FERREDOXIN LINKED DPN REDUCTION BY THE PHOTOSYNTHETIC BACTERIA CHROMATIUM AND CHLOROBIUM*

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Received August 24, 1965

Extracts of the photosynthetic bacteria Chromatium and Chlorobium have been found to catalyze the ferredoxin (Fd) dependent reduction of DPN. In Chromatium molecular hydrogen is a suitable reductant and the H_2-DPN reaction is probably of major importance to the cell when molecular hydrogen is available as electron donor in place of H_2S. Using extracts of Chlorobium H_2 was a suitable reductant only if a small amount of clostridial hydrogenase was added to insure a supply of reduced ferredoxin. It is interesting to speculate that in both of these organisms electrons from H_2 flow directly into the major branch of the "photosynthetic" electron transport chain terminating with DPN reduction. The H_2-DPN system would then share with the "photosynthetic" chain the final enzyme of the series which we have tentatively called Fd-DPN reductase. This enzyme couples Fd with DPN. Fig. 1 summarizes these ideas and served as a working hypothesis for the present experiments.

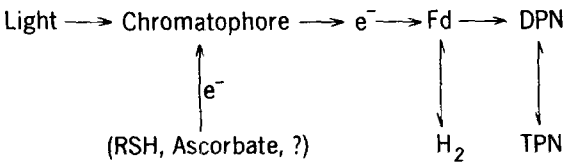

Fig. 1. Hypothetical electron flow pattern of photosynthetic bacteria leading to pyridine nucleotide reduction. See introduction and summary for discussion.

* Supported by grants from the National Science Foundation.

For completeness in Fig. 1 we have included the general "light" reactions which may take place in Chromatium or Chlorobium extracts; the present work is concerned mainly with the mechanism of the Fd-DPN reaction, a "dark" reaction.

Results and Experimental

Chromatium strain D was grown in 10 l carboys as described by Arnon et al., 1963. Extracts were prepared daily by crushing 15 g freshly harvested cells in the Hughes Press. The extract, in 12 ml of 0.01 M mercaptoethanol and 0.05 M potassium phosphate, pH 7.2, containing 5 μg/ml DNase to digest DNA, was clarified by centrifugation at 14,000 x g for 10 min. Extracts were stored under H_2 at 0°C. Frozen extracts lost about 25 per cent of their activity in 24 hr.

Chlorobium thiosulfatophilum was grown as described by Evans and Buchanan, 1965, and fresh extracts were prepared daily as above.

Crude hydrogenase and highly purified ferredoxin from Clostridium pasteurianum were prepared as described earlier (Mortenson et al., 1962).

The H_2-DPN reaction was carried out in anaerobic cuvettes stoppered with serum caps and flushed with H_2 for 10 min through a needle in the cap. A typical reaction vessel contained 3.8 μmoles DPN (or TPN), 100 μmoles potassium phosphate buffer at pH 7.2 and usually 80 μg ferredoxin. Mercaptoethanol (30 μmoles) was added in an attempt to reduce the initial "lag" period for DPN reduction often observed with different extracts; for certain aged extracts the lag was as long as 20 min. Final volume was 3.0 ml and incubation was at 24°C. The reaction was initiated by addition of enzyme with a syringe through the serum cap and DPNH synthesis was measured using a Zeiss spectrophotometer. A reaction vessel flushed with N_2 in place of H_2 served as control.

The first experiments were designed to test the pyridine nucleotide specificity with hydrogen gas as reductant using Chromatium extracts. Fig. 2A shows that crude extracts catalyze DPN reduction more than 3

times the rate of TPN reduction. This suggests that DPN is the primary reductant from H_2 and that TPN may be reduced in a secondary reaction. Note also in Fig. 2A that a small amount of DPN "sparked" the reduction of TPN implying the presence of pyridine nucleotide transhydrogenase in the extracts.

