FERREDOXIN LINKED DPN REDUCTION BY PYRUVATE IN EXTRACTS OF CLOSTRIDIUM ACIDI-URICI

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The pathway of formiminoglycine conversion to acetate by the purine-fermenting organism, Clostridium acidi-urici has been outlined by Sagers, Benziman, and Gunsalus (1961). In this pathway methenyl-tetrahydrofolate is reduced to the methylene derivative by a TPNH specific dehydrogenase. The one-carbon unit is then condensed with glycine to form serine, which is converted to pyruvate. Pyruvate is oxidized by a DPN-specific enzyme system and acetyl phosphate is generated, providing a source of energy for endergonic reactions by coupling to the ATP generating system via acetokinase.

In this report we present data showing the DPN specificity of pyruvate oxidation, and the dependency of DPN reduction upon ferredoxin. To our knowledge this is the first report of a ferredoxin-linked DPN reduction by pyruvate. We further present evidence that TPN can be reduced by pyruvate in extracts of C. acidi-urici, but only when the extracts are supplemented with catalytic amounts of DPN. Thus the transhydrogenase reaction could provide the necessary coupling between the DPN mediated pyruvate oxidation and the TPN mediated methylene tetrahydrofolate dehydrogenase systems. The coupled reactions can be visualized as follows:

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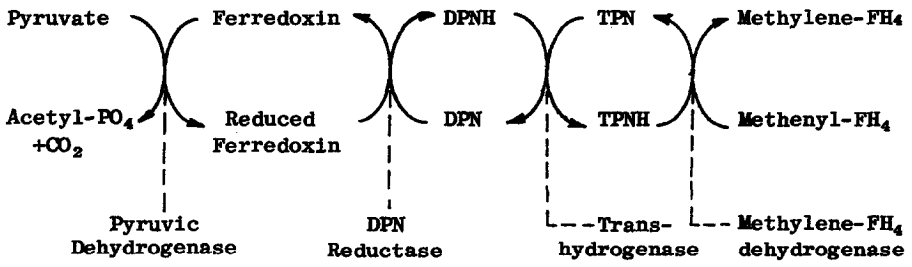

C. acidi-urici was grown on the urate medium as described by Benziman, Sagers, and Gunsalus (1960). Extracts were prepared by crushing the freshly harvested cells in a Hughes press. The crushed cells (8 g) were suspended in 30 ml of water at 0°C which contained 75 μg DNAase to reduce the viscosity of the extract. Cell debris was removed by centrifugation at 15,000 x g for 15 min. 30 ml of the amber-colored extract containing 30 mg of protein per ml were passed through a DEAE-cellulose (phosphate) colume (1 x 4 cm) to remove ferredoxin. The eluate prepared in this manner no longer carried out the reduction of DPN by pyruvate but the reducing activity was restored by addition of ferredoxin from either C. acidi-urici or C. pasteurianum. In Fig. 1A the upper curve shows the rate of DPN reduction in extracts supplemented with 183 μg of ferredoxin. The lower curve shows the rate without ferredoxin. Fig. 1B shows the rate of DPN reduction to be linearly proportional to ferredoxin concentrations between 0 and 183 μg. The ferredoxin from C. acidi-urici prepared by DEAE-cellulose chromatography and used in these experiments contained 60 pyruvate clastic units per mg (Mortenson, Valentine, and Carnahan, 1962).

As shown in Fig. 2, DPN but not TPN served as primary electron acceptor for pyruvate oxidation. However, when 0.029 μmoles of DPN were added to the reaction mixture containing 2.8 μmoles of TPN the reduction proceeded at a rate of 0.11 μmole per min., indicating the presence of transhydrogenase in the extracts.

