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D-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IN PHOTOSYNTHETIC CELLS

I. THE REVERSIBLE LIGHT-INDUCED ACTIVATION *IN VIVO* OF NADP-DEPENDENT ENZYME AND ITS RELATIONSHIP TO NAD-DEPENDENT ACTIVITIES

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SUMMARY

1. The possibility of the existence in photosynthetic tissues of a single D-glyceraldehyde-3-phosphate dehydrogenase enzyme, active with NAD and NADP, was investigated. By $(NH_2)_4SO_4$ precipitation a fraction active only with NAD and containing 40% of the total units was isolated from another fraction active with both coenzymes. Repetitive salt fractionation of this latter fraction failed to achieve further resolution of the two activities.

2. The lack of additivity of NAD- and NADP-dependent activities, always verified in pea leaves crude extracts, supports the idea that at least one glyceraldehyde-3-*P* dehydrogenase non-specific with respect to pyridine nucleotides is present in leaves.

3. The reversible light-induced activation *in vivo* of NADP-dependent glyceraldehyde-3-phosphate dehydrogenase was clearly shown to be independent from net protein synthesis.

4. The kinetic parameters of this activation phenomenon were examined both for the oxidative and reductive reaction. The activation was found to correspond to a reversible increase of the v_{max} of the NADP- and NADPH-dependent activities, expressed on a protein basis. Neither the v_{max} of NAD^+ and NADH linked activities, nor any of the apparent K_m values of substrates are significantly affected.

INTRODUCTION

NADP-dependent glyceraldehyde-3-phosphate dehydrogenase activity (D-glyceraldehyde-3-phosphate: $NADP^+$ oxidoreductase, EC 1.2.1.13) in leaf tissue is commonly associated with the CO_2 reductive cycle[1,2]. An NAD-linked glyceraldehyde-

3-phosphate dehydrogenase activity (D-glyceraldehyde-3-phosphate:NAD^+ oxidoreductase, EC 1.2.1.12) is also present in leaf tissue and this is believed to be associated with the glycolysis system.

SCHULMAN AND GIBBS[3] recently reported that the two activities cannot be separated by various differential precipitation procedures, by column chromatography, or by starch gel electrophoresis: they conclude that the glyceraldehyde-3-*P* dehydrogenase activities present in leaves are catalyzed by very similar proteins or possibly by a single enzyme. This latter possibility had also been proposed by us[4] on the basis of competition kinetics observed when the overall reduction of an equimolar mixture of $NADP^+$ and NAD^+ was measured in crude extracts of pea leaves. In this communication we show that a fraction enriched in NAD-dependent activity and containing 40% of the total units can be separated (by $(NH_4)_2SO_4$ precipitation) from another fraction active with both coenzymes.

Ziegler's group[5] at Darmstadt found that the NADP-linked activity is considerably enhanced by a short exposure of fully differentiated leaves to light, while the NAD-linked activity appears to be only slightly affected; the activation is rapidly reversed when the leaves are placed again in the dark. This phenomenon has since been observed in many algae[6,7] and all higher plants examined thus far. In order to gain further insight into the activation phenomenon, we have examined the kinetic parameters of both NAD- and NADP-dependent activities both in extracts from pea leaves previously exposed to light and control leaves maintained in the dark. This phenomenon was found to correspond merely to a reversible increase of the apparent v_{max} value of the NADP-dependent activities, both in the oxidative and reductive directions of the reaction.

MATERIALS AND METHODS

Reagents

NADH, NADPH, 3-phosphoglyceric acid, glyceraldehyde-3-phosphate, free acid, glyceraldehyde-3-*P* diethyl-acetal dibarium salt, from which glyceraldehyde-3-*P* was prepared, glyceraldehyde-3-*P* dehydrogenase, chloramphenicol, puromycin, cycloheximide, Trizma-base, collidine, reduced glutathione and L-cysteine were purchased from Sigma Chemical Co. (St. Louis, Mo.). $NADP^+$, NAD^+, and 3-phosphoglycerate kinase were obtained from Boehringer (Mannheim). $(NH_4)_2SO_4$, enzyme grade, was produced by Mann Research Laboratories.

Materials

The pea plants (*Pisum sativum*, cultivar Provenza) were grown in a greenhouse on vermiculite and sand plates at 23 ± 2°. In order to elicit the activation response to light, the plants were placed under a bank of three fluorescent lamps (about 3500 lux) for 8 h. The dark activity was measured using leaves of plants exposed to fluorescent light for 12 h and subsequently kept in the dark for 6 h.

