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Chloroplast Respiration[1]

A MEANS OF SUPPLYING OXIDIZED PYRIDINE NUCLEOTIDE FOR DARK CHLOROPLASTIC METABOLISM

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ABSTRACT

A spinach (*Spinacia oleracia* var. America) chloroplast particle fortified with ferredoxin, fructose-1,6-bisphosphate, or ribose-5-phosphate and NADP has been shown to generate NADPH by the oxidation of glyceraldehyde-3 phosphate to glycerate-3-phosphate (PGA) and to reduce ferredoxin with the NADPH. The resulting reduced ferredoxin can reduce O_2 to H_2O_2, nitrite to ammonia, or protons to H_2. Hydrogen production was the result of adding hydrogenase from *Chlamydomonas reinhardii* to the chloroplast preparation. The predicted stoichiometry of 1 PGA:1 O_2 in the absence of and 2 PGA:1 O_2 in the presence of catalase was observed indicating H_2O_2 as the end product of O_2 reduction. The predicted stoichiometry of 3 PGA:1 nitrite:1 ammonia was also observed. A scheme is presented to account for a sustained generation of NADP and ATP necessary for the dissimilation of starch in the darkened chloroplast. The unifying term chloroplast respiration is introduced to account for those reactions in which reduced ferredoxin interacts with physiological acceptors other than NADP or nitrite, hydrogen, or O_2 respiration when nitrite, protons, or O_2 is the ultimate electron acceptor.

Starch granules within the chloroplast are degraded through phosphoclastic and hydroclastic reactions resulting in the formation of glucose-1-P and free sugars such as maltose and glucose as the initial products (15, 23, 27, 31). The conversion of these compounds to dihydroxyacetone-P and PGA,[4] the major species exported from the chloroplast to the cytoplasm, is facilitated by the reactions of either glycolysis (Embden-Meyerhof pathway) or of the oxidative pentose-P pathway (16) coupled to glycolysis. The operation of these multienzyme systems demands a constant supply of ATP (phosphate cycle) and oxidized pyridine nucleotide (hydrogen cycle). The rate of starch breakdown within the chloroplast is restricted by the limited movement of adenylate and nicotinamide nucleotides between the organelle and the cytoplasm. Since chloroplasts lack an operating mitochondrial-type oxidative electron transport chain in the dark, ATP formation through substrate level phosphorylation and completion of the hydrogen cycle related to starch breakdown must be accomplished by another route.

When glucose-6-P or G3P is oxidized to gluconate-6-P or PGA, respectively, the hydrogen is accepted by NAD(P) and the reduced pyridine nucleotide must be oxidized before the process can continue. In complete glycolysis, acetaldehyde or pyruvate which serves as the physiological hydrogen acceptor is derived from PGA. The chloroplast seems to have an incomplete glycolytic system in that PGA is not further metabolized but is exported to the cytoplasm. There is no available evidence that the necessary dismutating enzymes, alcohol and lactate dehydrogenases, are localized within the chloroplast (17).

A dicarboxylic acid shuttle involving malate, OAA, chloroplastic and extra-chloroplastic malate dehydrogenase is known and is available to complete the hydrogen cycle (14). In this study, we have turned our attention to mechanisms located within the chloroplast which may balance the chloroplastic hydrogen cycle. To demonstrate a chloroplastic hydrogen cycle, we have utilized an envelope-free chloroplast preparation as a model system. These preparations were obtained by rupturing whole spinach chloroplasts in 15 mM $MgCl_2$. The resulting particles have been shown to be associated with high level activity of aldolase, G3P dehydrogenase, PGA kinase, triose-P isomerase, and the enzymes catalyzing the conversion of R5P to G3P (unpublished data). We have selected G3P dehydrogenase as the oxidative reaction because it functions with both pyridine nucleotides and plays a key role in starch breakdown inasmuch this enzyme generates the ATP needed for the phosphorylation of fructose-6-P through oxidative substrate phosphorylation. In addition to the dicarboxylic acid shuttle of malate and OAA, we found that the hydrogen from NADPH generated by the G3P dehydrogenase reaction could be transferred to nitrite, O_2, or protons resulting in the formation of ammonia, H_2O_2, or hydrogen gas, respectively. The rate of reduced pyridine nucleotide oxidation was monitored indirectly by measuring the rate of PGA formation from substrate levels of FBP or R5P in the presence of a catalytic amount of pyridine nucleotide. A preliminary report of this study has been presented (19).

