GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IN PHOTOSYNTHETIC TISSUES: KINETIC EVIDENCE FOR COMPETITIVITY BETWEEN NADP AND NAD.

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It is well established that photosynthetic tissues possess two different glyceraldehyde-3-phosphate dehydrogenase activities respectively dependent from NAD and NADP. The former, which is located in chloroplasts (Heber et al. 1963), is generally thought to be involved with the CO_2 reduction cycle. The latter, equally distributed between the cytoplasm and the plastids, is concerned with glycolysis. Accordingly only the NAD-dependent activity is present in non-photosynthetic structures, such as roots and seeds (Gibbs 1952).

Recently some data have accumulated that may suggest a light-promoted conversion of the NAD-dependent enzyme to the NADP-dependent enzyme (Ziegler et al. 1965 and 1968). The experiments reported here prove that in a pea leaf extract the glyceraldehyde-3-P dehydrogenase reaction occurs with both pyridine nucleotide coenzymes and that these are competitive to each other.

Materials and Methods

Pea plants (Pisum sativum, cultivar Provenza) were grown in a greenhouse on vermiculite and sand plates at 23 ± 2°; before the experiments the plants were placed for 12 hours under a bank of

three fluorescent lamps (about 3500 lux). The leaves were ground in a mortar in the cold with 15 to 20 volumes of 0.03 M tris HCl pH 8.5 containing 0.01 M EDTA, and the homogenate was centrifuged for 2 minutes at 1500 x g to remove the cell debris. The extract was used for kinetic experiments without further treatment. The glyceraldehyde-3-P dehydrogenase activity was assayed according to Gibbs (1955), using a Cary Mod. 15 spectrophotometer; glyceraldehyde-3-P was prepared from the diacetal derivative (SIGMA Chemical Company, St. Louis). The glyceraldehyde-3-P concentration was measured following the reduction of NAD promoted by an excess of muscle dehydrogenase (SIGMA); the NAD concentration was determined by means of the same reaction in presence of an excess of glyceraldehyde-3-P, and the NADP one using the glucose-6-P dehydrogenase reaction.

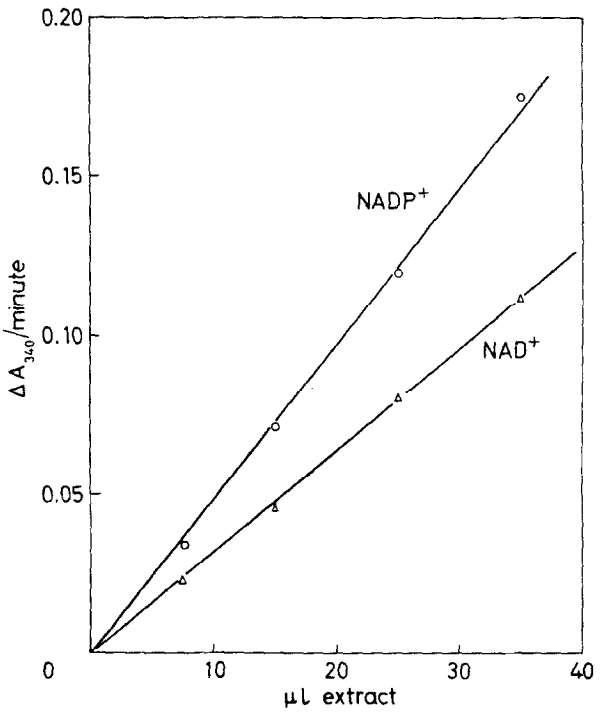

Figure 1. Linearity between the rates of glyceraldehyde-3-P dehydrogenase reaction and the amount of pea leaf extract added. Assays contained in a final volume of 1.2 ml: tris HCl pH 8.5 40 µMoles, NaF 24 µMoles, Na_2HAsO_4 20.4 µMoles, Cysteine 4.8 µMoles, NAD or NADP 0.4 µMoles, 3-GAP 1.2 µMoles.

Results

As shown in Figure 1 the rate of reduction of NADP or NAD was linear with the amount of extract added to the reaction mixture. The results of a typical experiment are shown in Figure 2. Each experiment was performed using the same extract; both the activities were absolutely stable during the course of the experiments. The NADP- and NAD-dependent activities, when separately measured, follow a Michaelis type kinetics for a wide range of coenzyme concentrations; the following kinetic constants are calculated: for NADP, K_s = 0.17 mM, and V_s = 3550 uMoles/h/g of leaf, and for NAD, K_s = 0.14 mM, and V_s = 1150 uMoles/h/g of leaf. In

