FERREDOXIN-DEPENDENT SYNTHESIS OF LABELLED PYRUVATE FROM LABELLED ACETYL COENZYME A AND CARBON DIOXIDE

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When the oxidation-reduction potential of ferredoxins was found to be close to that of hydrogen gas, i.e. about 100 mV more electronegative than that of pyridine nucleotides (Tagawa and Arnon, 1962), it became a matter of conjecture whether there are enzyme systems that use ferredoxins directly as reductants in carbon assimilation. Soon thereafter, such an enzyme system, which requires a reductant stronger than reduced DPN or TPN, was found, first in extracts of the non-photosynthetic bacterium C. pasteurianum (Bachofen et al., 1964), and more recently, in extracts of the photosynthetic bacteria Chromatium (Buchanan et al., 1964) and Chlorobium thiosulfatophilum (Evans and Buchanan, 1965). The enzyme, tentatively named pyruvate synthase, has now been purified about 30-fold (Evans and Buchanan, 1965). It catalyzes a reductive synthesis of pyruvate from CO_2 and acetyl-CoA, using reduced ferredoxin as the reductant (Eq. 1).

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

In previous investigations, the evidence for the pyruvate synthase reaction was based on the incorporation of labelled CO_2 into pyruvate. This reaction is distinguished from the well-known exchange reaction between bicarbonate and the carboxyl group of pyruvate (Wilson et al., 1948; Wolfe and O'Kane, 1955) by a requirement for a strong reducing potential and by a requirement for substrate amounts of acetyl-CoA. Despite these distinctions, it seemed desirable to document further

the difference between the pyruvate synthase and the CO_2 exchange reactions by measuring not the reductive incorporation of labelled CO_2 but the reductive incorporation of the labelled acetyl moiety.

We report here new evidence for the pyruvate synthase reaction (Eq. 1) based on the synthesis of pyruvate from (a) labelled acetyl-CoA and unlabelled CO_2, and (b) labelled acetate and unlabelled CO_2 in cell-free extracts of C. pasteurianum and Chromatium. Mortlock and Wolfe (1959) have shown earlier a synthesis of pyruvate from labelled acetyl phosphate and CO_2 in extracts of Clostridium butyricum supplied with a non-physiological reductant, sodium hydrosulfite.

In previous experiments with extracts of C. pasteurianum (Bachofen et al., 1964), acetyl-CoA was not supplied directly but as acetyl phosphate which was converted to acetyl-CoA by transacetylase (in the presence of CoA). Chromatium extracts have, in addition, the capacity, in the presence of ATP, to activate acetate (Losada et al., 1960; Fuller et al., 1961) to the coenzyme A thiolester. We have, therefore, substituted, in some of the present experiments, labelled acetate (in the presence of ATP and CoA) for labelled acetyl-CoA.

METHODS

Cell-free extracts of C. pasteurianum and Chromatium were prepared and treated with DEAE-cellulose to remove ferredoxin by methods previously described (Bachofen et al., 1964; Buchanan et al., 1964). Other methods, including the determination of pyruvate and amino acids, were also described in these earlier publications. C^{14}-acetyl-CoA and C^{14}-acetate were obtained from commercial sources.

RESULTS AND DISCUSSION

Table 1 shows the requirements for the incorporation of C^{14}-acetyl-CoA into pyruvate by DEAE-treated extracts of C. pasteurianum. Reduced ferredoxin was prepared by photoreduction with spinach chloroplast fragments; carbon monoxide was used as the gas phase to inhibit hydro-

genase (Bachofen et al., 1964). Under these conditions, the synthesis of pyruvate was dependent on CO_2 and reduced ferredoxin. Pyruvate was identified by paper chromatography as the 2,4-dinitrophenylhydrazone derivative (Bachofen et al., 1964).

TABLE I

PHOTOREDUCED FERREDOXIN AND THE REDUCTIVE SYNTHESIS OF PYRUVATE FROM C^{14}-ACETYL-CoA AND CO_2 BY EXTRACTS OF CLOSTRIDIUM PASTEURIANUM

Treatment	C^{14}-Acetyl-CoA fixed as pyruvate (cpm)
1. Complete	1,560
2. Complete, ferredoxin not reduced	30
3. Minus ferredoxin	270
4. Minus CO_2	540

The complete system contained DEAE-treated cell-free extract of C. pasteurianum (5 mg protein); 200 µg of spinach ferredoxin; heated chloroplast fragments equivalent to 0.5 mg chlorophyll; and the following in µmoles: potassium phosphate buffer, pH 7.3, 300; $MgCl_2$, 5; sodium ascorbate, 20; DPIP, 0.1; $KHCO_3$, 10; acetyl-1-C^{14}-CoA (125,000 cpm), 2.5. Final volume, 3.0 ml. Gas phase, carbon monoxide. Light intensity was 10,000 lux. The reaction was carried out at 30° for 15 min.