MATERIALS AND METHODS

Plants. *Spinacia oleracia* var. America was grown in pots placed in a growth chamber with a 12:12 light to dark photoperiod at 20:16°C. Chloroplasts were prepared from fully expanded leaves taken from 4- to 6-week-old plants.

Treatment of Chloroplasts with $MgCl_2$. Intact chloroplasts were isolated by a previously described procedure (20). They were washed once in the isolation medium and incubated in a medium containing 15 mM $MgCl_2$, 1 mM Na_2EDTA, and 50 mM Tricine-NaOH (pH 8.5). The osmotically ruptured $MgCl_2$-treated chloroplasts were centrifuged at 12,000*g* for 10 min. The resulting pellet was resuspended in 5 ml of the disrupting fluid.

Enzymes and Biochemicals. Ferredoxin was purified from an acetone powder preparation of spinach by the San Pietro and

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[4] Abbreviations: PGA, glycerate-3-phosphate; G3P, glyceraldehyde-3-phosphate; OAA, oxaloacetate; R5P, ribose-5-phosphate; FBP, fructose-1,6-bisphosphate; GSH, reduced glutathione; GSSG, oxidized glutathione.

Lang procedure (28). Hydrogenase from *Chlamydomonas reinhardii* was partially purified by $(NH_4)_2SO_4$ precipitation and DEAE cellulose chromatography as previously described (10). PGA kinase, α-glycerol-P dehydrogenase, NAD-G3P dehydrogenase, triose-P isomerase, and other materials were from Sigma.

PGA Formation. The basic 4 ml reaction mixture contained 50 mM DTT, 20 mM sodium arsenate, 5 mM KCl, 20 mM $MgCl_2$, 1 mM Na_2EDTA, 2 mM FBP or R5P, 50 μM NADP, and 14.5 μM ferredoxin. MgP containing 100 to 300 μg Chl was added to start the reaction. The incubations were carried out in darkness under an atmosphere of air or N_2.

Estimation of PGA. PGA was estimated by measuring the oxidation of NADH through the combined enzymic activities of PGA kinase, NAD-G3P dehydrogenase, triose-P isomerase and α-glycerol-P dehydrogenase. Samples of 0.8 ml were taken from the reaction mix and pipetted into 0.2 ml 20% $HClO_4$. The samples were then centrifuged at 3,000*g* for 5 min and the precipitate was discarded. The supernatant fraction was brought to neutrality with 2 M $KHCO_3$ and centrifuged at 3,000*g* for 5 min. An aliquot (usually 0.2–0.4 ml) was added to a medium containing 100 μmol Hepes-HCl (pH 7.8), 20 μmol $MgCl_2$, 2 μmol DTT, 2 μmol ATP, and 0.24 μmol NADH in a final volume of 1.0 ml. Ten units of triose-P isomerase and 5 units of α-glycerol-P dehydrogenase were added to consume the triose-P present in the aliquot taken from the reaction mixture. The PGA was measured by adding 10 units of NAD-G3P dehydrogenase and 10 units of PGA kinase. Reaction mixtures were maintained at 25°C and the change in *A* at 340 nm was followed.

Estimation of Nitrite and Ammonia. Nitrite was determined by the method of Miflin (24). Ammonia was removed from the reaction mixtures by microdiffusion into 1 N H_2SO_4, followed by a colorimetric assay developed with sodium nitroprusside and alkaline hypochlorite (6).

