COFACTOR EFFECTS ON ENHANCEMENT IN NADP REDUCTION BY CHLOROPLASTS[1]

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SUMMARY

Enhancement of NADP[2] reduction by isolated chloroplasts is strongly affected by the purity of cofactors used in the reaction mixture. The crude enzyme preparation (PPNR) supports significant enhancement. The enhancement effect is much reduced in the presence of purified cofactors. These observations appear to explain some contradictory results on enhancement in the literature.

Following the discovery of enhancement in eucaryotic photosynthesis (1,2) a number of studies were made to find whether this effect could be observed in NADP reduction by H_2O in isolated chloroplasts (3-8). Two groups of data have emerged from these studies. Govindjee, Govindjee and Hoch (3,4), Joliot, Joliot and Kok (7) and Ben-Hayyim and Avron (8) report significant enhancement while McSwain and Arnon (6) failed to see enhancement under a wide variety of conditons. Gibbs (5) also failed to observe enhancement in NADP reduction by isolated chloroplasts. On the basis of recent work from this laboratory (9,10) we proposed a possible explanation for these contradictory results. From our work it appears that stroma lamellae of green plant chloroplasts contain only PS 1 and are enriched in long wavelength absorption compared to grana lamellae. We suggested that if the action spectrum of PS 1 in stroma lamellae is shifted toward longer wavelengths than PS 1 in grana lamellae, coupling between the PS 2 in grana and PS 1

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[2]Abbreviations: Fd, ferredoxin; NADP, nicotinamide adenine dinucleotide phosphate; PC, plastocyanine; PPNR, photosynthetic pyridine nucleotide reductase; PS 1, photosystem 1; PS 2, photosystem 2.

in stroma lamellae may be necessary before enhancement in NADP reduction can be observed. Chloroplasts isolated under conditions unfavorable for retention of a factor involved in coupling might be expected not to demonstrate enhancement in NADP reduction.

The experiments reported here, while not differentiating between this model and energy transfer models (11) do indicate that a factor or factors other than purified Fd, PC, NADP reductase, and inorganic ions is required for observation of large enhancement effects in reduction of NADP by chloroplasts.

MATERIALS AND METHODS

Chloroplasts were isolated from spinach obtained at local market. 150 g of leaves were homogenized in a Waring blendor for 60 seconds with 250 ml of 0.5 M sucrose, 0.05 M $(K)PO_4$ (pH 7.4), 0.01 M KCl, 0.01 M $MgCl_2$ to yield class 2 chloroplasts. The brei was passed through 8 layers of cheesecloth and was centrifuged for 5 minutes at 200xg. The supernatant was recentrifuged for 10 minutes at 1000xg and the pellet obtained was washed once in 0.3 M sucrose, 0.05 M $(K)PO_4$(pH 7.4), 0.01 M KCl and 0.01 M $MgCl_2$ and was finally resuspended in this medium. The chloroplasts isolated in this manner were almost totally class 2 when examined with the light microscope using phase optics. Chlorophylls were determined according to the method of Arnon (12). Purified ferredoxin and reductase were prepared according to the methods of Arnon and coworkers (13,14). PPNR was obtained by the acetone precipitation procedure of San Pietro and Lang (15). PPNR was not further purified by protamine sulfate treatment.

A Cary 14 spectrophotometer equipped with a scattered transmission accessory was used to monitor NADP reduction at 340 nm. The 650 nm actinic beam was provided by a Bausch and Lomb 500 mm monochromator. A Tiyoda microscope lamp operated at maximum current was used as a source for 708 nm light. The light from the lamp was passed through a CS 1-69 cut off and 708 nm interference filter. A neutral optical density filter (1.3 O.D.) was used

as a beam splitter. The combined beams--the 708 nm light reflected from the beam splitter and the 650 nm light passed through the beam splitter--were passed through a 2-61 red cut off filter and focused on the side of the sample cuvette. The actinic beam passed through 3 mm of sample and the analyzing beam passed through 10 mm of sample. The incident intensity of 708 nm light was 2.8 nanoeinsteins/cm^2/sec and the incident intensity of 650 nm light was 0.35 nanoeinsteins/cm^2/sec. These intensities are at the lower end of the range of intensities studied by McSwain and Arnon (6). Higher incident intensities diminished the enhancement effect. Enhancement in NADP reduction was calculated as described by McSwain and Arnon (6).

RESULTS AND DISCUSSION

Several variations in the reaction mixture were tried to observe NADP reduction. The basic reaction mixture contained 0.05 M PO_4 (pH 7.4), 0.3 M sucrose, 0.01 M KCl, 0.01 M $MgCl_2$, 0.2 mM NADP and sufficient chloroplasts to give 15-20 µg Chl/ml. PPNR or cofactors were added in saturating amounts.

TABLE 1

Enhancement in NADP reduction. NADP reduction rates as (ΔOD_{340} per min) x 10^3. Data from Fig. 1. Average forward reactions are corrected for average back reactions.

