COMPLEX FORMATION OF FERREDOXIN-NADP REDUCTASE WITH FERREDOXIN AND WITH NADP*

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Ferredoxin-NADP reductase was first crystallized by Shin et al. (1963) and is a functional chloroplast enzyme participating in photosynthetic NADP reduction by illuminated chloroplasts (Keister et al., 1962). This enzyme catalyzes reversible electron transfer between ferredoxin and NADP (Shin and Arnon, 1965; Lazzarini and San Pietro, 1962).

In this communication, it will be shown that ferredoxin-NADP reductase forms a complex with spinach ferredoxin and also with NADP. The interaction between ferredoxin and ferredoxin-NADP reductase has been reported by Foust and Massey (1967) and also by Nelson and Neumann recently (1968).

Ferredoxin-NADP reductase was prepared from spinach leaves by the method of Shin et al. (1963); spinach and clostridial ferredoxin by published methods (Tagawa and Arnon, 1962; San Pietro and Lang, 1958).

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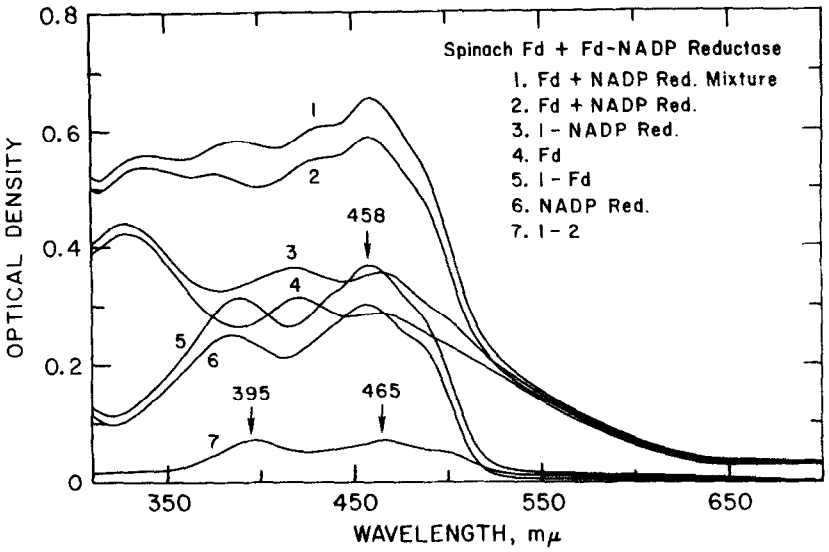


Figure 1- Formation of complex between ferredoxin and ferredoxin-NADP reductase. The proteins were dissolved in 0.05M Tris-HCl buffer, pH 7.5, at room temperature. Curve 1- Absorption spectrum of complex; Curve 2- Additive absorption spectrum of the individual proteins (curves 4 + 6); Curve 3- Difference spectrum of curve 1 minus curve 6; Curve 4- Absorption spectrum of ferredoxin; Curve 5- Difference spectrum of curve 1 minus curve 4; Curve 6- Absorption spectrum of ferredoxin-NADP reductase; Curve 7- Difference spectrum of curve 1 minus curve 2.

