JOURNAL OF BACTERIOLOGY, Feb. 1975, p. 743-745

Vol. 121, No. 2
Printed in U.S.A.

Comparison of Two Ferredoxins from *Rhodospirillum rubrum* as Electron Carriers for the Native Nitrogenase

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Received for publication 15 October 1974

In coupling the reducing power of illuminated chloroplasts to the nitrogenase from photosynthetically grown *Rhodospirillum rubrum* cells, one of the native ferredoxins, FdI, was found to be three times more effective than FdII.

Shanmugam et al. (6) discovered that the photosynthetic bacterium *Rhodospirillum rubrum* produces two types of ferredoxin. One (FdI) is formed only when the cells are grown photosynthetically in the light, and the other (FdII) is formed when the cells are grown either in the light or (heterotrophically) in the dark. This discovery was confirmed and extended by Yoch, Sweeney, and Arnon (submitted for publication) who found that FdI was an 8 Fe-8 S ferredoxin and FdII was a 4 Fe-4 S ferredoxin, with molecular weights of about 9,000 and 15,000, respectively.

Since FdI was found to be formed only in the light, it seemed likely that it participated in reactions that were directly or indirectly linked to photosynthesis. When this possibility was tested with respect to cyclic photophosphorylation, FdI and FdII were found to be about equally effective in restoring this light-dependent reaction of *R. rubrum* chromatophores (5). We have now found, however, that FdI is greatly superior to FdII in promoting the activity of nitrogenase isolated from photosynthetically grown *R. rubrum* cells. Only photosynthetically grown *R. rubrum* cells have a capacity for nitrogen fixation, a process that requires two products that these cells form at the expense of light energy—reduced ferredoxin and adenosine 5'-triphosphate.

A high level of nitrogenase activity (measured by acetylene reduction [3]) was induced in *R. rubrum* cells by growing them in a nutrient medium containing a limiting concentration (5 mM) of NH_4^+. The exhaustion of this supply of NH_4^+ activated the synthesis of nitrogenase in the cells (see ref. 1, 4, 2). The synthesis of nitrogenase was accompanied by a light-dependent evolution of H_2.

Extracts with nitrogenase activity were prepared from cells suspended in an equal volume of 0.33 M Tricine buffer, pH 8.0 (containing 1 mg of deoxyribonuclease), and disrupted in a Ribi cell fractionator. The high buffer concentration was used to prevent a drastic drop in the pH of the disrupted cells—a pH drop which, if allowed to occur, resulted in loss of nitrogenase activity. The disrupted cells were centri-

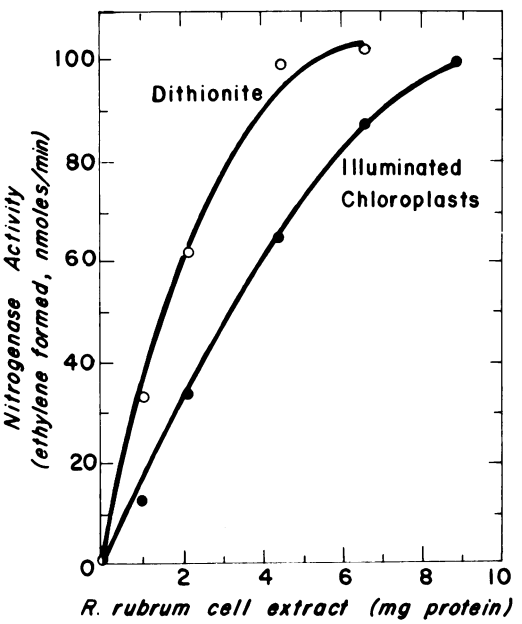

FIG. 1. *A comparison of dithionite and illuminated chloroplasts as sources of reducing power for nitrogenase activity in extracts of R. rubrum. Nitrogenase activity was measured as acetylene reduction. The reaction mixture (final volume, 1.5 ml) contained (micromoles): N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid buffer (pH 7.4), 50; $MgCl_2$, 5; creatine phosphate, 40; adenosine 5'-triphosphate, 4; and creatine phosphokinase, approximately 5 μg. sodium dithionite (10 μmol) or illuminated spinach chloroplasts (300 μg of chlorophyll) supplemented with ascorbate (10 μmol) and 2,6-dichlorophenol indophenol (0.05 μmol were used to supply reducing power (see ref. 7). Conditions: light intensity, 9000 ft.-can.; gas phase, 73% argon and 27% acetylene; temperature, 31 C; reaction time, 20 min.*

fuged at 270,000 × *g* for 1 h, and the residue containing cell debris and chromatophores was discarded.

The supernatant extract showed good nitrogenase activity when the reducing power needed for nitrogenase activity was supplied either by sodium dithionite or by illuminated chloroplasts, which were linked to the nitrogenase (7) through endogenous electron carriers present in the extract (Fig. 1). An indication that the endogenous electron carriers included the ferredoxins of *R. rubrum* was obtained by passing the extract through a diethylaminoethyl-cellulose column, a treatment that removed ferredoxins. An extract so treated no longer showed any nitrogenase activity when combined with illuminated spinach chloroplasts. Activity was restored, however, by eluting (with 0.8 M NaCl contained in 20 mM phosphate buffer, pH 7.3) the adsorbed "electron carrier fraction" from the diethylaminoethyl-cellulose column and adding aliquots of the eluate to the reaction mixture (Fig. 2). The restored nitrogenase activity was proportional to the amount of added electron carrier fraction.

