ANTIBODIES TO PLANT FERREDOXIN

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1. Introduction

Ferredoxin is a major electron transporting component in plants and in some bacterial species [1, 2]. Specific antibodies to ferredoxin can help in the elucidation of the sites of action of ferredoxin. Antibodies to ferredoxin from *Clostridia* species were prepared by Mitchell et al. [3–5] who studied also the antigenic specificity of the protein.

We report here the preparation of specific precipitable antibodies to Swiss chard ferredoxin. No cross reaction was detected with *Clostridial* ferredoxin. The plant ferredoxin antibodies are shown to specifically inhibit three different ferredoxin dependent reactions: NADP photoreduction, cytochrome *c* (cyt-*c*) photoreduction and the flavoprotein and ferredoxin catalysed reduction of cyt-*c* by NADPH. These antibodies were also shown to inhibit endogenous cyclic photophosphorylation.

2. Materials and methods

Ferredoxin and ferredoxin-NADP-reductase were isolated and purified from Swiss chard leaves essentially as described [6, 7]. *Clostridium* ferredoxin was kindly supplied by Dr. J. Neumann, of the Tel-Aviv University.

Six rabbits were immunized intradermally in multiple sites with the purified ferredoxin (1.5 mg per rabbit) emulsified in complete Freund's adjuvant. The immunization was repeated after an interval of ten days, using the same emulsified materials. Sera were collected weekly, starting a week after the second immunization.

Precipitation reactions were carried out as described earlier [8]. The immunoglobulin fraction (Ig) of immune and normal serum were fractionated by repeated precipitation in ammonium sulfate at 40% saturation. The precipitated immunoglobulins were dissolved in 0.01 M sodium phosphate pH 7.4, 0.15 M NaCl, and dialyzed against the same solution.

Lettuce chloroplasts were prepared as described previously [9].

Spectrophotometric measurements were performed in a Cary 14 spectrophotometer. NADP reduction was followed at 350 nm and cytochrome *c* reduction at 550 nm. Samples were illuminated by a 500 W slide projector through a Schott RG 645 filter.

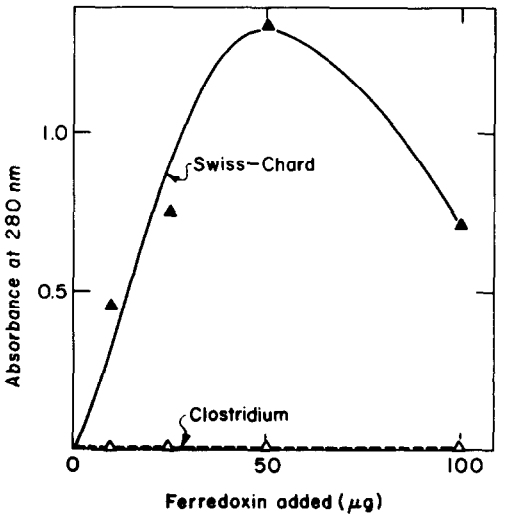

Fig. 1. Precipitation reactions of ferredoxin specific immunoglobulins (12 mg) with increasing amounts of Swiss chard or *Clostridium* ferredoxin. Details as described under Methods.

3. Results and discussion

All six immunized rabbits responded to ferredoxin by the production of precipitable antibodies (0.1–1 mg precipitable antibodies/ml serum). A typical precipitation curve is shown in fig. 1. The figure also illustrates that *Clostridium* ferredoxin did not cross precipitate with these antibodies. Anti-ferredoxin antibodies neither cross precipitated with the flavoprotein ferredoxin-NADP-reductase (which may be present in minute amounts in the purified ferredoxin), nor did they inhibit the flavoprotein activity in catalyzing dichlorophenolindophenol reduction from NADPH.

Fig. 2a describes the effect of ferredoxin antibodies on the photoreduction of NADP via endogenous ferredoxin. The reaction was severely inhibited by the immunoglobulin fraction from immune sera, but not by immunoglobulin from normal rabbit serum.

The enzymic reduction of cyt-*c* by NADPH catalyzed by the flavoprotein together with ferredoxin was also inhibited only by the immunoglobulins from immune sera. It was apparent from several experiments that higher extents of inhibition could be achieved in the enzymic system than in the chloroplast system. This difference may be due to some residual ferredoxin, bound to the chloroplast membrane, which may be inaccessible to the antibodies.