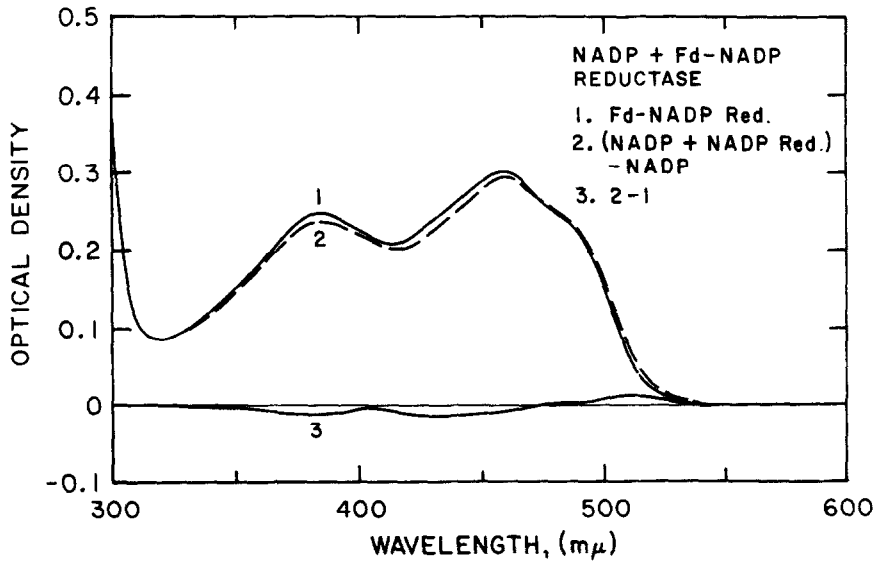
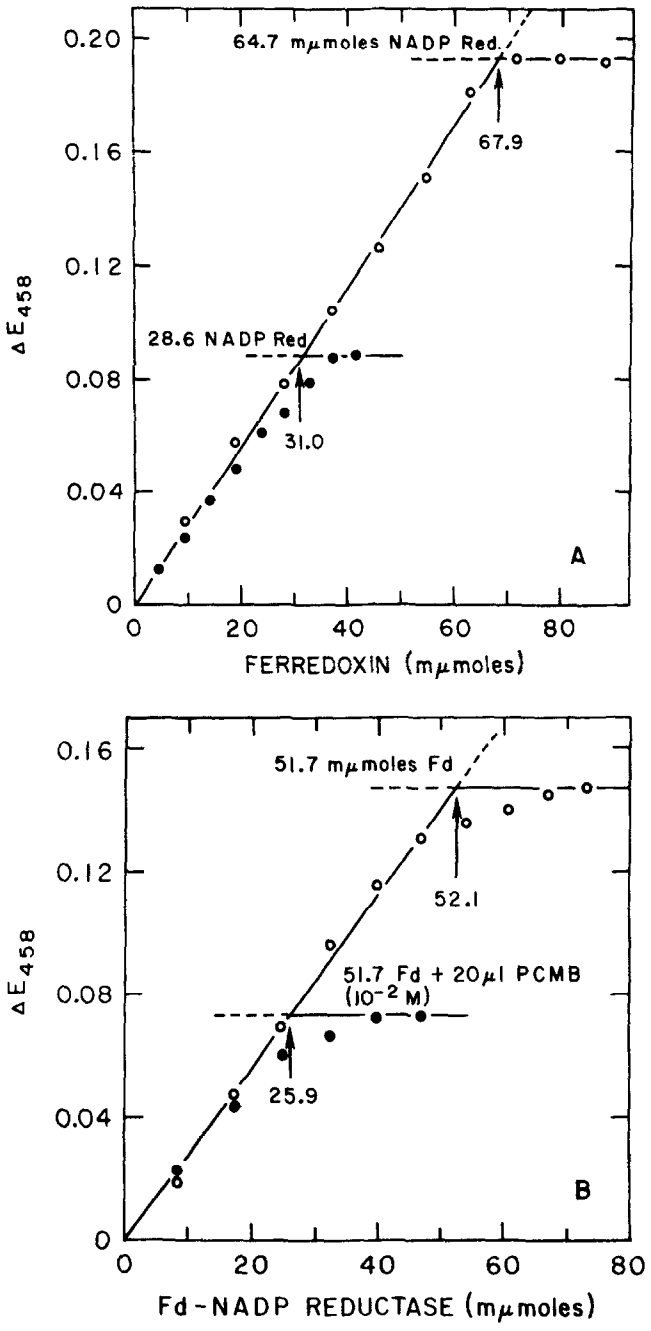


Figure 2- Formation of complex between NADP and ferredoxin-NADP reductase. The protein and NADP (1.19×10^{-3}M) were in 0.05M Tris-HCl buffer, pH 7.5. Curve 1- Absorption spectrum of ferredoxin-NADP reductase; Curve 2- Difference spectrum of the mixture of NADP and ferredoxin-NADP reductase minus NADP; Curve 3- Difference spectrum of curve 2 minus curve 1.