	NADP reduction rates	
	In the presence of PPNR	In the presence of purified Fd
708 nm light alone - (A)	7.2	5.8
650 nm light alone - (B)	3.4	3.7
708 nm light and 650 nm light together - (C)	14.5	9.6
Enhancement calculated as $\frac{C}{A+B}$	1.4	1.0

The addition of methylamine, an uncoupler of photophosphorylation, had no effect on enhancement or rate of NADP reduction. Table 1 and Fig. 1 compare the enhancement observed in presence of PPNR and purified Fd. These observations demonstrate that PPNR contains a factor or factors which greatly increase the enhancement effect. This is readily evident by comparing the effect of turning the 708 light on and off in the presence of the 650 beam. The back reactions were generally greater in the presence of the crude (PPNR) enzyme preparation. When PPNR was replaced by purified Fd, negligible enhancement, if any, was observed. The addition of NADP reductase and PC

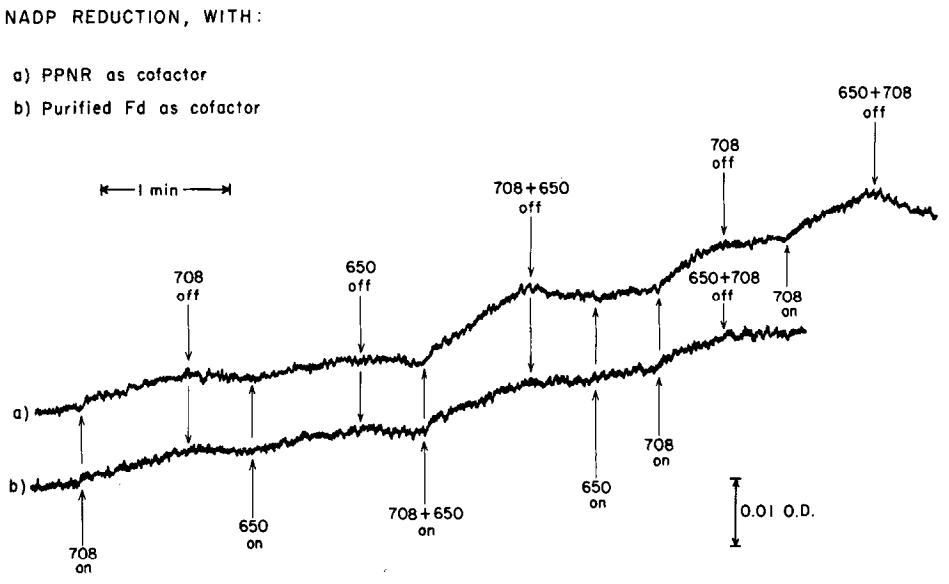

Fig. 1. Comparison of effects of PPNR and purified cofactors on enhancement of NADP reduction by chloroplasts. Reaction mixtures as described under Methods.

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had no effect on either enhancement or rates of NADP reduction. These two cofactors were apparently not rate limiting at the low light intensities we used. The presence of 0.01 M Mg^{++} yielded a small increase in enhancement. These experiments were repeated many times and clearly indicated that PPNR was essential to obtain significant enhancement. We also studied the rate of NADP reduction by the reaction mixture containing either PPNR

or purified Fd as a function of 680 nm light intensity. We observed that the rates of NADP reduction at a given absorbed intensity were similar in both cases. As purified cofactors were added to the reaction mixture containing PPNR it became increasingly difficult to demonstrate enhancement. These results on purified cofactors are in agreement with those of McSwain and Arnon (6) who also failed to see any enhancement in presence of purified cofactors. Govindjee, Govindjee and Hoch (3,4), who have reported enhancement in NADP reduction by chloroplasts, used PPNR as cofactor. Thus our conclusion that presence of PPNR is essential for seeing enhancement is in agreement with both the reports. Our results could be explained in several ways.

1. There are two kinds of PS 1 in chloroplasts, only one of which is a long wavelength form of PS 1. The PPNR contains a factor which couples the long wavelength form of PS 1 to PS 2 and, therefore, enhancement in NADP reduction is mediated only by the long wavelength PS 1. We have previously proposed that PS 1 present in the stroma lamellae may be coupled to PS 2 present in the grana by a diffusible carrier. A rate limiting reaction at the carrier step could account for the absence of enhancement in NADP reduction at higher light intensities.

2. Large enhancement would be expected when one photoreaction is limiting and the other is overdriving. The action spectra results from Avron's laboratory (11) on ferricyanide reduction have shown that transfer of energy can occur from either photosystem to the other. If experimental conditions are such that transfer of energy can occur between the two photosystems, enhancement would be expected to be lower. It is possible that purified Fd facilitates such transfers of energy between PS 2 and PS 1 and, therefore, we are unable to see significant enhancement using this cofactor. Such a hypothesis could be tested by studying the magnitude of variable fluorescence in presence of PPNR or Fd.

The experiments reported here cannot differentiate between these pos-

sibilities or tell the extent to which each may be involved in enhancement. Nevertheless, the experiments do show that significant enhancement occurs only at relatively low incident intensities and in the presence of a factor or factors present in PPNR.

Preliminary experiments on fractionation of PPNR, using acetone and $(NH_4)_2SO_4$ precipitation show that enhancement capacity is not equally distributed among fractions derived from PPNR.

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