Ferredoxin dependent synthesis of α-ketoglutarate and pyruvate by extracts of the green photosynthetic bacterium Chloropseudomonas ethylicum.

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Carbon dioxide fixation in the obligately autotrophic green sulphur bacterium Chlorobium thiosulphatophilum and in the purple non sulphur bacterium Rhodospirillum rubrum has been shown to involve a reductive carboxyllic acid cycle (Evans et al, 1966; Buchanan et al, 1967). This cycle involves two ferredoxin dependent CO_2 fixation reactions, the synthesis of α-ketoglutarate from succinyl CoA (Equation 1) and of pyruvate from acetyl CoA (Equation 2).

1) Succinyl CoA + CO_2 + Fd_{red} —— α-ketoglutarate + CoA + Fd_{ox}

2) Acetyl CoA + CO_2 + Fd_{red} —— Pyruvate + CoA + Fd_{ox}

Extracts of the green photosynthetic bacterium Chloropseudomonas ethylicum grown with ethanol and CO_2 have now been found to contain both pyruvate and α-ketoglutarate synthases. Ferredoxin has also been purified from this organism.

Cps. ethylicum can grow either as an autotroph or as a photoheterotroph with acetate or ethanol as carbon source and electron donor. Callely and Fuller (1968) have reported the presence of many enzymes of the tricarboxyllic acid cycle, in extracts of Cps. ethylicum but they did not assay pyruvate synthase or α-ketoglutarate synthase. Doman (1968) has recently reported short exposure experiments on acetate, malate and CO_2 assimilation by Cps. ethylicum grown on acetate or ethanol and found evidence for the presence

of pyruvate synthase in cells grown under both condition and of α-ketoglutarate synthase in cells grown on ethanol but not in cells grown on acetate, indicating the operation of the reductive carboxyllic acid cycle only in cells which have "excess" reductant available.

Materials and Methods.

A culture of Cps. ethylicum was obtained from Dr. J.M. Olson. It was maintained in a modified Pfennig's medium (Evans and Buchanan, 1965). The sulfide concentration was reduced to 0.02%, and 2% sodium chloride and 0.4% ethanol were added. After harvesting, the unwashed cell paste was stored at -20° until required. Ferredoxin was prepared essentially as described previously for Chromatium (Evans et al, 1968). Cps. ethylicum ferredoxin shows a very high affinity for DEAE cellulose and the final chromatography was carried out using 0.43 M NaCl in 0.01 M potassium phosphate buffer pH 7.3. Cps. ethylicum ferredoxin had a spectrum similar to that of other bacterial ferredoxins with peaks at 280 mμ and 390 mμ with a shoulder at 310 mμ. The best preparations obtained had a 390 : 280 mμ ratio of 0.53. It was not reduced by dithionite.

Extracts for assay of pyruvate and α-ketoglutarate synthases were prepared by suspending frozen cell paste 1:2 w/v in 0.02 M potassium phosphate buffer pH 7.3. The cells were broken by passing the extract through a French Press. The crude extract was centrifuged for 1 hr. at 150,000g. to remove chlorophyll containing particles. The clear supernatant (from 5.0g. of cells) was made 0.2 M with NaCl and passed through a 1 x 3 cm. DEAE cellulose column equilibrated with 0.2 M NaCl in 0.02 M potassium phosphate buffer pH 7.3 to remove ferredoxin. 5.0 ml. of the treated extract were then passed through a 1.5 x 15 cm. column of Sephadex G 50 to remove endogenous substrates.

Assay procedures were essentially as described previously (Evans and Buchanan, 1965; Buchanan and Evans, 1965).