For inhibitor experiments, leaves were excised and placed in Petri dishes containing tap water; after 3 h in darkness they were transferred to the inhibitor solution. After 90 min of contact with inhibitor in darkness, the leaves were exposed to light (6000 lux, fluorescent lamps) on a mechanical shaker for 2 h (or, in the controls, kept

in the dark). Suitable control experiments were simultaneously performed with leaves maintained continuously in tapwater.

Methods

To prepare extracts, pea leaves were ground in a mortar (kept at 0–4°) with 15–20 vol. of the appropriate buffer, and the homogenate was centrifuged for 2 min at 1500 × *g* to remove cell debris. The glyceraldehyde-3-*P* dehydrogenase reaction was studied in both directions, *i.e.*, oxidation of glyceraldehyde-3-*P* and reduction of 1,3-diphosphoglyceric acid. For the "oxidative" direction, the activity was assayed as described[4], except that a six minute preincubation in the presence of all reagents (except glyceraldehyde-3-*P*) was allowed before each measurement. The "reductive" reaction was measured according to HEBER *et al.*[8], except that the final reaction volume was 1.20 ml and an excess of 3-phosphoglycerate kinase from yeast was added. The buffers used for the preparation of the extracts were 0.03 M Tris–HCl (pH 8.5) containing 0.01 M EDTA for assays in the "oxidative" direction and 0.05 M 2,4,6-collidine (pH 7.2) for the "reductive" reaction.

Salting out experiments

Pea leaves (15 g, w.w.) were blended for 1 min at maximum speed in a Virtis homogenizer in presence of 50 ml of 0.03 M Tris–HCl (pH 8.5) containing 0.01 M EDTA. The homogenate was filtered through cheesecloth and centrifuged for 10 min at 10 000 × *g*.

A saturated solution of $(NH_4)_2SO_4$ at 2°, brought to pH 8.5 with NH_4OH, was added dropwise to the supernatant fluid up to 30% saturation. The suspension was centrifuged, after a 20-min incubation in the cold, and the supernatant fluid was used as starting material for salting out experiments. The solution was subdivided into several equal portions (1.6 ml) and different volumes of buffer and saturated $(NH_4)_2SO_4$ solution (pH 8.5) were added to vary the $(NH_4)_2SO_4$ saturation between 30% and 80%, in a final volume of 5.6 ml. Precipitation was allowed to proceed for 30 min in an ice bath; the suspensions were then centrifuged for 15 min at 10 000 × *g* and the individual supernatant fluids were assayed for NAD- and NADP-dependent triose-phosphate dehydrogenase activities.

RESULTS

Partial resolution of NAD-linked activity by salt precipitation

Differential fractionation tests with $(NH_4)_2SO_4$ at pH 8.5 were undertaken as preliminary tests indicated that this separation procedure was effective in partially fractionating the NAD- and NADP-dependent activities. As shown in Fig. 1a, upon salting out with $(NH_4)_2SO_4$ the NADP-linked activity disappears from the supernatant fraction (and can be recovered in the precipitate) with a pattern different from the NAD-linked activity. The latter activity is distributed in a broader peak displaced toward the highest $(NH_4)_2SO_4$ concentration (Fig. 1b).

Obviously, this precipitation pattern is compatible with our interpretation of the competition between NAD- and NADP-linked activities (*i.e.* the presence of a pyridine nucleotide non-specific enzyme) only if the broad peak of the "NAD" activity