Complex formation was observed spectrophotometrically as shown in Figure 1. The difference spectrum of the ferredoxin-ferredoxin-NADP reductase complex (curve 7) was obtained by subtraction of the additive absorption of ferredoxin and ferredoxin-NADP reductase (curve 2) from the mixture of the two proteins (curve 1) using a Cary recording spectrophotometer, Model 14. Two reference cells each with 1 cm. light path were placed in series in the reference beam; one contained ferredoxin and the other contained ferredoxin-NADP reductase. In the sample compartment, two sample cells in series contained the mixture which was made by mixing an equal volume of each protein solution. The difference spectrum showed two peaks; one at 395 mμ and the other at 465 mμ. The complex formation seemed to be specific for spinach ferredoxin and spinach flavoprotein. When either clostridial ferredoxin was used in place of spinach ferredoxin, or glucose oxidase (from Penicillium; kindly supplied by Dr. K. Kusai, Nagase and Co. Ltd., Amagasaki, Japan) for ferredoxin-NADP reductase, no significant absorption change was observed. It should be noted that Foust and Massey have observed complex formation between clostridial ferredoxin and ferredoxin-NADP reductase in 0.03 M Tris, pH 7.5 and at 12°C (personal communication). Our inability to observe a similar complex was due probably to the higher buffer concentration (ionic strength) and temperature used in our experiments.

An interaction of ferredoxin-NADP reductase with NADP was also observed in the same manner (Fig. 2.). Complex formation between NADP and reductase is seen as absorption peaks at 380, 430 and 510 m in the difference spectrum (Fig. 2, curve 3).

The properties of complex formation with ferredoxin were investigated quantitatively by titration of ferredoxin-NADP reductase with a concentrated solution of ferredoxin and also by the



Figures 3A and 3B- Increase in absorbancy at 458 mμ due to formation of ferredoxin-ferredoxin-NADP reductase complex. Reaction mixture contained ferredoxin-NADP reductase in A, and ferredoxin in B, in the amount indicated and 50 μmoles of Tris-HCl buffer, pH 7.5, in 1 ml. Titration was carried out at room temperature. The amount of protein was calculated from the reported millimolar extinction coefficients, 10.740 at 456 mμ for ferredoxin-NADP reductase (Nelson and Neumann, 1968) and 9.68 at 420 mμ for ferredoxin (Tagawa and Arnon, 1968).

reverse titration. Complex formation could be followed by the increase in optical density at 458 mμ (see curve 4 in Fig. 1). The same amount of ferredoxin (or ferredoxin-NADP reductase) was added from a syringe microburet (Micro Metric Instrument Co., Model No. SB2) to both the reference and sample cells which contained 1 ml of 0.05 M Tris-HCl buffer, pH 7.5 and 1 ml of ferredoxin-NADP reductase (or ferredoxin) in the same buffer, respectively. The increase at 458 mμ was found to be a linear function of the amount of ferredoxin (or ferredoxin-NADP reductase) added. The data in Figure 3 illustrates this linear relationship and shows further that ferredoxin and ferredoxin-NADP reductase react in the molar ratio of one to one. This same stoichiometry was observed by Foust and Massey (1967). It was found further that PCMB inhibits complex formation. Ferredoxin reacts with 9 equivalents of PCMB (Fry and San Pietro, 1963); however, the ability for complex formation was completely lost when 8 equivalents of PCMB were added. In addition, PCMB treated ferredoxin-NADP reductase did not form a complex with native ferredoxin. The results of these experiments will be published elsewhere.

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