiosulfatophilum, Chromatium and R. rubrum have PEP synthase and can synthesize PEP directly from pyruvate and ATP.

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