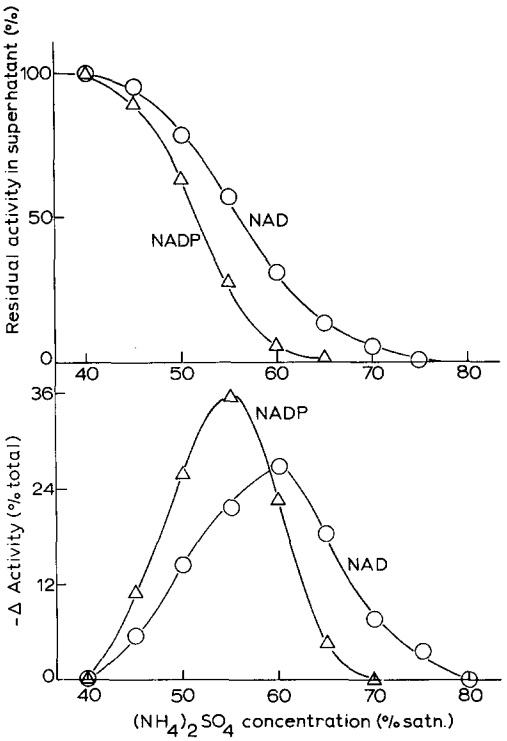


Fig. 1. Precipitation profile of NADP- and NAD-dependent glyceraldehyde-3-*P* dehydrogenase activities in pea leaf extracts during $(NH_4)_2SO_4$ fractionation. For experimental details, see MATERIALS AND METHODS.

TABLE I

EFFECT OF REPEATED SALT FRACTIONATIONS ON THE NADP- AND NAD-DEPENDENT GLYCERALDEHYDE-3-*P* DEHYDROGENASE ACTIVITIES OF A PEA LEAF CRUDE EXTRACT

40 ml of crude leaf extract were prepared as described in *Methods*; a saturated $(NH_4)_2SO_4$ solution (pH 8.5) was added up to 40% satn. and the precipitate discarded. The $(NH_4)_2SO_4$ concentration in the supernatant fluid was brought to 60% satn. and the enzymatic activities were assayed in both the supernatant fluid and in the precipitate, (dissolved in 25 ml of 0.05 M Tris–HCl (pH 8.5), 0.01 M EDTA). The residual salt present in the precipitate solution was determined conductimetrically, and the 60% fractionation was repeated. The salt fractionation was performed a third time under identical conditions.

Cycle no.		*Total activity (μmoles/h)*		*NADP/NAD activity ratio*
		NADP-dependent	*NAD-dependent*	
I	40% satd. supernatant	15530	7100	2.19
	60% satd. precipitate	9746	4704	2.07
	60% satd. supernatant	1159	3014	0.38
II	60% satd. precipitate	7470	3675	2.03
	60% satd. supernatant	804	412	1.95
III	60% satd. precipitate	4460	2236	2.00
	60% satd. supernatant	849	408	2.08

can be shown to consist of two overlapping components, one of which is coincident with the NADP-linked activity peak. If the "NAD" peak, however, is due to a single protein precipitating over broader $(NH_4)_2SO_4$ concentration range, it would be expected that a repetitive salt fractionation would gradually deplete the precipitated fraction of the NAD-dependent activity. A 40–60% $(NH_4)_2SO_4$ fraction was subjected to repeated cycles of precipitation with 60% $(NH_4)_2SO_4$ (after measuring the residual salt concentration by conductimetry), and it was found that the ratios of the two activities in both precipitates and supernatant fluids converged to a common value and that the only fraction significantly enriched in NAD-linked activity was the first 60% supernatant (Table I).

These tests are therefore consistent with the hypothesis that two distinct dehydrogenase enzymes dependent on NAD are present in pea leaves. It appears that one of the enzymes precipitates in the 60–80% $(NH_4)_2SO_4$ fraction, while the other behaves like the NADP-linked enzyme with respect to salt fractionation.

The activation by light of NADP-linked activity

Insensitivity to inhibitors of protein synthesis

The possible involvement of *de novo* enzyme synthesis in the light-stimulated enhancement of NADP-linked glyceraldehyde-3-*P* dehydrogenase activity was definitely excluded by experiments with inhibitors of protein synthesis. As a measure of inhibitor activity *in vivo*, effects on the greening of etiolated pea leaves were examined. The concentrations employed in the present experiments were at least three times greater than those which cause a 90% inhibition of chlorophyll synthesis[9]. The data shown in Table II extend the results of ZIEGLER AND ZIEGLER[5] and demonstrate, in addition, that chloramphenicol exerts no effect on the activation mechanism when added at concentrations still inhibitory for chlorophyll biosynthesis but lower than used by these authors.

TABLE II

EFFECTS OF SOME INHIBITORS OF PROTEIN SYNTHESIS ON THE *in vivo* LIGHT-INDUCED ACTIVATION OF THE NADP-DEPENDENT GLYCERALDEHYDE-3-*P* DEHYDROGENASE ACTIVITY

Excised leaves were placed for 3 h in the dark in Petri dishes containing tap water. The inhibitors were supplied in the dark for 90 min, after which time some leaves were exposed to light and some maintained in the dark for 2 h in presence of inhibitor (treated). In the control experiments the leaves were maintained continuously in tap water and subjected to the same light and dark regimens.