The requirements for the assimilation of C^{14}-acetate by DEAE-treated extracts of the photosynthetic bacterium Chromatium are presented in Table 2. In these experiments ferredoxin was reduced by hydrogen gas and the native hydrogenase present in the extract. The reductive assimilation of C^{14}-acetate required ferredoxin, CO_2, CoA, ATP, DPN, and $MnCl_2$. No substantial C^{14}-acetate incorporation occurred when hydrogen gas was replaced by argon, with or without the addition of reduced DPN. These results are in accord with those reported previously, when the activity of the pyruvate synthase system was measured by the incorporation of $C^{14}O_2$ (Buchanan et al., 1964).

TABLE II

FERREDOXIN AND THE REDUCTIVE ASSIMILATION OF C^{14}-ACETATE AND CO_2 BY CHROMATIUM EXTRACTS

Treatment	Total C^{14}-acetate assimilated (cpm)
1. Complete	10,150
2. Minus ferredoxin	2,300
3. " $MnCl_2$	5,800
4. " $KHCO_3$	3,500
5. " CoA	2,100
6. " ATP	2,300
7. " DPN	2,000
8. Complete, but H_2 replaced by argon	2,250
9. Complete, but H_2 replaced by argon and DPN replaced by $DPNH_2$	2,100

The complete system contained DEAE-treated cell-free extract of Chromatium (23 mg protein); 400 µg of spinach ferredoxin; and the following in µmoles: potassium phosphate buffer, pH 6.5, 200; $MnCl_2$, 4; DPN, 2; $KHCO_3$, 10; ATP, 10; CoA, 0.1; sodium acetate-1-C^{14} (500,000 cpm), 10. In treatment 9, DPN was omitted and 7.5 µmoles of $DPNH_2$ were added. Final volume, 3.0 ml. Gas phase, H_2, except as indicated. The flasks were preincubated for 60 min before tipping. The reaction was carried out at 30° for 90 min in the dark.

In these earlier experiments the accumulation of pyruvate was not observed unless a pyruvate trapping reagent was present. In the absence of a pyruvate trapping system, and in the presence of DPN, $MnCl_2$, and an amino donor (an amino donor was required only after the DEAE-treated extract was extensively dialyzed), these extracts converted the pyruvate to amino acids, mainly glutamate, aspartate and alanine. Since a ferredoxin-dependent reduction of DPN with hydrogen gas was also found to be catalyzed by these extracts, it was tentatively concluded that the reduced DPN produced in this manner was used in reductive amination reactions that gave rise to one or more of these amino acids (Buchanan et al., 1964).

In the present experiments, incorporation of C^{14}-acetate into pyruvate led also to the formation of the same amino acids as were found in the earlier experiments with unlabelled acetate derivatives and $C^{14}O_2$ (Buchanan et al., 1964). The compounds were chromatographically identified as aspartate, glutamate and alanine. In addition, the chromatogram contained two radioactive, ninhydrin-insensitive compounds which were not further identified.

The present results provide direct evidence that in certain photosynthetic bacteria the ferredoxin-dependent pyruvate synthase system can function as a mechanism for the assimilation of acetate as well as of CO_2. These results confirm and extend the original observations made with extracts of C. pasteurianum and Chromatium (Bachofen et al., 1964). Recently, this mechanism for the assimilation of acetate and CO_2 has been found in other bacteria: in extracts of Clostridium kluyveri (Andrews and Morris, 1965), and with a partly purified enzyme isolated from the purine fermenting heterotroph, Clostridium acidi-urici (Raeburn and Rabinowitz, 1965).

SUMMARY

Evidence is presented for a ferredoxin-dependent reductive synthesis of pyruvate from CO_2 and C^{14}-acetyl-CoA or C^{14}-acetate by extracts of C. pasteurianum and Chromatium.

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