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*Short Communication*

# A Mechanism for the Indirect Transfer of Photosynthetically Reduced Nicotinamide Adenine Dinucleotide Phosphate from Chloroplasts to the Cytoplasm[1]

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## ABSTRACT

**A triose phosphate/3-phosphoglycerate shuttle for the indirect transfer of photosynthetically reduced NADP from chloroplasts to the cytoplasm has been demonstrated *in vitro*. Triose phosphate, formed from 3-phosphoglycerate in the chloroplast, was oxidized back to 3-phosphoglycerate outside the chloroplast by the nonreversible D-glyceraldehyde 3-phosphate dehydrogenase reaction which is specific for NADP. The 3-phosphoglycerate could presumably return to the chloroplast to complete the shuttle. The properties of nonreversible D-glyceraldehyde 3-phosphate dehydrogenase are considered particularly suitable for effective operation of this shuttle system.**

During photosynthesis in the chloroplast, NADPH and ATP are synthesized and may be subsequently utilized in the Calvin cycle for the conversion of 3-P-glycerate to D-glyceraldehyde-3-P. It is generally agreed that photosynthetically generated NADPH and ATP are not directly available for reactions outside the chloroplast, since neither of these compounds can penetrate the envelope of whole chloroplasts in significant amounts (5, 6). However, a shuttle system involving triose-P and 3-P-glycerate, both of which easily penetrate the chloroplast envelope (2, 4), has been demonstrated for the indirect transfer of reducing power and ATP from chloroplasts to the cytoplasm (3, 9, 10). Operation of this shuttle system requires the oxidation of D-glyceraldehyde-3-P to 3-P-glycerate in the cytoplasm by two enzymes, namely, NAD-linked reversible D-glyceraldehyde-3-P dehydrogenase and 3-P-glycerate kinase and therefore is dependent on the levels of adenine nucleotides, Pi, and NAD(H).

From recent work in this laboratory, it was proposed that an analogous shuttle system may exist for the indirect transfer of NADPH from chloroplasts to the cytoplasm utilizing nonreversible D-glyceraldehyde-3-P dehydrogenase (8). This enzyme seemed particularly suitable for this role, since evidence was obtained that it is located outside the chloroplast *in vivo* and that the affinity for the substrates is quite high. The participation of nonreversible D-glyceraldehyde-3-P dehydrogenase in such a shuttle system has now been demonstrated *in vitro*.

## MATERIALS AND METHODS

Chloroplasts were isolated from leaves of commercially grown spinach (*Spinacia oleracea* L.) by a modification of the method of Jensen and Bassham (7): 10 g of chopped leaves were homogenized for 2 sec in 40 ml of a solution, pH 6.8, containing 0.33 M sorbitol, 50 mM HEPES-KOH buffer, 2 mM EDTA, 1 mM PPi, 1 mM $MgCl_2$, and 1 mM $MnCl_2$. The homogenate was filtered through Miracloth (Calbiochem), centrifuged at 2500*g* for 80 sec, and the pellet of chloroplasts was resuspended in 2 ml of the solution used above, except that the pH was 7.2.

A partially purified preparation of nonreversible D-glyceraldehyde-3-P dehydrogenase was obtained from the cotyledons of pumpkin (*Cucurbita pepo* L., var. Spookie) after germination in the dark for 9 days at 22 C. The purification procedure was as described previously for the enzyme from pea shoots (8), but with the following modifications: the Sephadex step was omitted, the buffer used for the second dialysis and on the diethylaminoethyl cellulose column was HEPES-NaOH, pH 7.7, and the elution of the enzyme from the diethylaminoethyl cellulose required a gradient of NaCl concentration between 0.05 M and 0.3 M. The enzyme preparation contained fructose-1,6-diP aldolase and a high activity of triose-P isomerase, but was free of reversible D-glyceraldehyde-3-P dehydrogenases (both NAD- and NADP-linked) and NADPH oxidase.

Reaction mixtures were of the composition described in Table I and were contained in a 1-ml compartment connected to a Clark-type oxygen electrode which had been previously calibrated. NADPH was estimated enzymatically: 0.5 ml of reaction mixture (after removal of chloroplasts) and 2 μmoles of pyruvate were combined in a final volume of 1 ml, and the change in absorbance of 340 nm was recorded following the addition of 14 units of lactic acid dehydrogenase.

## RESULTS AND DISCUSSION

Nonreversible D-glyceraldehyde-3-P dehydrogenase effectively catalyzed the indirect transfer of photosynthetically reduced NADP from chloroplasts. In the complete reaction mixture at least 1 mole of NADP was reduced outside the chloroplast for each mole of oxygen evolved during photosynthesis.

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Table I. *Demonstration of Shuttle System for the Indirect Transfer of NADPH across the Chloroplast Envelope*

The complete reaction mixture contained, in a final volume of 1 ml, 0.33 mmole of sorbitol, 50 μmoles of HEPES-KOH buffer, 2 μmoles of EDTA, 1 μmole of PPi, 1 μmole of $MgCl_2$, 1 μmole of $MnCl_2$, 1 μmole of 3-P-glycerate, 0.1 of μmole NADP, pumpkin-cotyledon preparation containing 0.1 unit nonreversible D-glyceraldehyde-3-P dehydrogenase, and spinach chloroplasts containing 25 μg of Chl. The final pH was 7.8. Oxygen evolution was followed during 15 min of photosynthesis with a light intensity of 1500 ft-c. The reaction mixture was then removed, centrifuged, and NADPH in the supernatant was estimated enzymatically with pyruvate and lactic acid dehydrogenase. Chl was determined by the method of Arnon (1).

| Reaction Mixture | Oxygen Evolved | NADP Reduced |
|---|---|---|
| | *μmoles/mg Chl·hr* | |
| Complete | 10.9 | 14.1 |
| Minus 3-P-glycerate | 4.1 | 2.9 |
| Minus NADP | 6.4 | 0.3 |
| Minus nonreversible D-glyceraldehyde-3-P dehydrogenase | 5.7 | 1.8 |
| Minus chloroplasts | −0.5 | 0 |
| Dark control | −3.3 | 0.5 |

The results (Table I) show that this NADP reduction was largely dependent on all components of the reaction mixture and was completely light-dependent; the amount of NADPH formed in the complete system was over four times greater than that formed when any one component was omitted. The ratio of NADP reduced to oxygen evolved was also four times greater in the complete system. However, this ratio was relatively high in the absence of 3-P-glycerate, suggesting that this compound was being generated from an endogenous source.