Inhibitor	*Enzyme activity (μmoles/h per mg protein)*			
	Light-exposed leaves		*Dark-maintained leaves*	
	Control	*Treated*	*Control*	*Treated*
Cycloheximide (15 μg/ml)	47.5	43.0	27.2	26.5
Puromycin (400 μg/ml)	35.0	40.0	19.8	18.5
Chloramphenicol (1 mg/ml)	43.0	48.0	23.6	23.0

Insensitivity to dilution and gel-filtration

The activation *in vivo* of the NADP-linked glyceraldehyde-3-*P* dehydrogenase appears to involve some kind of stable and reversible rearrangement of the enzyme

TABLE III

EFFECT OF FILTRATION THROUGH SEPHADEX G-75 ON THE NADP- AND NAD-DEPENDENT GLYCERALDEHYDE-3-*P* DEHYDROGENASE ACTIVITIES OF AN ACTIVATED PEA LEAF EXTRACT

2 ml of crude extract, prepared as described in *Methods*, were applied to a Sephadex G-75 column (1 cm × 45 cm) and eluted with 0.05 M Tris–HCl (pH 8.5), 0.01 M EDTA. The fraction of eluate immediately following the void volume was assayed for the enzymatic activities.

Extract	*NADP-dependent activity (μmoles/h per mg protein)*	*NAD-dependent activity (μmoles/h per mg protein)*	*NADP/NAD activity ratio*
Crude	47.4	28.1	1.69
Gel-filtered	124.0	69.5	1.78

structure, rather than a single allosteric effect. This conclusion has been reached on the basis of several criteria.

Although the leaf extract is diluted about 1000-fold during the kinetic measurements in comparison with the concentrations *in vivo*, the presence of possible effectors in the homogenates were tested by mixing experiments. The activity of an equal volume mixture of dark- and light-treated leaf homogenates was compared to those of the individual extracts measured separately. Complete additivity was observed, indicating the absence of positive or negative effectors in either of the two preparations.

The same conclusion was reached on the basis of failure to obtain any differential decrease in NADP-dependent reaction, by dialysis or gel-filtration (Sephadex G-75) of extracts from light-exposed leaves. The results of a typical gel-filtration experiment are shown in Table III.