RESULTS AND DISCUSSION

Oxidation of Reduced Pyridine Nucleotide by Malate Dehydrogenase and Glutathione Reductase. There is substantial evidence for a malate/OAA shuttle between the chloroplast and the cytoplasm to account for the reoxidation of chloroplastic-reduced pyridine nucleotides (14). The recent discovery of a NADP-linked GSH reductase in the spinach chloroplast (32) suggests a similar role for GSH and GSSG. Our initial experiments were carried out to estimate the rate of pyridine nucleotide turnover by dismutating systems which react directly with pyridine nucleotide for comparison with indirect electron acceptors such as O_2, nitrite, and protons.

Table I shows that the addiiton of NAD and OAA together stimulated PGA formation approximately 20-fold over the control with NAD or OAA alone. This result indicated that oxidation of G3P to PGA could be coupled to OAA reduction in our preparation and also substantiated our earlier unpublished findings that the MgP was relatively free of cofactors such as NAD(P) and substrates. NADP in the absence of OAA was 3-fold more effective than NAD, but this difference was eliminated when air was replaced with N_2, indicating the presence of a NADP catalyzed system independent of OAA reduction. When NADP replaced NAD in the presence of OAA, the rate fell by two thirds. This lower rate with NADP was not due to G3P dehydrogenase since the ratio of NADP to NAD-G3P dehydrogenase in our chloroplast particles is 2:1 (data not shown).

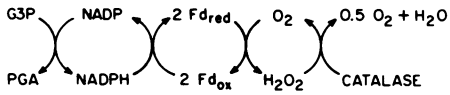
Table I. *Effect of OAA and GSSG on NAD(P)-Dependent PGA Formation*

The control reaction mixture of 2 ml contained 50 mM Tricine-NaOH (pH 8.5), 4 mM DTT, 20 mM sodium arsenate, 5 mM KCl, 20 mM $MgCl_2$, 1 mM Na_2EDTA, 14.5 μM ferredoxin, 2 mM FBP and particles containing 210 μg Chl. An aliquot of 0.8 ml was removed after 20 min for PGA determination. The experiment was carried out under air.

Additions	PGA Produced
	μmol/mg Chl·h
None	0.60
NAD, 50 μM	0.90
OAA, 2 mM	1.66
NAD, 50 μM + OAA, 2 mM	22.28
NAD, 50 μM + OAA, 4 mM	22.16
NADP, 50 μM	3.33
NADP, 50 μM + OAA, 2 mM	7.03
NADP, 50 μM + GSSG, 2 mM	8.90

The rate of PGA formation with GSSG and NADP was comparable to that observed with OAA. NAD did not substitute for NADP in the presence of GSH. Ascorbate or DTT each at 2 mM in the presence of NADP increased the rate about 30 and 200% over the control. Cysteine was ineffective.

Ferredoxin-Dependent PGA Formation. Several investigators (4, 5, 30) have demonstrated that NADPH can transfer its low potential electrons to oxidized ferredoxin with NADPH; ferredoxin oxidoreductase serving as catalyst. Ferredoxin has been reported to reduce O_2 readily with the formation of H_2O_2 (9), thus, PGA formation coupled to O_2-uptake is a potential pathway for connecting G3P oxidation to O_2 as a terminal electron acceptor.



The findings presented in Figures 1 and 2 and Table II supported this scheme. In the absence of catalase, the predicted stoichiometry is 1 PGA:1 O_2 while in its presence, the ratio is 2 PGA:1 O_2.

