ACTIVITY OF PPNR IN FERREDOXIN-DEPENDENT REACTIONS OF CLOSTRIDIUM PASTEURIANUM *

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The similarity in properties and function of PPNR[1-3] (photosynthetic pyridine nucleotide reductase) and ferredoxin[4-6] a new electron transport catalyst isolated from clostridia, prompted us to compare them in several ferredoxin-dependent reactions in clostridia. Both carriers have redox potentials close to that of the hydrogen electrode[7], and ferredoxin was shown by Tagawa and Arnon[7] to replace PPNR in TPN reduction by illuminated chloroplasts. These workers also confirmed our earlier demonstration that ferredoxin in conjunction with hydrogenase will catalyze the evolution of H_2 from aqueous dithionite.

Cells and crude extracts of Clostridium pasteurianum, W-5, were prepared as described by Carnahan et al[8]. Extracts were used within five hours of preparation. The ferredoxin as well as the ferredoxin-free extract containing hydrogenase, phosphoroclastic enzymes, TPN-reductase and other enzymes was prepared as described by Mortenson et al[4], and Valentine et al[5]. The specific activity of the ferredoxin was 24-30 units per mg of protein[4]. PPNR was purified from spinach as described by San Pietro[2] through the protamine sulfate step and then by chromatography

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on DEAE-cellulose according to Hill and Bendall[9]. The preparation was red-brown in color, had a specific activity of 35 units per mg of protein[2] (ultraviolet absorption procedure)[10], and was homogeneous as tested by ultracentrifugation and electrophoresis. The most purified preparation of ferredoxin obtained by Mortenson et al[4] had a specific activity of 120. Assuming this to be a pure preparation, the ferredoxin used here would be approximately 25 per cent pure. Since the method of assay and assay units for PPNR and for ferredoxin are different, the values expressed below have more qualitative than quantitative significance.

Protein in crude extracts was determined by the biuret assay[11]; protein in ferredoxin preparations was determined by the Lowry method[12]. Acetyl phosphate was measured as acethydroxamic acid[13]. Ammonia was determined by the Conway diffusion method[14].

RESULTS

A comparison of the activity of spinach PPNR and ferredoxin in the cleavage of pyruvate (reactions a and b) using a resolved extract of Clostridium pasteurianum is shown in Table I.

TABLE I

Comparison of PPNR and ferredoxin in the cleavage of pyruvate by Clostridium pasteurianum

a) $\text{pyruvate} + \text{Pi} + \text{Ferredoxin (OX)} \xrightarrow[\text{CoA, DPT}]{\text{Pyruvic Dehydrogenase}} \text{Acetyl}\sim\text{P} + \text{Ferredoxin (RED)} + CO_2$

b) $\text{Ferredoxin (RED)} \overset{\text{Hydrogenase}}{\rightleftarrows} \text{Ferredoxin (OX)} + H_2$

Electron Carrier Added	Acetyl Phosphate Formed (μmoles)
None	0.1
PPNR (0.4 mg)	1.7
Methyl Viologen (0.5 μmole)	6.5
Ferredoxin (0.5 mg)	8.5

Each test tube contained, in 1 ml, 50 μmoles potassium phosphate buffer at pH 6.5; 0.12 μmole coenzyme A; 100 μmoles sodium pyruvate, and 5.4 mg of ferredoxin-free extract. Incubation was at 31° for 20 minutes.

We have confirmed the observation of Tagawa and Arnon[7] that PPNR will mediate electrons from dithionite (reactions c and b).

c) Ferredoxin (OX) $\xrightarrow{Na_2S_2O_4}$ Ferredoxin (RED)

Several electron carriers were tested for activity in the H_2-TPN reactions of C. pasteurianum (reactions b and d).

d) Ferredoxin (RED) + TPN $\xrightarrow{\text{TPN Reductase}}$ TPNH + H^+ + Ferredoxin (OX)

Figure 1 shows that the rate of H_2 uptake was about 10 times as great with ferredoxin as with PPNR. Since only 0.25 mg of PPNR was used, ferredoxin was 5 times more effective than PPNR on a weight basis. Neither ferredoxin nor PPNR mediates the reduction of DPN from H_2 with an extract of C. pasteurianum.

