INTERACTION BETWEEN FERREDOXIN AND FERREDOXIN-NADP REDUCTASE FROM CHLOROPLASTS

Nathan Nelson and Joseph Neumann
Department of Botany, Tel-Aviv University
Tel Aviv, Israel

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The photochemical reduction of pyridine nucleotides in isolated chloroplasts was shown to proceed via ferredoxin and ferredoxin-NADP reductase (Shin et al., 1963). The latter enzyme is a flavoprotein which can also mediate the transfer of electrons in reverse, from NADPH to ferredoxin (Lazzarini and San Pietro, 1962), or to other acceptors such as: dyes (Avron and Jagendorf, 1956), NAD (Keister et al., 1960) and chloroplast cytochrome f (Zanetti and Forti, 1966).

This communication reports observations indicating that ferredoxin inhibits several activities of the flavoprotein and forms a complex with it.

MATERIALS AND METHODS

Ferredoxin was isolated from swiss chard leaves and purified according to the procedure of Böger et al. (1966). The purest fraction had a ratio of absorbance at 330 mμ to 277 mμ of 0.60. Ferredoxin-NADP reductase was isolated from swiss chard of lettuce chloroplasts, following the method of Shin et al. (1963). The purified enzyme had a ratio of absorbance at 456 mμ to 275 mμ of 0.085. Protein determinations were performed according to Lowry et al. (1951). The concentrations of the purified proteins were calculated on the basis of the following reported molar extinction coefficients: 8,240 at 465 mμ for ferredoxin (Keresztes-Nagy and Margoliash, 1966) and 10,740 at 456 mμ

for the flavoprotein. Glucose 6-phosphate dehydrogenase was purchased from Bohringer. All other reagents were of analytical grade.

RESULTS AND DISCUSSION

Figure 1 illustrates that ferredoxin inhibited the diaphorase activity of the flavoprotein, namely the transfer of electrons from NADPH to ferricyanide. At 2.5 μM NADPH, ferredoxin inhibited the rate of the reaction by 50%. This inhibition was not due to a ferredoxin

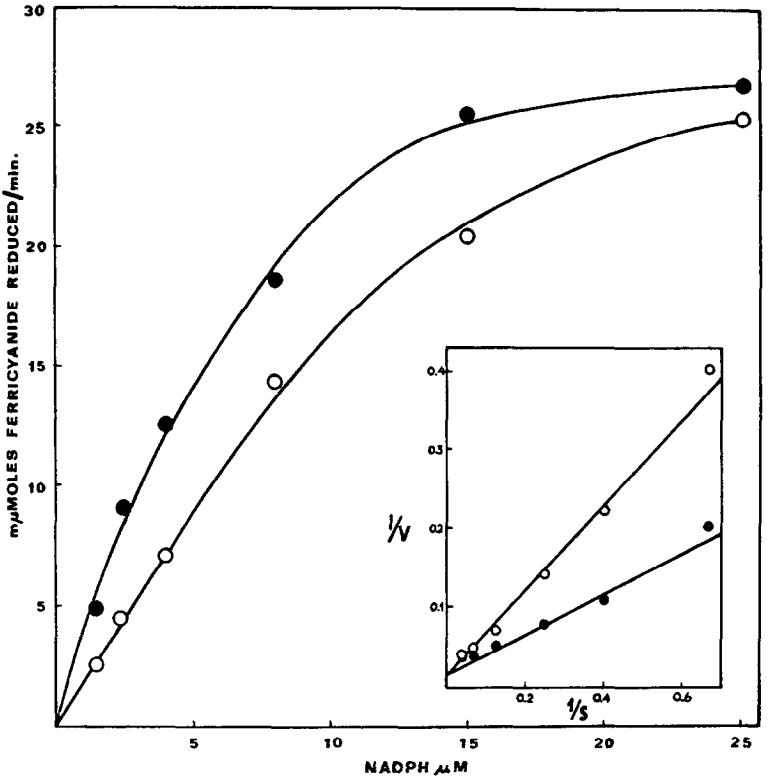

Figure 1. Effect of ferredoxin on the diaphorase activity. The reaction mixture contained in μmoles: buffer "Tris" 50, at pH 8.0; potassium ferricyanide 0.35; glucose 6-phosphate 5; ferredoxin 0.0072 where indicated; various concentrations of NADP, 0.7 units of glucose 6-phosphate dehydrogenase and 0.47 μg of the purified flavoprotein in a total volume of 1.0 ml. The incubation was carried on for 5 minutes, at room temperature. Ferricyanide reduction was assayed by measuring the decrease in O.D. at 420 mμ. Full circles, control; open circles, plus ferredoxin.