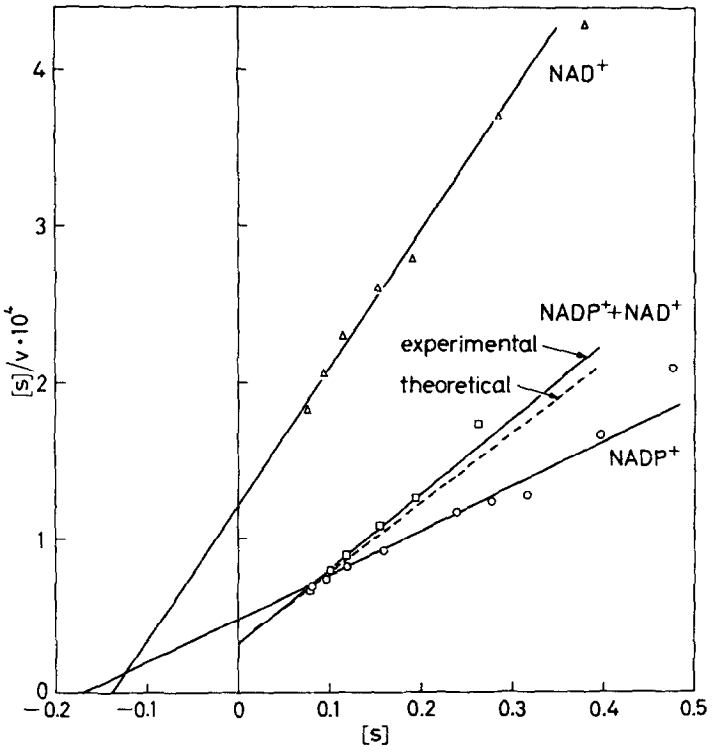

Figure 2. Effect of NAD, NADP, or of an equimolar mixture of both on the glyceraldehyde-3-P dehydrogenase activity of a pea leaf extract. Condition as in Figure 1. [s] corresponds to the mM concentration of one coenzyme either added separately or mixed. v corresponds to the reaction rate expressed in µMoles/h/g of leaf. The protein content of the extract was 51.5 mg/g of leaf.

the presence of both NADP and NAD, at equal concentrations and over a wide range of concentrations, the rates observed are considerably lower than the sum of the rates with the two acceptors measured separately (Fig. 2). The V_s in these conditions is 2200 uMoles/h/g of leaf. The experimental data are in quite good agreement with the theoretical pattern, as calculated assuming a mutual competitivity of the two coenzymes on the same protein site (Dixon and Webb, 1965).

The additivity of the NADP- and NAD-dependent activities, when the purified NAD-dependent muscle enzyme is added to the reaction mixture, was observed in a separate experiment as shown in Tab. 1.

Table 1

Additivity of the reaction rates obtained with the plant extract and with purified muscle enzyme. Conditions: as in Figure 1.

Muscle Enzyme	Pea Leaf Extract	Δ A / min NADP	Δ A / min NAD	Δ A / min NADP + NAD
10 µl	---	---	0.036	0.036
---	7 µl	0.070	0.036	0.055
10 µl	7 µl	---	0.071	0.090

Discussion

The results presented here strongly suggest that the NADP- and the NAD-dependent glyceraldehyde-3-P dehydrogenase reaction is catalyzed by the same enzyme in pea leaf extract.

Gibbs (1965) undertook the purification of the NADP-dependent enzyme from pea leaves, and succeeded in obtaining a preparation devoid of the NAD activity. Only the last step of his purification, involving a loss of over than 95% of the total activity, resulted in an absolute specificity for NADP. This might suggest

that the loss of NAD acticity might be due to inactivation rather than separation of two proteins.

Recently Hood and Carr (1967) have reported evidence that in the blue-green alga Anabaena variabilis a single enzyme is responsible for both NAD- and NADP-dependent activities.

Hudock and Fuller (1965) were unsuccessful in separating the NAD- and NADP-dependent activities in Chlamydomonas reinhardi. The same authors have demonstrated that in Chromatium a single protein, NAD-dependent, can be changed in its structure by the conditions of growth of the microorganism, so as to favor the oxidation of glyceraldehyde-3-P or the reduction of 1,3-diphosphoglycerate. Autotrophic conditions favor the reductive version of the enzyme (low K_s for 1,3-DiPGA), whilst heterotrotrophic conditions favor the "glycolytic" structure (large K_s for 1,3-DiPGA).

All these results prove that the lower organisms possess a single protein connected with photosynthesis and glycolysis. On the basis of our results, this situation could be extended also to the photosynthetic tissues of higher plants. The possibility that a single enzyme is endowed with glyceraldehyde-3-P dehydrogenase activities, dependent from both NAD and NADP, suggests the occurrence of a strict correlation between the photosynthetic and glycolytic pathway and offer a potential site of metabolic regulation.

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