PHOTOCONTROL OF FORMATION OF RED KIDNEY BEAN LEAF TRIPHOSPHOPYRIDINE NUCLEOTIDE LINKED TRIOSEPHOSPHATE DEHYDROGENASE [1]

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INTRODUCTION

The enzymatic oxidation of glyceraldehyde-3-phosphate is mediated by either DPN or TPN. The reaction involving DPN is present in both mammalian (6) and plant tissues, (11, 15) while that involving TPN (equation 1) has been found only in plant leaves 1) D-glyceraldehyde-3-phosphate + TPN^+ + phosphate $\rightleftarrows$ 1,3-diphosphoglyceric acid + TPNH + H^+ (8, 12). Hageman and Arnon (12) showed that the TPN triosephosphate dehydrogenase appeared only in leaves of seedlings that had been exposed to light. Since such exposure results in the formation of chlorophyll, it was suggested (2, 12) that the TPN enzyme was associated with photosynthesis.

The present investigation was designed to ascertain the nature of the light requirement for the formation of the TPN triosephosphate dehydrogenase. The possibility was considered that, like many of the light-controlled plant processes, (3, 7, 14) radiation in the red region would be the controlling factor. Such control by the photomorphogenic reaction would result in the formation of TPN triosephosphate dehydrogenase in the absence of chlorophyll. The results to be reported support this suggestion.

MATERIALS AND METHODS

The experiments were carried out with red kidney beans grown in sand (7). Flats were kept in a dark room for four days. On the fifth day, two flats were removed and given continuous light from two 40-watt fluorescent tubes (70 ft-c). Two other flats were given 5 minutes of red radiation (0.3×10^{-3} watts per cm^2) (7) on four successive days (5th through 8th) and returned to the dark immediately thereafter. A third set of flats was given 5 minutes of red radiation followed immediately by 5 minutes of far-red radiation (0.3×10^{-3} watts per cm^2) for four successive days. A fourth set of four flats was kept in the dark throughout. On the 11th day two of the flats in the fourth set were exposed to continuous light for 6 hours. The leaves were then rapidly removed from the various sets and blended with 7 volumes of acetone. After filtering on a Buchner funnel an aliquot was removed from the acetone filtrate for chlorophyll estimation and the residue was blended again with 4 volumes of acetone. The suspension was filtered and dried in vacuo over P_2O_5 for 24 hours. The acetone powders were stored at −20° C and were assayed within a week after preparation. The powders were extracted by grinding in a mortar with 10 volumes of ice-cold 0.03 M phosphate buffer pH 8.5, containing 0.009 M ethylene diamine tetraacetic acid and the suspension was centrifuged for 20 minutes at 13,000 × G. The supernatant liquid was diluted appropriately with distilled water and assayed immediately for triosephosphate dehydrogenase activity.

The assay mixture for triosephosphate dehydrogenase is given in table I. Aldolase and alcohol dehydrogenase were crystalline preparations (Worthington). Protein was determined by the biuret reaction (10) and chlorophyll was estimated from the absorbance at 652 mμ (1).

RESULTS

To ascertain whether the formation of TPN triose dehydrogenase required the concomitant formation of

TABLE I

PHOTOCONTROL OF TPN TRIOSE PHOSPHATE DEHYDROGENASE FORMATION

SAMPLE	μG PROTEIN	CHANGE IN OD_{340}		RATIO TPN / DPN
		DPN	TPN	
Complete light	90	0.079	0.070	0.89
Complete dark	250	0.147	0.031	0.21
Red light	75	0.063	0.051	0.81
	340	0.168	0.134	0.80
Red-far red	60	0.060	0.013	0.22
	270	0.167	0.040	0.24
Dark + 6 hrs light	230	0.156	0.029	0.19

The complete system contained 150 μM tris buffer pH 8.5, 10 μM cysteine, 50 μM sodium arsenate, 50 μM sodium fluoride, 0.2 μM DPN or TPN, 100 γ aldolase, acetone powder extract and 2 μM fructose 1,6 diphosphate in 3.0 ml. The reaction was started by addition of fructose diphosphate.

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chlorophyll, one preliminary experiment was performed comparing light-grown leaves and leaves of plants given red radiation. The plants were allowed to grow 13 days and the leaf-acetone powders were prepared by the procedure of Hageman and Arnon (12), which differs from the procedure described above in requiring a preliminary homogenization in phosphate-versene. The leaves from plants given red radiation were elongated and showed no visible signs of chlorophyll. When assayed for triosephosphate dehydrogenase (see legend of table I for assay conditions) the extract of the red-treated leaves was found to have somewhat greater activity with TPN than with DPN. The light-grown leaves showed about 30 percent more activity with TPN. It was quite apparent, however, that the TPN enzyme had been formed in the red-treated leaves under conditions where essentially no chlorophyll was formed.

