SOME PROPERTIES OF PHOTOSYNTHETIC PYRIDINE NUCLEOTIDE REDUCTASE FROM SPINACH*

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A reddish-brown protein - called "photosynthetic pyridine nucleotide reductase (PPNR)" - was prepared from spinach by San Pietro and Lang, who found that it catalyzed reduction of TPN, but not DPN, by illuminated chloroplasts (San Pietro and Lang, 1956 & 1958; San Pietro, 1958). Davenport and Hill (1960) purified a similar protein from pea leaf etc. and demonstrated that it stimulated photoreduction of methaemoglobin and cytochrome c, but not ferricyanide. The physiological function of this protein remains to be clarified.

With spinach, we found that San Pietro et al's preparation procedure is well reproducible with high yield up to at least their protamine sulfate precipitation step. Spinach PPNR can be purified further by chromatography on a DEAE-cellulose column, equilibrated with 0.5-M Tris(hydroxymethyl)aminomethane-HCl buffer (pH 8.0). More than 80 per cent of the PPNR present in the starting sample for chromatography can be collected in a fraction with purity estimated to be 25% higher than that described previously (San Pietro, 1961) on the basis of absorban-

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cies; e.g., ratio of $A_{422\ m\mu}$ to $A_{275\ m\mu}$ (Fig. 1). Concentrated PPNR, so purified, is reddish, and completely homogeneous ultracentrifugally, S_{20} 1.67 s. It is found that PPNR has one atom of iron in each 18,800 g. of protein. Since the molecular weight of the protein has been given as 17,000 (Appella and San Pietro, 1962) and 19,000 (Davenport and Hill, 1960), these data indicate that one molecule of PPNR contains one iron atom.

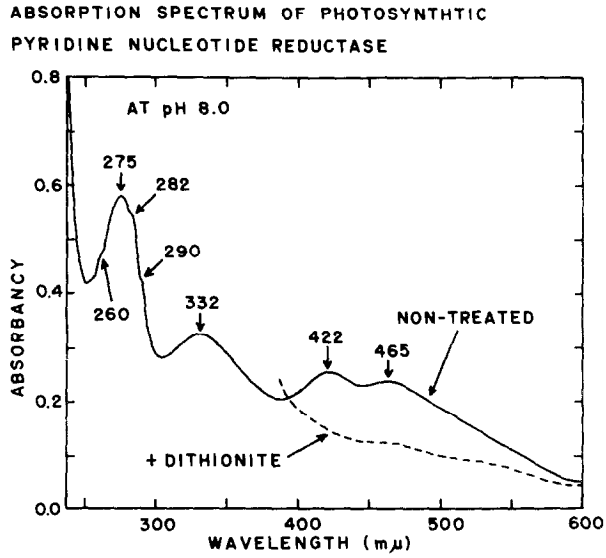

Fig. 1. Absorption spectrum of photosynthetic pyridine nucleotide reductase from spinach.
In 0.1-M Tris(hydroxymethyl)aminomethane-HCl buffer of pH 8.0, approximately 390 μg. of PPNR was dissolved per ml. Measurements were carried out at room temperature (24° C) with a Cary, model 14-R, spectrophotometer; Samples contained in cuvette of 1.0-cm. optical path under conditions designated in figure.

PPNR is bleached completely by illuminated chloroplasts to a leuco form; at the end of reaction, no further color change occurs upon addition of dithionite (Fig. 2). It seems likely that PPNR is photoreduced to its leuco form, concomitant, perhaps, with valency change of the protein-bound iron. However, the bleached PPNR is not restored to its original color by addition of TPN in darkness immediately after illumination.

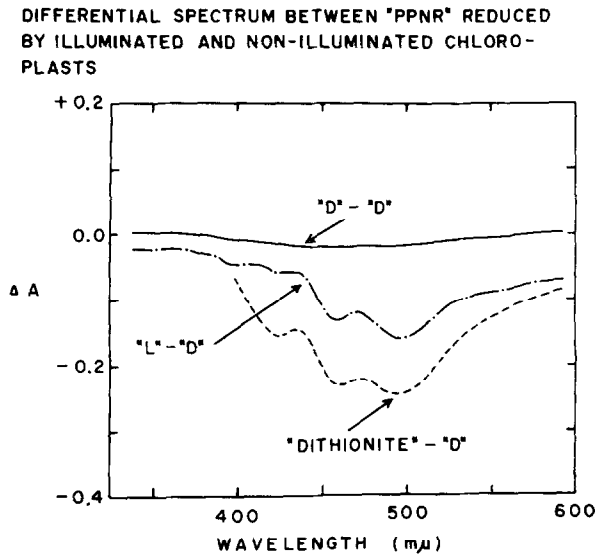

Fig. 2. Difference spectrum for spinach PPNR treated with illuminated and non-illuminated spinach chloroplasts.

Components of the reaction mixture were as follows: in a total volume of 1.50 ml. in cuvette of 1.0-cm. optical path, 125 μ-moles of Tris(hydroxymethyl)aminomethane-HCl buffer of pH 7.8, 670 μg. of PPNR, and spinach chloroplasts (equivalent to 35 μg. of chlorophyll). The components were mixed at an ice-water temperature in darkness, and incubated at 22° C for two minutes in darkness ("D"). Then, one cuvette was illuminated (approximately 1,000 ft-candles) at 22° C for 15 minutes ("L"), and at the end of reaction a trace amount of solid dithionite was added ("DITHIONITE"). Difference spectra were measured aerobically, as developed under these conditions. A prolonged period of illumination caused "L" to be changed almost linearly to the same extent as for "DITHIONITE".

The well-washed spinach chloroplasts, if supplemented with PPNR and illuminated, can catalyze reduction of TPN but not DPN, in agreement with reports of others (San Pietro and Lang, 1956; San Pietro, 1958). Photoreduction of ferricyanide is notably stimulated by the addition of PPNR as well as of cytochrome c_2 (Horio and Kamen, 1961). The degree of stimulation depends upon the extent of washing of the chloroplasts; with unwashed or incompletely washed chloroplasts, photoreduction of ferricyanide is not or little affected by PPNR.

Furthermore, PPNR acts as a Hill reagent. In the presence of PPNR in substrate amount, but in the absence of TPN, the

illuminated chloroplasts synthesize ATP from ADP and orthophosphate; the rate of ATP formation parallels the rate of bleaching of PPNR. Under the same experimental conditions, no ATP formation occurs if PPNR is absent. On the other hand, if PPNR is present in much smaller amounts, cytochrome c_2 and TPN are effective activators for ATP formation, but not in the absence of PPNR.

These results suggest that PPNR is functional as one of the oxidation-reduction components in the electron-transferring system of the Hill reaction of the chloroplasts and plays a key-role in electron transport to cytochrome f (Hill and Scarisbrick, 1951) on the one hand, and to TPN on the other.

Full details of these and of other experiments will be reported elsewhere.

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