catalysed electron transfer from NADPH to oxygen, since such an activity was found to be negligible. Also, the inhibition of the diaphorase by ferredoxin could be visualized by measuring the rate of disappearance of either ferricyanide or NADPH. The activity of glucose 6-phosphate dehydrogenase, which was used in the regeneration of NADPH, was not affected by ferredoxin. As might be seen from the reverse plot of

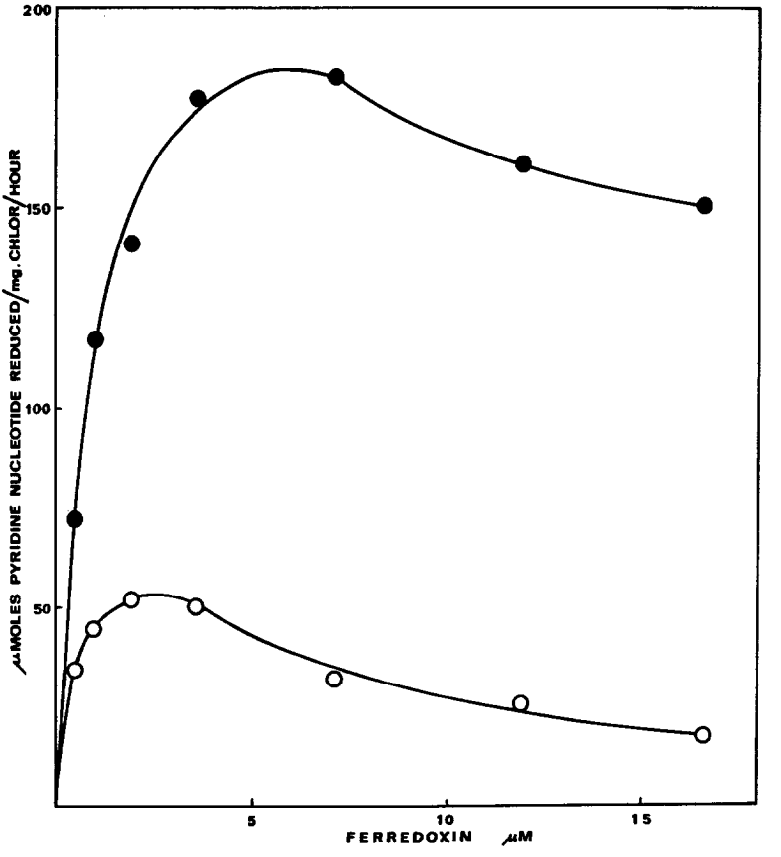

Figure 2. Effect of ferredoxin on photoreduction of pyridine nucleotides by chloroplasts.
Lettuce chloroplasts were isolated as reported (Neumann and Drechsler, 1967) and once washed in "Tris" 0.001 M, pH 8.0. The reaction mixture contained in μmoles "Tris" 20, pH 8.0; NaCl 35; NADP 0.25 or NAD 2.5 and chloroplasts equivalent to 6 μg chlorophyll in a total volume of 1.0 ml. The reaction mixture was illuminated by a 150-Watt bulb shielded by a water bath providing 3500 ft-c at the level of the test tubes. Pyridine nucleotide reduction was measured by increase in OD at 340 mμ. Full circles, NADP reduction; open circles, NAD reduction.

figure 1, the inhibition was competitive with NADPH. The calculated apparent K_m and K_i were 10 μM and 6 μM, respectively.

Denaturation of ferredoxin and removal of its iron by heat or by o-phenanthroline abolished its inhibitory activity.

Ferredoxin was also found to inhibit the transhydrogenase activity. In this reaction, evidence was obtained that ferredoxin inhibits both at the site where hydrogens are donated and at the site where hydrogens are accepted. Assuming that the site where NAD is reduced during the transhydrogenase reaction is identical with the site where pyridine nucleotides are photoreduced in the Hill reaction, the latter reaction was re-investigated.