To relate the formation of the enzyme more directly to the photomorphogenic control, a complete series of experiments was performed (see Materials and Methods). The leaves used in these experiments were removed after 11 days in order to prevent starvation effects in the non-photosynthesizing sets. The leaves were blended directly in acetone so that a direct measure of the chlorophyll content might be obtained. The results are given in change of optical density at 340 mμ between 0.5 and 3.5 minutes. Because the change in optical density was not completely proportional to protein concentration, comparisons were made with similar quantities of protein. Representative data are shown in table I. When calculated as the ratio of activity with TPN to that with DPN, the conclusion is seen clearly. Thus, both the light-grown leaves and those treated with red radiation show TPN activity approaching that of DPN as compared to those grown in the dark. If the red radiation is followed by far-red radiation there is apparently a reversal of the promotive action of the red. Leaves grown for 11 days in the dark followed by 6 hours in the light have about five times as much chlorophyll per weight of leaf as the red-treated leaves. Yet the TPN activity is similar to those grown completely in the dark, further substantiating the lack of association of chlorophyll and the TPN triose dehydrogenase. The larger samples taken in the dark and far-red series were necessary in order to obtain measurable changes with TPN.

The possibility that the TPN reduction in the experiment with red-treated leaves might be due to a side reaction, (i.e., the dephosphorylation of fructose diphosphate with subsequent reduction of TPN by glucose-6-phosphate dehydrogenase) was eliminated by finding that with the enzyme concentrations used, almost no reduction of TPN was obtained in the absence of arsenate. (With higher concentrations of enzyme a reaction in the absence of arsenate was more evident.) In addition, preincubation of the extract with 0.01 M iodoacetate for 5 minutes (9), completely destroyed the capacity to reduce TPN. An additional possibility, namely, that the TPN reduction was due to primary reduction of DPN followed by transfer to TPN via transhydrogenase (5) was eliminated by two types of experiments (table II). In the first experiment, yeast alcohol dehydrogenase was allowed to reduce DPN with ethanol (16). No increase in optical density was obtained when TPN and leaf extract were added. In the second experiment, the extract (which contains TPN isocitric dehydrogenase) was allowed to reduce TPN with isocitrate. Further addition of DPN did not result in increased absorption at 340 mμ. In either case, if the extract had contained transhydrogenase, the total increase in optical density upon addition of both DPN and TPN should have exceeded that obtained from 0.10 ml of the reacting nucleotide. The data obtained show that only the specific nucleotide is reduced and that the extract does not catalyze a transfer from DPNH to TPN.

Discussion

It appears from these data that the formation of TPN triosephosphate dehydrogenase is brought about by low-energy red radiation. The photocontrol of chlorophyll formation has been shown to occur in at least two stages (17). In the first stage, protochlorophyll is formed as the result of low-energy red radiation while the subsequent conversion to chlorophyll requires longer periods of high-intensity red radiation. Thus, one might be tempted to consider an association of TPN triosephosphate dehydrogenase formation with synthesis of protochlorophyll. The subsequent dark period necessary for formation of the enzyme, however, as contrasted with the dark

Table II

Absence of Transhydrogenase in Red-Radiated Leaf Preparations

Substrate	Addition	Δ OD^{340}
Ethanol	Extract + DPN (0.10 ml)	0.000
	Extract + TPN (0.10 ml)	0.000
	Extract + DPN (0.10 ml) + ADH	0.398
	Extract + TPN (0.10 ml) + ADH	0.000
	Extract + DPN (0.03 ml) + TPN (0.10 ml) + ADH	0.127
Isocitrate	Extract + DPN (0.10 ml)	0.000
	Extract + TPN (0.10 ml)	0.337
	Extract + TPN (0.03 ml) + DPN (0.10 ml)	0.107

In the experiment with ethanol, the complete system contained 2.8 ml of buffered semicarbazide (16) and 0.02 ml ethanol. Additions were 0.0015 M DPN or TPN, 0.01 ml yeast alcohol dehydrogenase and 300 γ of extract of leaves grown with red radiation in a final volume of 3.0 ml. In the experiment with isocitrate, the complete system contained 100 μM of tris buffer pH 7.5, 40 μM D,L-isocitrate and 10 μM of $MgCl_2$. Additions were 0.0015 M DPN or TPN and 300 γ of extract in 3.0 ml. The optical density at 340 mμ was followed until there was no further change.

period required for synthesis of protochlorophyll suggests there is a common controlling mechanism rather than a direct association between these processes.

Another observation which may be more pertinent is the finding that plastid development (fusion of vesicular elements into lamellar structures) is initiated by low-intensity red radiation (13). Although complete plastid development was observed only under conditions allowing chlorophyll synthesis (high intensity light) the authors did not examine plastids of leaves kept in the dark after a period of low-intensity red radiation. Since brief low-intensity red radiation has been shown to initiate plastid development, it is reasonable to assume that complete development may require only a subsequent dark period of sufficient duration. With this possibility in mind, the formation of TPN triosephosphate dehydrogenase might be directly correlated with plastid development. Such a correlation would be consistent with various lines of evidence indicating that the enzyme plays a significant role in the photosynthetic process (4, 18).

Summary

Extracts of red kidney bean leaves grown with brief exposure to red light show considerable TPN triosephosphate dehydrogenase, although no visible chlorophyll is present in the leaves. If the red radiation is followed by exposure to far-red, the promotive effect of the red light is nullified. It is concluded that TPN triosephosphate dehydrogenase formation is controlled by the photomorphogenic reaction.

Supplementary Note

After completing this work, it was brought to the author's attention that Fuller and Gibbs (Abstract of 1956, Plant Physiology meetings 1956 xxxi) reported the presence of TPN triose phosphate dehydrogenase in a genetic albino barley mutant. This finding supports the contention discussed above that the formation of this enzyme is not directly related to chlorophyll formation.

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