# A CHLOROPLAST CYTOPLASMIC SHUTTLE AND THE REDUCTION OF EXTRAPLASTID NAD

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Summary: A triosephosphate-phosphoglyceric acid shuttle system that transfers photosynthetically generated reducing power from intact chloroplasts to the surrounding medium is described. The reduction of exogenously added NAD by isolated intact chloroplasts was found to be light and PGA dependent.

A number of physiological activities show light stimulations that have been linked in some cases to photosynthetically trapped energy but not directly to $CO_2$ reduction. For example, ADP and ATP appears to pass readily through the plastid envelope under certain conditions (8,9) and light stimulated potassium absorption by leaf tissue is associated with ATP generated by cyclic photophosphorylation (7).

In addition to photosynthetically produced ATP and carbohydrates that may move out of the chloroplast and participate in cytoplasmic reactions, a glycolate-glyoxylate shuttle that would transfer reducing power out of chloroplasts has been proposed by Kisaki and Tolbert (6).

The direct transfer of NADPH from the chloroplast appears to be very slow (4,8). This results in a compartmentation of pyridine nucleotides between the cytoplasm and the chloroplast, and the question then arises of the possible existence of a mechanism that would facilitate the transfer of hydrogen from chloroplasts to the cytoplasm. Heber and Santarius (4) discussed some possible shuttle mechanisms but concluded that their results indicate that the efficiency of a hydrogen shuttle system must be rather low.

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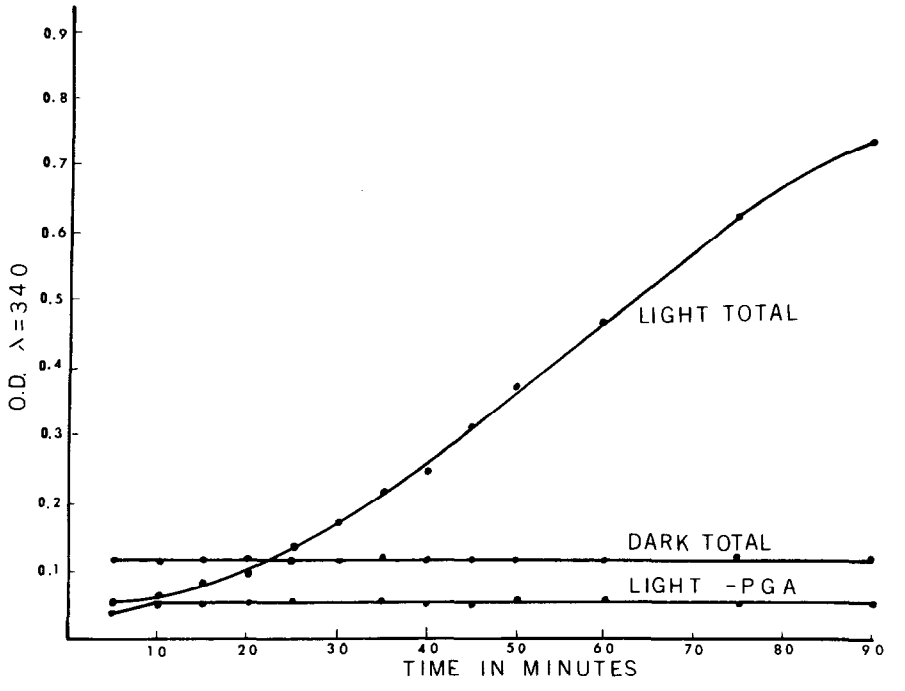


Figure 1. Influence of light and phosphoglyceric acid on the reduction of exogenous NAD by isolated chloroplasts. For details of reaction conditions see Table 1.

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The present communication contains evidence of a light stimulated reduction of extraplastid NAD *in vitro* through the action of a phosphoglyceric acid-triosephosphate shuttle system.

Methods: Chloroplast preparations containing a high percentage of intact plastids were isolated from spinach (*Spinacia oleracea* L.) leaves by the methods of Jensen and Bassham (5) and Walker and Hill (10). Oxygen evolution was determined with a Gilson Oxygraph.

Results and Discussion: The outer envelopes of intact, isolated chloroplasts are permeable to exogenously added PGA (1,2,8). In addition, when such plastids are fed $C^{14}O_2$ in the light, several carbon cycle intermediates