Figure 2 describes the effect of various ferredoxin concentrations on the rate of photoreduction of pyridine nucleotides by chloroplasts. In agreement with well-documented data, this reaction depends on the addition of ferredoxin. However, as seen in this figure, high concentrations of ferredoxin are inhibitory. It should be noted that the inhibition of NAD reduction sets in at a lower concentration of ferredoxin than that of NADP reduction. Consequently by using proper ferredoxin concentrations, high rates (up to 60 μmoles per mg chlorophyll per hour) of NAD reduction can be achieved. These results are at variance with the accepted view that the rate of NAD photoreduction is negligible in comparison with that of NADP. The latter data represent only the case where high ferredoxin concentrations are added.

The fact that ferredoxin inhibits the electron transfer reactions mediated by the flavoprotein suggests that a complex between the two proteins was formed. Spectrophotometric measurements indicate that such a complex does indeed exist. Figure 3 presents the difference spectrum obtained by measuring the difference in absorption between ferredoxin and ferredoxin-NADP reductase mixed in the same cuvette in a molar ratio approximately of two to one, and the two enzymes added

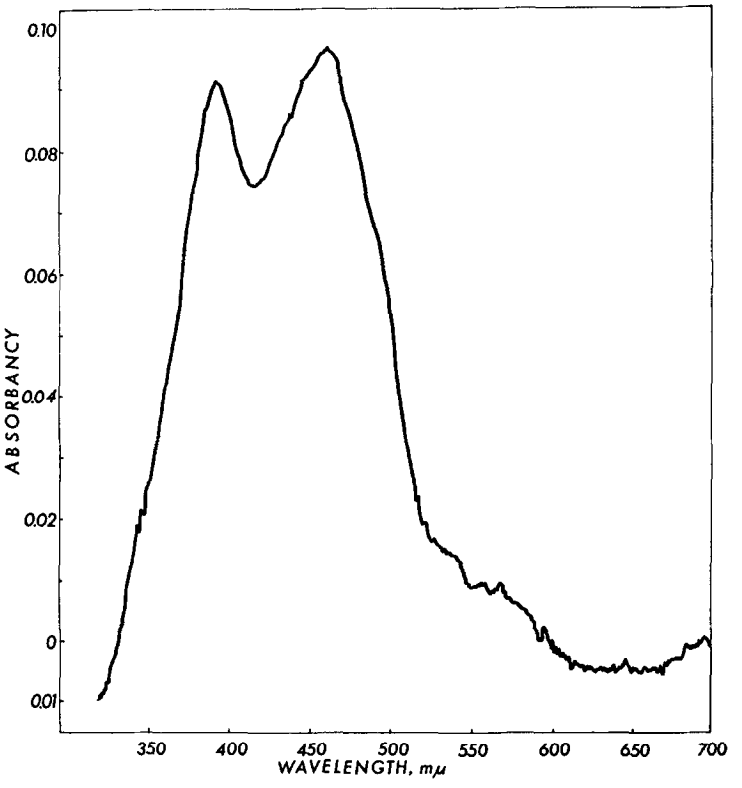

Figure 3. Difference spectrum obtained by mixing ferredoxin and ferredoxin-NADP reductase.
The mixture contained 95 mμmoles of ferredoxin and 57 mμmoles of the flavoprotein in 0.05 M "Tris" pH 8.0, 0.08 M NaCl. In the reference cell two cuvettes were lined up, one behind the other, each containing one of the proteins at the same concentration as in the mixture. The difference spectrum was measured in a Cary-14 spectrophotometer.

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separately and serving as a blank. the difference spectrum has two conspicuous peaks at 395 and 465 mμ.

It could be postulated that the ferredoxin, ferredoxin-NADP reductase complex has a functional significance in electron transfer. Pertinent to this suggestion is the observation that in contrast to other flavoproteins which contain metals, including non-heme iron, the isolated ferredoxin-NADP reductase has not been shown to contain metals (Zanetti and Forti, 1966).

The detailed analysis of the mode of interaction between ferredoxin and the flavoprotein will be presented separately.

Acknowledgment

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