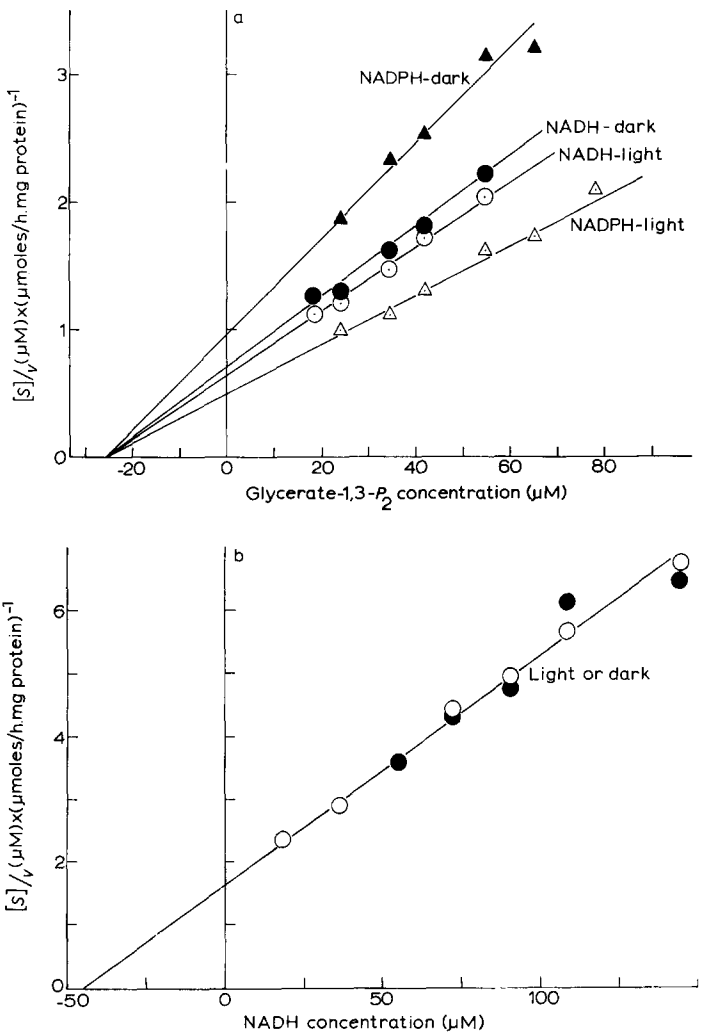
*Kinetic experiments**

The reversible light induced increase of the NADP-dependent glyceraldehyde-3-*P* dehydrogenase activity was examined extensively by kinetic methods. The purpose of these experiments was to examine the kinetic aspects of the activation in conditions as close as possible to the situation *in vivo*. For this reason, no attempt was made to partially purify the enzymes and crude extracts were used directly. A detailed study of the kinetic properties of plant glyceraldehyde-3-*P* dehydrogenases requires the use of homogeneous enzyme preparations and is currently in progress in this laboratory. The enzymatic activity of the crude extracts was stable for several hours, and the values of the kinetic parameters were reproducible. Since the enzymatic reactions to be studied were bimolecular in nature, the K_m and v_{max} values measured

* The K_m values given here are slightly different from values previously reported due to the fact that glyceraldehyde-3-*P* prepared from the Sigma diethylacetal derivative is inhibitory at high concentrations (>0.6 mM), its effect appearing as a decrease of the apparent affinity for $NADP^+$ and NAD^+ with increasing glyceraldehyde-3-*P* concentration. Other preparations of glyceraldehyde-3-*P* diethylacetal dibarium salt (Boehringer) were observed to be competitively inhibitory against the NAD reaction alone. These difficulties can be avoided by using the glyceraldehyde-3-*P* free acid preparation from Sigma. Furthermore, studies with purified preparations of the enzyme measured in the "oxidative" reaction have shown that the apparent K_m values are dependent on concentration of the "fixed" substrate in a complex way (data not shown). The conclusions that have been drawn from the kinetics, however, are not invalidated by these findings, provided that identical concentrations of the fixed substrate are always used (as was the case); small variations in affinity, which might escape detection, could not, in any case, account for the gross change in the reaction rates under light *versus* dark conditions.

for one substrate are a function of the concentration of the other substrates. The concentration of the "fixed" substrates were, therefore, maintained constant throughout any given set of experiments. Thus, even though one cannot ascribe an absolute meaning to the kinetic parameters measured, these parameters appear to be satisfactory for comparisons of the enzymatic reactions under light and dark physiological conditions. It should be emphasized moreover that all the reaction velocities in the "oxidative" reaction were measured with arsenate replacing phosphate; this substitution might have a significant effect on the kinetic properties of the reaction.

The kinetic parameters measured in the reductive reaction are shown in Fig. 2. The light triggered enhancement of NADPH-dependent dehydrogenase activity is shown to depend on an increase of the maximum velocity, whereas the apparent K_m's of NADPH and glycerate-1,3-P_2 remain unchanged (within the limits of experimental