Illuminated chloroplasts reduce cytochrome *c* [10]. This reduction is enhanced by added exogenous ferredoxin. As depicted in fig. 3a anti-ferredoxin immunoglobulin specifically inhibited the enhancement of the photoreduction of cyt-*c* due to the added ferredoxin. However, there was no specific effect on the same reaction in the absence of ferredoxin (fig. 3b). No

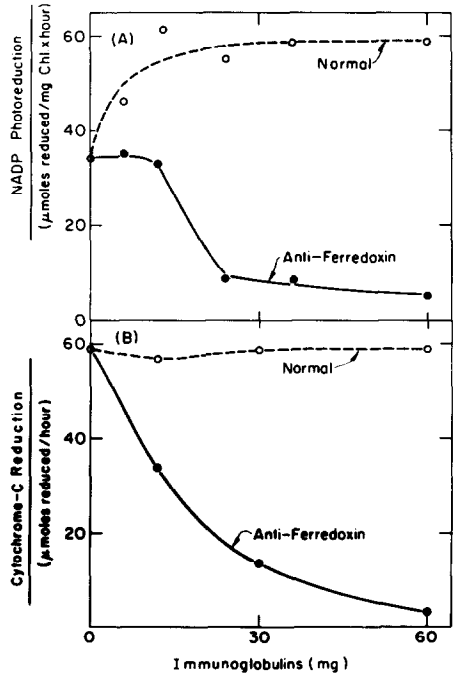

Fig. 2. Inhibition by ferredoxin specific immunoglobulin of NADP photoreduction and enzymic cyt-*c* reduction. Ferredoxin samples (45 μg) were incubated for 5 min at room temp. with the indicated amounts of immunoglobulin solution (60 mg/ml). They were then adjusted to a total volume of 3 ml containing: (a) Tricine-maleate, pH 7.5 (25 mM), KCl (20 mM), NADP (0.33 mM) and lettuce chloroplasts containing 31 μg of chlorophyll. (b) Tricine-maleate, pH 7.5 (25 mM), KCl (20 mM), cyt-*c* (0.033 mM) and saturating amounts of ferredoxin-NADP-reductase.

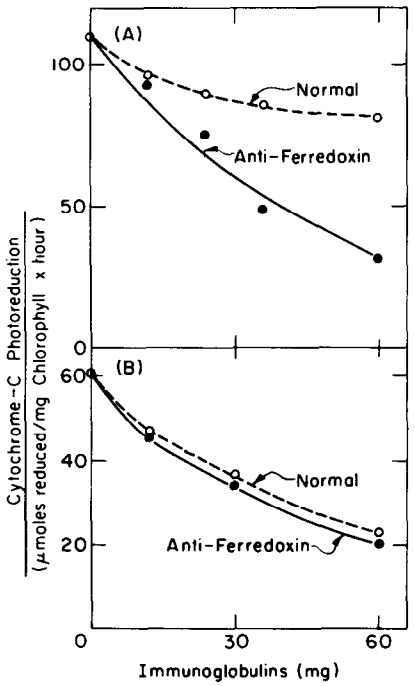

Fig. 3. The effect of ferredoxin specific immunoglobulins on the photoreduction of cytochrome *c* in the presence of and absence of added ferredoxin. (a) Ferredoxin samples (45 μg) were incubated with immunoglobulins as described in fig. 2 and then adjusted to a total volume of 3 ml containing Tricine-maleate, pH 7.5 (25 mM), KCl (20 mM), cyt-*c* (0.033 mM), and lettuce chloroplasts containing 14 μg of chlorophyll; (b) similar to (a) but omitting added ferredoxin.

effect was observed also on the photoreduction of ferricyanide or diquat. It is not clear whether photoreduction of the unaffected electron acceptors bypasses the ferredoxin site, or is dependent on bound ferredoxin. The photoreduction of ferredoxin was also inhibited by the anti-ferredoxin immunoglobins (not shown).

During the preparation of this report Hidemann-Van Wyk et al. [11] reported also on the preparation of anti-plant ferredoxin serum and studied the localization of ferredoxin in the thylakoid membrane.

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