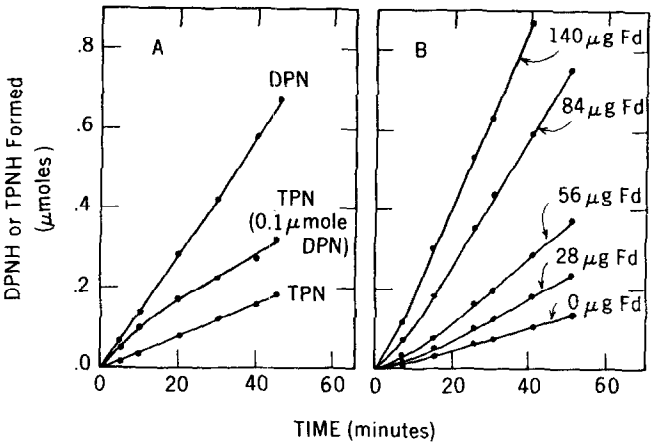

Fig. 2A. Pyridine nucleotide specificity with H_2 gas as reductant in extracts of Chromatium. Each vessel contained 3.8 μmoles DPN or TPN except one vessel which contained 3.8 μmoles TPN and 0.1 μmole DPN; other conditions as in text. Enzyme was 8 mg protein of crude extract dialized for 5 hr against 0.001 M mercaptoethanol to remove pyridine nucleotides.

Fig. 2B. Ferredoxin requirement for DPN reduction by Chromatium. Assay conditions as in text. Highly purified clostridial ferredoxin was used for this experiment. Enzyme was 5.0 mg protein of 100,000 x g supernatant (see below) freed of ferredoxin by passage through DEAE-cellulose column.

Next, extracts were treated with DEAE-cellulose to test for the requirement of ferredoxin in the H_2-DPN reaction of Chromatium. As shown in Fig. 2B, passage of extracts through a DEAE-column markedly reduced their ability to carry out the H_2-DPN reaction. This activity was restored by ferredoxin as seen in Fig. 2B. Both clostridial and chromatium ferredoxins were found to be active.

It was of interest to test the association of the H_2-DPN enzyme system with the particulate chromatophore fraction of Chromatium. For

this experiment crude extracts were sedimented at 100,000 x g for 90 min in the Spinco centrifuge. The pigmented chromatophore fraction sedimenting at the bottom of the tube and the clear amber colored supernatant remaining at the top of the tube after centrifugation were assayed for the H_2-DPN reaction. As summarized in Fig. 3 after sedimentation, the H_2-DPN system remained in the 100,000 x g supernatant and was readily separated from the pigmented chromatophore fraction, which contained only a small amount of activity.

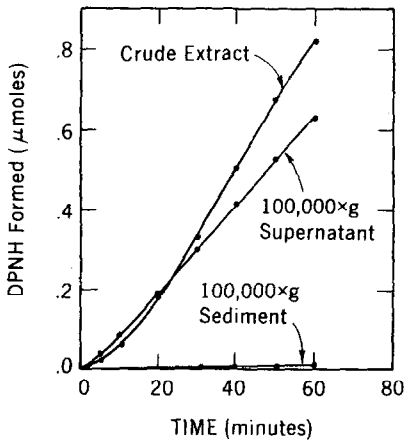

Fig. 3. Localization of the H_2-DPN reaction in the 100,000 x g supernatant of Chromatium. Standard assay; enzyme 5.0 mg protein of 100,000 x g supernatant and 3.9 mg protein chromatophore fraction added as indicated. Chromatophore fraction resuspended in original volume with 0.001 M mercaptoethanol after sedimentation.

In an attempt to generalize our finding to other photosynthetic bacteria, extracts of the green sulfur bacterium Chlorobium thiosulfatophilum were prepared and assayed for the H_2-DPN system as above. Several freshly prepared extracts were found to be inactive for this reaction; in these experiments it was also observed that the Chlorobium extracts possessed very low levels of hydrogenase activity and thus may have lacked the ability to generate reduced ferredoxin needed for DPN reduction.

Addition of a small quantity of clostridial hydrogenase for generation of reduced ferredoxin was found to markedly accelerate DPN reduction in Chlorobium (Fig. 4A). Fig. 4B shows the requirement for ferredoxin in the reaction.