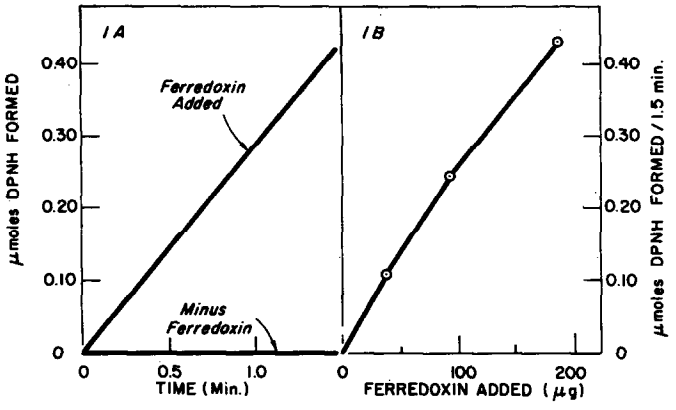

Figure 1A. Ferredoxin requirement for DPN reduction. 3 ml volume in an anaerobic cuvette contained in µmoles: coenzyme A, 0.1; thiamine pyrophosphate, 0.22; K-phosphate buffer, pH 7.3, 300; $FeSO_4$, 1; sodium pyruvate, 150; DPN, 2.8. Ferredoxin-free C. acidiurici extract (8.8 mg protein) and 183 µg ferredoxin from C. acidiurici were also added. The cuvettes were alternately evacuated and flushed five times with argon. The reduction of TPN was recorded continuously on a Cary model 14 recording spectrophotometer by following the increase in optical density at 340 mµ and the curves shown were taken from the Cary recording sheets.

Figure 1B. Effect of ferredoxin concentration on DPN reduction. Conditions were as for Fig. 1A; ferredoxin concentrations were as indicated on the graph.

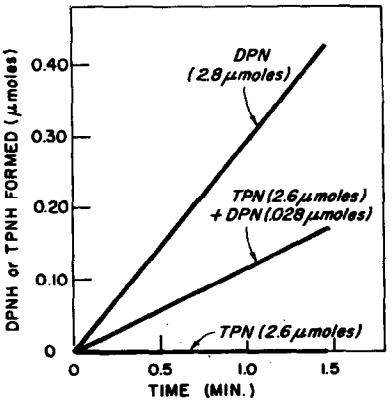

Figure 2. Pyridine nucleotide specificity and transhydrogenase activity. Conditions were as described for Fig. 1A; DPN or TPN concentrations are indicated on each curve; 183 µg of ferredoxin were added.

Table 1 shows the requirements for DPN and ferredoxin in the conversion of pyruvate to acetyl phosphate by extracts of C. acidi-urici.

Table 1

Ferredoxin and DPN Requirement for Acetyl Phosphate Production from Pyruvate

System	Acetyl Phosphate Formed
	(μmoles)
Complete	2.5
Minus Ferredoxin (91 μg)	0.4
Minus DPN (2.9 μmoles)	0.1
Minus Pyruvate (50 μmoles)	0.0

Complete system (1 ml) contained, in μmoles: $KHPO_4$, pH 7.3, 50; coenzyme A, 0.1; $FeSO_4$, 1.0; thiamine pyrophosphate, 0.3. Ferredoxin-free enzyme (6 mg protein) and other constituents as above. Incubated at 30 C for 15 min. Acetyl phosphate determined as acetylhydroxamate by the method of Lipmann and Tuttle (1945).

DISCUSSION

In the overall fermentation of urate by C. acidi-urici, ferredoxin may be a key electron carrier, mediating electron transfer between pyruvic dehydrogenase and uric acid reductase for the generation of xanthine (Bradshaw and Barker, 1960). A similar reaction has been described in Micrococcus lactilyticus (Valentine, Jackson, and Wolfe, 1962). We have been unable to couple formate oxidation with ferredoxin or with pyridine nucleotide reduction, whereas we have demonstrated that pyruvate is an active electron donor for ferredoxin reduction. Molecular hydrogen is not a urate fermentation product and hydrogenase is absent from extracts of C. acidi-urici, indicating that excess electrons are not produced during urate degradation. Two reductive steps occur in the overall fermentation of urate to acetate (one for the reduction of urate to xanthine, and one for the reduction of methenyl-FH_4 to methylene-FH_4). Thus, a source of electrons other than the oxidation of pyruvate would be required. This additional source could be supplied

by the DPN-specific oxidative cleavage of glycine as demonstrated by Sagers and Gunsalus (1958).

We have recently been interested in the ferredoxin-linked pyridine nucleotide reductions in the Clostridia because of the similarity of these reactions with the ferredoxin-TPN system of green plants (Tagawa and Arnon, 1962; Valentine, Brill, and Wolfe, 1962). In this communication we have described the pyruvate oxidizing system of C. acidi-urici, a new ferredoxin-linked reaction leading to the generation of DPNH. This reaction might serve as a model for the terminal electron transport step in photosynthetic bacteria leading to the generation of reduced DPN (Frenkel, 1961).

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