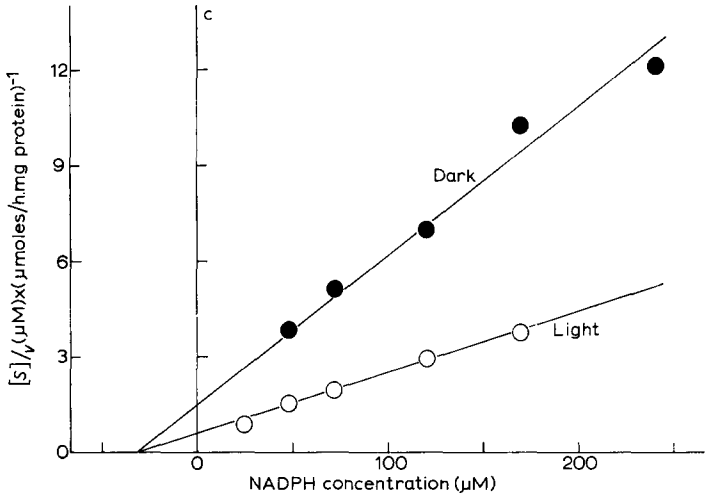


Fig. 2. Velocity of NADPH- and NADH-dependent reduction of 1,3-diphosphoglycerate as a function of substrate concentrations. The reaction mixture contained: 2,4,6-collidine buffer (pH 7.2), 40 mM; GSH, 1 mM; cysteine, 5.2 mM; $MgCl_2$, 8 mM; sodium 3-phosphoglycerate, 1.25–21 mM (Fig. 2a) or 21 mM (Figs. 2b and c); ATP, 1.1 mM; 3-phosphoglycerate kinase from yeast, in excess; NADPH or NADH, 0.15 mM (Fig. 2a) or as indicated (Figs. 2b and c); and pea leaf extract from dark-maintained or light-treated plants. The reaction was started, after 6 min preincubation, by addition of the coenzyme; variation in $A_{340\ nm}$ was followed for 30 sec with a Cary, Model 15 spectrophotometer. The concentration of 1,3-diphosphoglyceric acid was calculated assuming an equilibrium constant for the kinase reaction equal to $3 \cdot 10^{-4}$ (see ref. 10).

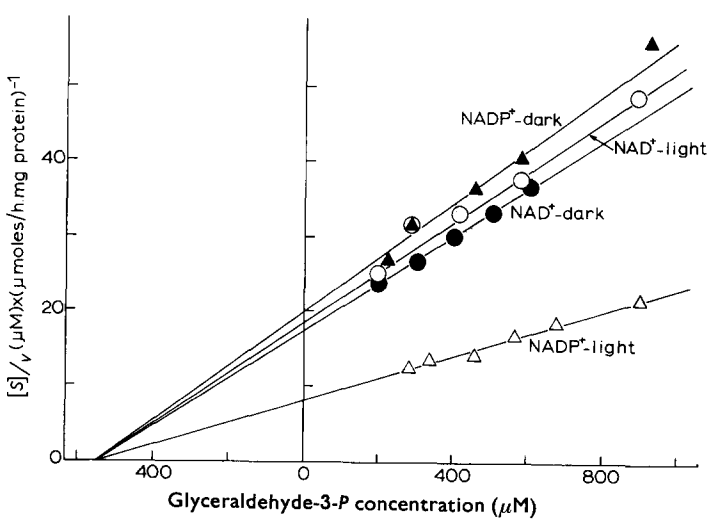


Fig. 3. Velocity of NADP- and NAD-dependent oxidation of glyceraldehyde-3-*P* as a function of substrate concentration. The reaction mixture contained: Tris–HCl buffer (pH 8.5), 67 mM; cysteine, 4 mM; NaF, 20 mM; sodium arsenate, 17 mM; $NADP^+$ or NAD^+, 0.4 mM; glyceraldehyde-3-*P*, 0.5 mM and pea leaf extract from dark-maintained and light-treated plants. The reaction was started after 6 min of preincubation, by the addition of glyceraldehyde-3-*P*.

error). Both v_{max} and K_m values of the NADH dependent reaction are virtually unaffected by the preillumination treatment.

Comparable results were obtained for the oxidative direction, as shown in Fig. 3. The K_m apparent values for glyceraldehyde-3-*P* in the reactions with the given concentrations of both coenzymes are similar and do not seem to vary in "light" *versus* "dark" experiments. The only marked change promoted by light is an increase of the v_{max} value, in the light, for the NADP-linked reaction.