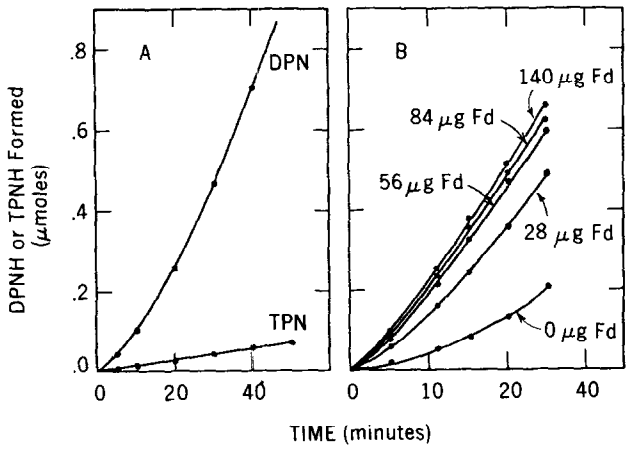

Fig. 4A. Specificity of the pyridine nucleotide reductase system of Chlorobium for DPN. Chlorobium extract (4.4 mg protein) was sedimented at 100,000 x g for 90 min and dialized for 4 hr against 0.001 M mercaptoethanol to remove pyridine nucleotides. Clostridial extract (2.1 mg protein) dialized as above. Assay conditions as in text.

Fig. 4B. Ferredoxin requirement for DPN reduction by Chlorobium. Chlorobium extract 4.4 mg protein and clostridial extract 3.0 mg protein freed of ferredoxin by treatment with DEAE-cellulose. Assay conditions as for Fig. 4A.

It should be pointed out that fresh clostridial extracts normally rapidly reduce TPN; the reaction appears to be highly specific for TPN since DPN is not reduced under any of the experimental conditions tested even when small amounts of TPN are added along with DPN to take advantage of any pyridine nucleotide transhydrogenase in the extract. We have concluded earlier (Valentine et al., 1962) that transhydrogenase was absent or not detectable in the strain of Clostridium pasteurianum used. However, the small quantity of clostridial extract used for Fig. 4A catalyzed only a very slow reduction of TPN alone and showed no

reduction of DPN. It thus appeared that the Chlorobium extract possessed a Fd-DPN reductase system directly coupling reduced ferredoxin with DPN and that DPN reduction via TPN and Chlorobium transhydrogenase probably played only a minor role in the extracts. These questions may be more fully answered when more highly purified enzyme preparations become available.

Discussion and Summary

The major products of bacterial photosynthesis are thought to be ATP and reducing power in the form of reduced pyridine nucleotides (for general reference see "Bacterial Photosynthesis", Gest, San Pietro, and Vernon, 1963). We have speculated earlier that the terminal reactions of pyridine nucleotide reduction by photosynthetic bacteria might be similar to the systems found in clostridia (Valentine, Brill, and Sagers, 1963). The experiments presented here tend to confirm this notion. Taking the findings with clostridia into account we have proposed the scheme of Fig. 1 as a working model for "noncyclic" electron flow in photosynthetic bacteria. The major feature of the scheme is that electrons activated during photochemical events are passed to Fd which in turn couples with DPN reductase to generate DPNH. Major support for the overall scheme comes from recent experiments by Evans and Buchanan (1965) who have presented evidence for the photochemical reduction of ferredoxin by a particulate (chromatophore) fraction of Chlorobium. Fd reduction was measured in a couple reaction with the Fd-linked pyruvate synthetase of Chlorobium. This interesting experiment represents the first example of photochemical Fd reduction by photosynthetic bacteria and is an important argument for the scheme of Fig. 1. A further clue to the mechanism of DPN reduction by Chromatium was furnished by Buchanan et al. (1964) who mentioned that extracts of Chromatium catalyzed the Fd-dependent reduction of DPN with H_2. We have studied the H_2-DPN

reaction of Chromatium in more detail and have found that the reaction is Fd dependent. The rate of DPN reduction using dialyzed extracts was 3 to 4 times that of TPN indicating that DPN was the primary electron acceptor. Extracts of the green sulfur bacterium, Chlorobium posssessed a Fd-DPN system which could be assayed only when clostridial hydrogenase was added to supply reduced Fd. The name Fd-DPN reductase is proposed for the enzyme coupling reduced Fd with DPN in these extracts. It is interesting to speculate that this enzyme plays a key role in photochemical DPN reduction.

Acknowledgment

We are grateful to Dr. M. Evans, Dr. N. Pfennig and Miss R. Kunisawa for kindly supplying cultures and for advice on culturing the photosynthetic bacteria. Mr. David Epstein kindly supplied ferredoxin.

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