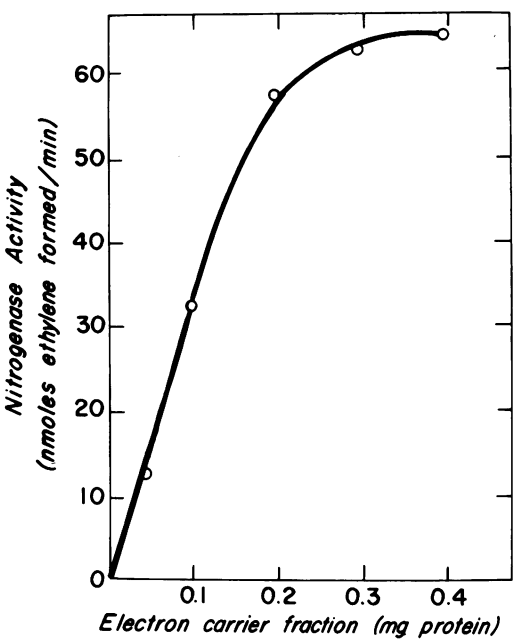

FIG. 2. *Nitrogenase activity as a function of protein concentration of the diethylaminoethyl-cellulose eluate from an R. rubrum cell extract. The eluate contained the electron carrier proteins adsorbed on diethylaminoethyl-cellulose. The reaction mixture (final volume, 1.5 ml) was that described for Fig. 1 with illuminated chloroplasts as the source of reducing power with the following exception. The source of nitrogenase was a sample (4.4 mg of protein) of a cell extract that had been passed over a diethylaminoethyl-cellulose column to remove the electron carriers. The crude electron carrier fraction eluted from diethylaminoethyl-cellulose with buffer containing 0.8 M NaCl was added as indicated. Other conditions were also as in Fig. 1.*

The two *R. rubrum* ferredoxins were purified from the eluate by methods essentially as described previously (6). The relative effectiveness of FdI and FdII in supporting the *R. rubrum* nitrogenase system is shown in Fig. 3. The 8 Fe-8 S ferredoxin (FdI) was about three times as effective in the nitrogenase reaction as was the 4 Fe-4 S ferredoxin (FdII).

Since both ferredoxins were found to be reduced at nearly equal rates by illuminated chloroplasts (Yoch et al., submitted for publication), FdI, found only in light-grown cells, appears to be the preferential electron carrier for nitrogenase, an enzyme also found only in light-grown *R. rubrum* cells. However, the role of FdI seems not to be limited to the nitrogenase system. Photosynthetically grown *R. rubrum* cells supplied with sufficient amonium nitrogen, and therefore not engaged in nitrogen fixation (4), also contain FdI.

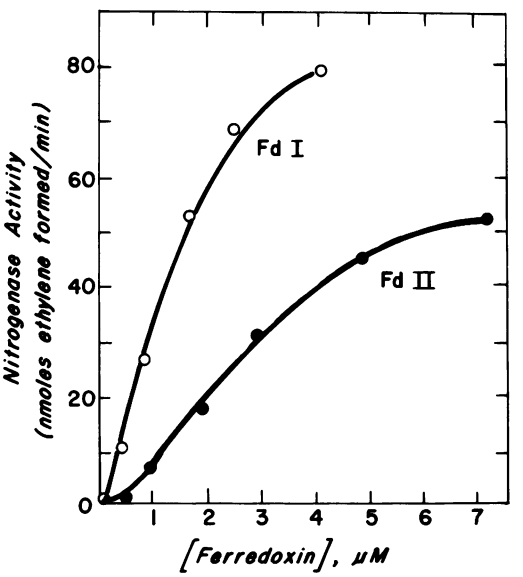

FIG. 3. *A comparison of R. rubrum FdI and FdII as electron carriers in coupling the reducing power generated by illuminated chloroplasts to R. rubrum nitrogenase. Reaction mixture and conditions of assay were as described in the legend to Fig. 2 with the exception that FdI and FdII served as the electron carriers to nitrogenase. The extinction coefficients used to calculate the concentration of FdI and FdII were 30 and 17.0 mM^{-1}/cm, respectively (Yoch, Sweeney, and Arnon, submitted for publication).*

TABLE 1. *Effectiveness of different ferredoxins in mediating electron transport between illuminated chloroplasts and R. rubrum nitrogenase*[a]

Electron carrier	Conc (μM)	Nitrogenase activity (ethylene formed, nmol/min)
None		0.0
R. rubrum FdI	2.0	70.0
R. rubrum FdII	2.0	22.3
C. pasteurianum Fd	2.0	58.5
Spinach ferredoxin	6.3	4.6

[a] Except for the electron carriers at the concentrations shown, the reaction mixture (containing 4.3 mg of protein of cell extract) and conditions of assay were as described in the legend to Fig. 2.

A comparison of the effectiveness of different ferredoxins in coupling the reducing power generated by illuminated chloroplasts to *R. rubrum* nitrogenase activity is shown in Table 1. Only *Clostridium pasteurianum* ferredoxin, an 8 Fe-8 S protein, approached the effectiveness of *R. rubrum* FdI, also an 8 Fe-8 S ferredoxin. The 4 Fe-4 S *R. rubrum* FdII was less than half as effective and the 2 Fe-2 S spinach ferredoxin was almost wholly ineffective.

We thank John R. Benemann for his advice concerning preparation of the nitrogenase extract.

This investigation was aided by National Science Foundation grant GB-30494X.

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