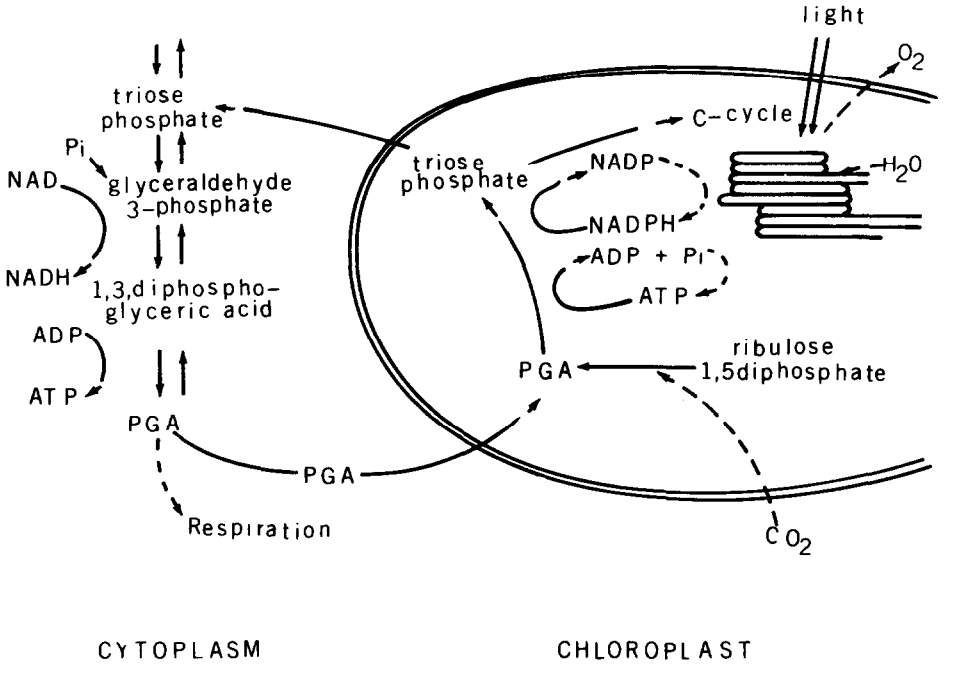


Figure 2. A diagrammatic representation of the chloroplast cytoplasmic shuttle system that results in the reduction of extraplastid NAD.

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including triosephosphates move through the plastid envelope into the external medium (3). Consequently it is conceivable that triosephosphates photochemically produced during the reduction of PGA could move out of the chloroplast and become oxidized (through glycolysis) to PGA which in turn could enter the chloroplast to repeat the cycle (Figure 2).

In order to test whether such a shuttle system might operate a light driven reduction of NAD, the effect of PGA on the reduction of NAD was studied with isolated chloroplasts.

A preparation containing broken and intact washed spinach chloroplasts is unable to reduce exogenously added NAD in the absence of added ferredoxin. However, when such chloroplasts are incubated in the presence of PGA, ADP and Pi, a light stimulated reduction of NAD occurs if glyceraldehyde-3-phosphate dehydrogenase and phosphoglyceroakinase are present in the reaction

Table 1

REDUCTION OF EXOGENOUS NAD AND NADP BY ISOLATED CHLOROPLASTS

| Treatment | O.D. Change at 340 nm |
|---|---|
| NADP, Complete, in the light | 0.130 |
| NAD, Complete, in the light | 0.403 |
| NADP, minus PGA | 0.180 |
| NAD, minus PGA | 0.093 |
| NADP, Complete, in the dark | 0.044 |
| NAD, Complete, in the dark | 0.034 |

The complete reaction mixture contained in a final volume of 3.3 ml, intact and broken chloroplasts containing 67 µg of chlorophyll and the following concentrations: sorbitol, 0.33M; $NaNO_3$, 4 mM; EDTA, 4 mM; Na isoascorbate, 4 mM; $MnCl_2$, 2 mM; $MgCl_2$, 2 mM; $K_2HPO_4$, 10 mM; 2 mM ADP; HEPES buffer (N-2-Hydroxyethyl Piperazine-N-2 Ethanesulfonic acid) pH 7.6, 0.05 M; $Na_4P_2O_7$, 5 mM. In addition the mixture contained 3 µmoles of PGA, 3 µmoles of NAD or NADP, 1.3 units of glyceraldehyde-3 phosphate dehydrogenase, and 2 units of 3-phosphoglyceric phosphokinase. The reaction was run 30 minutes. Light intensity was 1000 ft-c.

medium or if supernatant from the isolated plastids is added back to the preparation.

Figure 1 shows that this reduction of NAD is dependent on both PGA and light. In the absence of PGA, no reduction of NAD takes place. The nucleotide apparently is unable to pass readily through the envelope of intact plastids. The naked thylakoids from broken and washed plastids have lost their associated ferredoxin thus there is no light dependent reduction of NAD.

Exogenous NADP, being unable to penetrate rapidly into intact chloroplasts, does not act effectively as a Hill oxidant in these chloroplasts, and as would be expected, the triosephosphate-PGA shuttle system is not effective in bringing about the reduction of NADP in washed chloroplast preparations (Table 1).

Although the *in vitro* operation of this system (diagramed in Figure 2) can be observed, it does not follow that it is important *in vivo*. Whether or

not the shuttle system is important under certain physiological conditions as a mechanism of transfer of photochemically trapped energy from chloroplasts to cytoplasm must be determined by further studies.

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