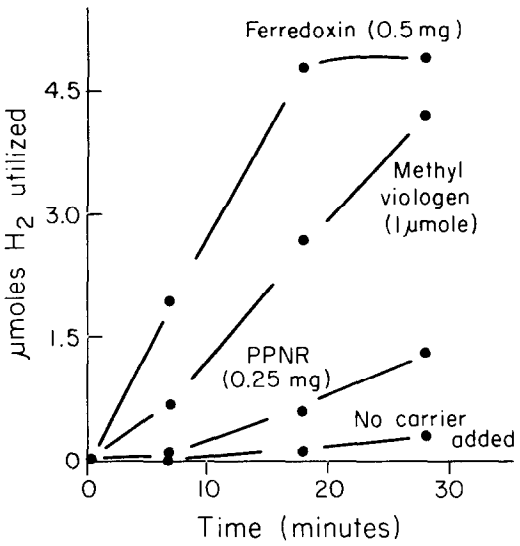


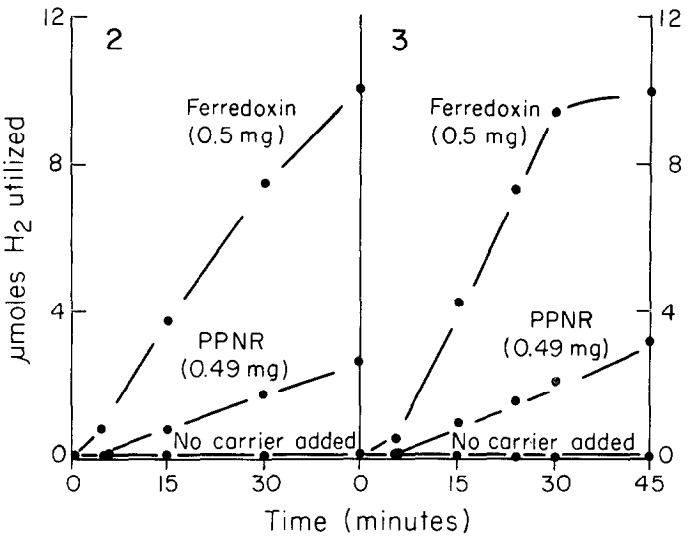
Figure 1. Comparison of PPNR and ferredoxin in the H_2-TPN reaction of Clostridium pasteurianum.
Each Warburg vessel contained, in 3 ml, 27 mg ferredoxin-free extract, 50 µmoles potassium phosphate buffer at pH 6.5, and 4 µmoles TPN. H_2 was the gas phase.

Both PPNR and ferredoxin function in the nitrite and hydroxylamine reductase systems of C. pasteurianum (reactions b, e, and f).

As shown in Figures 2 and 3, ferredoxin was 5 times more active than PPNR for nitrite and hydroxylamine reduction to ammonia with H_2

e) Ferredoxin (RED) + Nitrite $\xrightarrow{\text{Nitrite Reductase}}$ Ammonia + Ferredoxin (OX)

f) Ferredoxin (RED) + Hydroxylamine $\xrightarrow{\text{Hydroxylamine Reductase}}$ Ammonia + Ferredoxin (OX)



Figures 2 and 3. Nitrite (2) and hydroxylamine (3) reduction with H_2 by Clostridium pasteurianum.
Each Warburg vessel contained, in 3 ml, 10 μmoles sodium nitrite or 10 μmoles neutral hydroxylamine, 100 μmoles potassium phosphate buffer at pH 6.5, and 7.5 mg ferredoxin-free preparation containing hydrogenase and other enzymes. H_2 was the gas phase and incubation was at 31°.

The finding that PPNR substitutes for ferredoxin in the nitrite and hydroxylamine reductase systems of Clostridium pasteurianum focuses attention on the recent work of Huzisige and Satoh[15]. "Photosynthetic nitrite reductase" which they isolated may be similar to PPNR, and nitrite reduction by spinach could occur by a mechanism similar to that of the clostridia (reaction e), reducing power being generated during the light reaction.

The effectiveness of PPNR and ferredoxin as catalysts (or carriers) for the reactions tested should be compared on a molar basis rather than on a weight basis. If one assumes both

preparations are pure and corrects for their reported molecular weights, namely 17,000 for PPNR[3] and ca. 12,000 for ferredoxin[7] one would conclude that PPNR is approximately 30 per cent as effective as ferredoxin in these clostridial systems. Since ferredoxin at ca. 30 units per mg protein was used in the present experiments and calculations, and 120-unit material has been prepared[4], the 30 per cent effectiveness for PPNR on a molar basis is a maximal figure which would correct to less than 10 per cent, if 120-unit ferredoxin is pure. Tagawa and Arnon[7] have reported PPNR to be only ca. 10 per cent as effective as ferredoxin in the TPN-reducing, chloroplast, light system.

In view of the many differences in properties, it is interesting that PPNR can substitute for ferredoxin in several systems. The two preparations differ in: molecular weight[3,7]; spectral properties[1,7]; iron content[4,7,16]; and oxidized-minus-reduced difference spectra[7,17]. An analogy to the variation in properties of cytochromes may be pertinent; PPNR and ferredoxin being two examples of a new class of non-heme, iron-protein electron carriers.

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