TABLE 1

Requirements for pyruvate synthesis by extracts of Cps. ethylicum

	Pyruvate synthesised c.p.m.
Complete	17,000
minus Ferredoxin	180
minus Acetyl Phosphate	320
minus Coenzyme A	710
minus Thiamin Pyrophosphate	6780
minus Phosphotransacetylase	6870
minus Cps. ethylicum extract	0
Complete; Ferredoxin not reduced	310

The complete reaction mixture contained in a final volume of 3.0 ml 300 μ Moles N-2-Hydroxyethylpiperazine-N-2-Ethane Sulfonate pH 7.3; 50 μ Moles Acetyl Phosphate; 0.25 μ Moles Coenzyme A; 50 μ Moles 2-Mercaptoethanol; 100 μg Thiamin pyrophosphate; 100 μg Cl. welchii ferredoxin; 500 μg Chloroplasts (P_1S_1); and 5.0 μ Moles Na_2CO_3 100,000 c.p.m. / μ Mole. Extract 0.5 mg Protein. Gas phase Argon; Temp. 30°; Incubation time 30 mins.

Results.

Extracts of Cps. ethylicum were found to catalyse the synthesis of pyruvate from acetyl coenzyme A and CO_2 and of α-ketoglutarate from succinyl coenzyme A and CO_2. The synthesis of both keto acids required reduced ferredoxin.

Table 1 shows the requirements for synthesis of pyruvate; the system showed an absolute requirement for acetyl coenzyme A, (supplied as acetyl phosphate, coenzyme A and phosphotransacetylase), ferredoxin and the Cps. ethylicum enzyme fraction. If the ferredoxin was not reduced no pyruvate was synthesised.

Table 2 similarly shows the requirement for α-ketoglutarate synthesis; the system showed an absolute requirement for succinyl coenzyme A (supplied as succinate, coenzyme A and ATP), ferredoxin and the enzyme preparation. If the

TABLE 2

Requirements for α-Ketoglutarate synthesis by extracts of Cps. ethylicum

	α-Ketoglutarate synthesised c.p.m.
Complete	11,140
minus Ferredoxin	300
minus Succinate	1,160
minus ATP	700
minus $MnCl_2$	1,326
minus Coenzyme A	689
minus TPP	3,872
minus Cps. ethylicum extract	200
Complete; Ferredoxin not reduced	600

The reaction mixture was as described in Table 1, except that Acetyl phosphate and phosphotransacetylase were omitted and 10.0 μ Moles Sodium Succinate; 5.0 μ Moles ATP and 5.0 μ Moles $MnCl_2$ were added.

TABLE 3

DIFFERENT FERREDOXINS IN α-KETO ACID SYNTHESIS BY EXTRACTS OF Cps. ethylicum

	Pyruvate	α-Ketoglutarate
Source of Ferredoxin	c.p.m.	c.p.m.
None	700	300
Cl. welchii	29,000	40,000
Chromatium	54,000	78,000
Cps. ethylicum	44,500	86,000
Spinach	25,000	7,800

Reaction conditions were as described in Tables 1 and 2 except that Cl. welchii ferredoxin was omitted and 200 μ g of each ferredoxin added as indicated.

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ferredoxin was not reduced no α-ketoglutarate was synthesised. The α-ketoglutarate system showed an additional requirement for $MnCl_2$. This is

presumably required for the synthesis of succinyl coenzyme A by succinyl coenzyme A synthetase in the extract, as was the case in Chl. thiosulfatophilum. Both enzymes showed a requirement for thiamin pyrophosphate; this was also observed with sephadex treated extracts of R.rubrum (Buchanan et al, 1967) but not in the experiments with Chl. thiosulfatophilum (Evans et al, 1966).

Table 3 shows the effect of using four different ferredoxins in the synthesis of pyruvate and α-ketoglutarate. Ferredoxins from Cps. ethylicum and Chromatium were the most effective. Cl. welchii ferredoxin was less effective while spinach ferredoxin showed very low activity in the α-ketoglutarate system but was as effective as Cl. welchii ferredoxin in pyruvate synthesis. The greater effectiveness of ferredoxin from photosynthetic bacteria may be due to their very low redox potential.

The results presented here demonstrate the presence of ferredoxin, pyruvate and α-ketoglutarate synthase in Cps. ethylicum. Together with the results of Callely and Fuller (1968), and Doman (1968) they indicate the presence of the enzymes of the reductive carboxyllic acid cycle in Cps. ethylicum and are consistent with the functioning of these enzymes in CO_2 fixation during growth on ethanol. The effect of growth under varying conditions on the activity of enzymes of the cycle is under investigation.

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