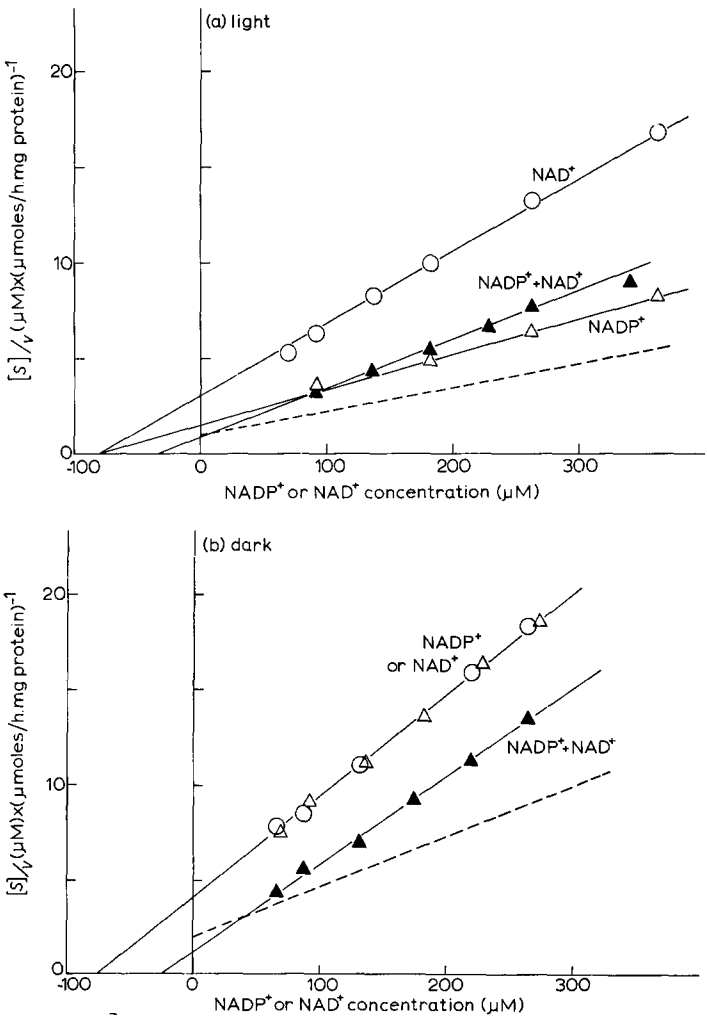


Fig. 4. Lack of additivity of NADP+- or NAD+-dependent glyceraldehyde-3-*P* dehydrogenase activities in crude extracts of light-treated (Fig. 4a) or dark-maintained (Fig. 4b) pea leaves. Conditions as in Fig. 3. In the mixed substrate experiments both NADP+ and NAD+ were present in the reaction mixture at equimolar concentrations and the overall reduction rate of pyridine nucleotide coenzymes was measured at 340 nm; in these experiments the concentration indicated in abscissa corresponds to that of one of the two coenzymes present. The dashed lines correspond to the theoretical reaction velocities calculated assuming complete additivity between the NAD+- and NADP+- dependent reaction rates.

Fig. 4 shows two experiments demonstrating competition between NAD^+ and $NADP^+$. As already reported[4], the reaction velocity measured in the presence of both $NADP^+$ and NAD^+ is markedly lower than the value calculated assuming a complete additivity of the two activities, measured separately (this last velocity is indicated with a dashed line in Fig. 4). The conclusion that can be drawn from these experiments is the same as in the case of the "reductive" reaction, namely, the affinities for the two coenzymes remain constant under light and dark conditions and the only noticeable difference is in the v_{max} value for $NADP^+$.

DISCUSSION

Partial resolution of NAD-linked activity by salt precipitation

Current views on the intracellular distribution of glyceraldehyde-3-*P* dehydrogenase enzymes assume that in photosynthetic tissues 40–60% of the NAD-dependent activity is located in the chloroplast, the remainder being present in the cytoplasm. These conclusions have been based on experimental evidence indicating that (a) NADP-dependent activity is entirely present in the plastid fraction, prepared with aqueous[11] or non-aqueous techniques[8], and it is associated with 40–60% of the NAD-linked activity; (b) the appearance of the NADP-dependent dehydrogenase is generally correlated with the chloroplast differentiation[9,12] and (c) the light promoted enhancement of the NADP-linked activity can also be observed in suspensions of isolated chloroplasts[13,14].

Our salting out experiments are in agreement with this evidence since they show that a fraction enriched in NAD-linked activity can indeed be isolated by $(NH_4)_2SO_4$ fractionation. It can be tentatively concluded that the 60–80% $(NH_4)_2SO_4$ fraction contains most of the NAD-specific enzyme present in the cytoplasm. The 40–60% fraction, on the contrary, contains nearly all of the NADP-dependent activity and a part of the NAD-linked activity, which are not separable by repetitive salt precipitation; this fraction should be considered to contain the plastid enzyme(s). Evidence is now available that the two NAD-dependent activities, separated by salt fractionation also exhibit different affinities for NAD^+ and different response to the presence of $NADP^+$ in the assay mixture (unpublished observations). This supports the concept that the NAD-dependent dehydrogenases present in the two separable fractions are indeed different enzymes.