We consider the doubling of the rate of $O_2$ evolution by a system which returns glyceraldehyde-3-P to 3-P-glycerate to indicate that the aldotriose, or perhaps glycerate-1,3-diP, regulates the metabolism of this preparation. However, glyceraldehyde-3-P in moderate concentrations has hitherto been demonstrated to stimulate photosynthetic $CO_2$ assimilation in chloroplast preparations (2). Finally, the extent of shuttling deserves comment. If all the carbon substrate were used to generate NADPH in this shuttle, the theoretical ratio would be 2 NADPH/$O_2$ evolved. In the complete system a ratio of roughly unity was observed, indicating that half of the glyceraldehyde-3-P was transported across the chloroplast envelope, resulting in a very effective mechanism of generating extra chloroplastic pyridine nucleotide.

A possible shuttle mechanism which could explain these results is shown in Figure 1. This mechanism is similar to that proposed by Stocking and Larson (10) except that the conversion of D-glyceraldehyde-3-P to 3-P-glycerate outside the chloroplast requires only one enzyme and NADP, rather than NAD, is reduced. Nonreversible D-glyceraldehyde-3-P dehydrogenase catalyzes an irreversible reaction and has a high affinity for the substrates, particularly for NADP (the *Km* values for D-glyceraldehyde-3-P and NADP were approximately 20 μM and 3 μM, respectively [8]). It, therefore, seems probable that *in vivo* any triose-P which passes out of the chloroplast would be rapidly oxidized to 3-P-glycerate providing NADP was available. Hence in the light this shuttle system may be expected to reduce essentially all NADP outside the chloroplast. This may, indeed, be true; Heber and Santarius (5) have provided evidence that most NADP in the cytoplasm of leaf cells occurs in the reduced state.

The nonreversible D-glyceraldehyde-3-P dehydrogenase reaction is neither readily reversible nor is it significantly affected by adenine nucleotide or Pi(8). Consequently, that portion of the NADPH shuttle which is outside the chloroplast is probably not influenced by the levels of adenine nucleotides and Pi and, being irreversible, may be relatively insensitive to the NAD(P)H/NAD(P) ratio. This situation would be in contrast to the regulatory effects of these metabolites on the shuttle system involving NAD-linked reversible D-glyceraldehyde-3-P dehydrogenase as shown by Krause (9). This latter shuttle system may, therefore, have the advantage of being more sensitive to the energy status of the cytoplasm and also more efficient in that, besides transferring reducing power from the chloroplast (10), ATP is also transferred (3, 9).

The relative roles of the two shuttle systems may, therefore, be considered. Previous experiments in this laboratory have shown that the activity of nonreversible D-glyceraldehyde-3-P dehydrogenase in crude plant extracts is clearly detectable and considerably greater than the activity of NAD-linked reversible D-glyceraldehyde-3-P dehydrogenase when the concentrations of triose-P and pyridine nucleotides approximate *in vivo* levels (8). These observations suggest that the shuttle forming NADPH (involving nonreversible D-glyceraldehyde-3-P dehydrogenase) could compete more than favorably with the shuttle forming NADH and ATP (involving NAD-linked reversible D-glyceraldehyde-3-P dehydrogenase). This by no means infers that the latter system is unimportant; at the onset of photosynthesis the triose-P/3-P-glycerate shuttle may be expected to transfer NADPH first, but as soon as most NADP outside the chloroplast is in the reduced state the shuttle could continue to transfer energy as NADH and ATP. In this way, NADPH for biosynthetic reactions would be given first priority while transfer of energy for other purposes could be controlled by the energy conditions outside the chloroplast.

Finally, an increasing NADPH/NADP ratio could be an effective means of controlling the flow of carbohydrate through the oxidative pentose phosphate cycle.

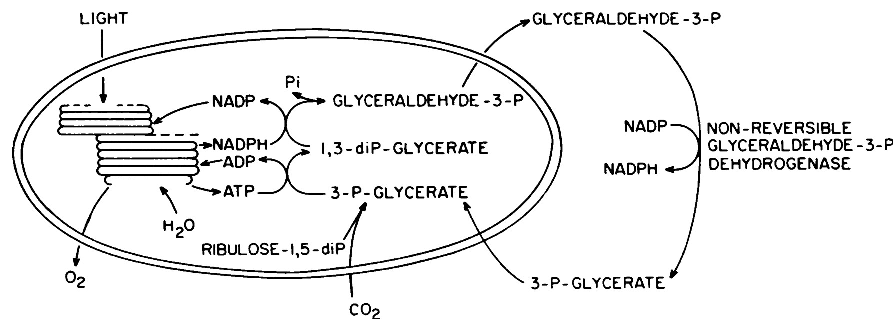


FIG. 1. Proposed mechanism for the indirect transfer of photosynthetically reduced NADP from chloroplasts to the cytoplasm.

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