On the other hand, the relative lack of additivity between NADP and NAD activities, always seen in preilluminated and in dark treated leaves, is rather direct evidence of the presence in crude extracts of a pyridine nucleotide non-specific enzyme. An alternative explanation could be looked for in an inhibitory effect of NADP- on NAD-dependent enzyme or *vice versa*. We at present prefer, however, the former interpretation on the basis of the facts that (a) a glyceraldehyde-3-*P* dehydrogenase, pyridine nucleotide non-specific, has been already reported in blue-green algae[15] and in *Alcaligenes faecalis*[16], and (b) other examples of non-specific dehydrogenases were described in different systems[17]. Moreover, the impossibility of separating completely the two activities, stated by other authors, who always used the 40–60% $(NH_4)_2SO_4$ fractions, correlates well with these conclusions. We propose, therefore, that at least one nucleotide non-specific glyceraldehyde-3-*P* dehydrogenase is present in the pea leaves, and it is located in chloroplasts. The localization of the NAD-specific and the

pyridine nucleotide non-specific enzymes in photosynthetic cells will be the topic of a subsequent paper.

The activation by light of NADP-linked activity

The experiments with inhibitors described above conclusively exclude any role of net protein synthesis on the reversible increase of NADP-dependent glyceraldehyde-3-*P* dehydrogenase activity promoted by light in plants. The explanation of the light activation must therefore be sought elsewhere, perhaps as a regulatory effect at the level of enzyme activity. The work done in ZIEGLER's laboratory[6,18] has clearly shown that the activation is strictly correlated with photosynthetic non-cyclic electron flow.

In our experiments the activation *in vivo* results in an increase of the v_{max} of the $NADP^+$- and NADPH-dependent activities expressed on a total protein basis, whereas neither the v_{max} of NAD^+- and NADH-linked reactions nor any of the apparent K_m values of the substrate are significantly affected. Moreover, within the range of concentrations used (from 0.03 up to 2 mM) we were unable to detect any evidence of cooperative substrate effects, *i.e.* kinetics higher than first order, either in NAD- or NADP-linked reactions measured in the oxidative and in the reductive directions.

On this basis, the mechanism of activation of the NADP-linked activity could be possibly explained either as a variation of the turn-over number of the NADP-linked enzyme, or by assuming that the fraction of the protein active with NADP is (reversibly) increased when leaves are exposed to light. MÜLLER *et al.*[13] have suggested a mechanism of conversion of the NAD-dependent enzyme of chloroplasts to a form specific for NADP. This hypothesis was originally put forward by FULLER AND HUDOCK[19] for the y-2 strain of *Chlamydomonas reinhardii*. This kind of conversion would involve a marked simultaneous decrease of the NAD-linked activity, for which, however, we have found no definite evidence. Hence, it can be suggested that, if a partial conversion does indeed take place, it will be between an enzyme form active solely with NAD to a form active with both pyridine nucleotides (as suggested by the low response of the NAD activity to illumination). In principle the analysis of a competition experiment offers the tool for a direct test of this hypothesis, since it is theoretically possible to measure directly the fraction of enzyme on which the two coenzymes compete. The numerical values of the "competitive fraction" we obtained in several experiments range between 0.5 and 0.7 in extract from leaves kept in the dark and around 0.7 to 0.9 for extract of preilluminated leaves. Although the extreme sensitivity of these calculations to experimental errors prevent us from overestimating their significance, we believe that the results of the competition experiments indicate a substantial constancy of the "competitive fraction". These data therefore do not support the conversion model, which seems not sufficient by itself to account for the activation phenomenon.

It has been suggested recently that NADPH (ref. 14) and ATP (ref. 20) are the natural activators *in vivo* of the NADP-dependent triosephosphate dehydrogenase reaction, acting as typical "allosteric effectors". This suggestion was based principally on the sigmoidal relation between reaction rate and concentration of the effectors, observed using a briefly sonicated chloroplast suspension preincubated for 20 min with NADPH or ATP. Another indication of the possible allosteric behavior of the enzyme was identified in the apparent desensitization of the enzyme by heat. However,

the lack of cooperative substrate effects, and the failure to obtain a differential decrease in the NADP linked activity by gel filtration or by mixing extracts derived from light and dark exposed leaves, must be considered in evaluating the possibility of NADPH or ATP acting as classical allosteric effectors. If the variation in NADP-linked activity is really due to a modifier effect by NADPH, or ATP, the unusual properties of the modulation should be noted: sluggishness of action[14,20], possible tight bindings of the modifier (*cf.* Table II), enhancement of turnover number in both oxidative and reductive reaction with very little change, if any, of K_m values (*cf.* Figs. 2–4).

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