As seen in Figures 1 and 2, the rate of PGA formation was dependent upon NADP, ferredoxin, and O_2. In the presence of all three, PGA was formed at a linear rate for up to 40 min (Fig. 1). The initial burst observed under the other conditions was the result of reduction of the 50 μM pyridine nucleotide originally present in the reaction mixture. The low rate in N_2 was due to the

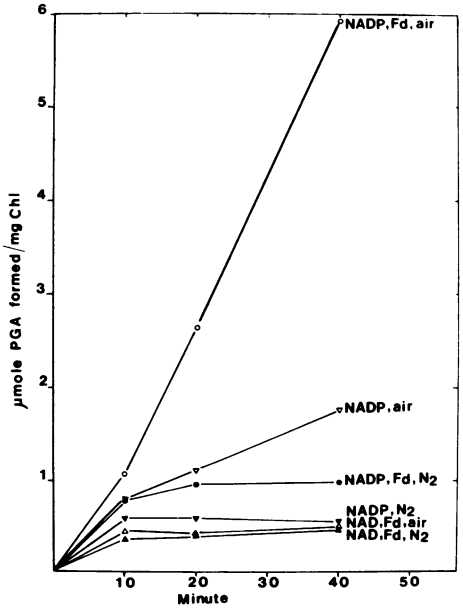


FIG. 1. Effect of cofactors on the time course of PGA formation. The reaction mixture and other conditions are described in Table I with the exception of 271 μg Chl/4.0 ml of reaction medium. The concentration of NAD was 50 μM.

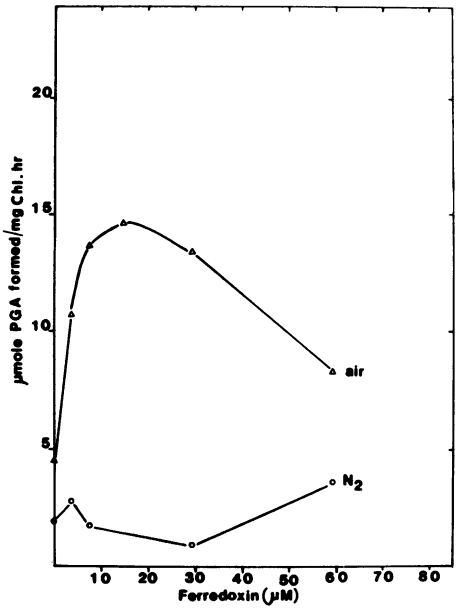


FIG. 2. Effect of ferredoxin concentration and atmosphere on PGA formation. The reaction mixture is described in Table I. The Chl concentration was 226 µg Chl/4.0 ml reaction mixture and PGA was determined in the 20 to 40 min interval. Air or N_2 was bubbled for 10 min before MgP was added to start the reaction. Bubbling was continued throughout the experiment.

Table II. *Stoichiometry of PGA Formation and O_2 Consumption*

The endogenous O_2 consumption rate was recorded in a reaction mixture of 1 ml containing 50 mM Tricine-NaOH (pH 8.5), 2 mM DTT, 20 mM sodium arsenate, 5 mM KCl, 20 mM $MgCl_2$, 1 mM Na_2EDTA, 14.5 µM ferredoxin, particles containing 199 µg Chl, 2 mM FBP or 2 mM R5P, and 550 units catalase where indicated. After 5 min, 50 µM NADPH was added to start the reaction and the increased rate of O_2 uptake was recorded. After another interval of 5 or 10 min 0.8 ml was taken from the electrode chamber and injected into 0.2 ml 20% $HClO_4$ for the estimation of PGA. The rate of O_2 consumed was calculated by the difference between the rate before and after the addition of NADPH. O_2 uptake was monitored with a Clark type electrode and air-saturated water was taken as containing 0.28 mM O_2.

Time	Substrate	O_2 Consumed	PGA Formed	PGA Formed to O_2 Consumed
min		*nmol/ml aliquot*		*ratio*
0–10	FBP	104.0	97.9	0.94
0–10	FBP	75.0	76.3	0.98
0–10	FBP + catalase	57.5	127.6	2.22
0–10	FBP + catalase	33.2	67.8	2.04
0–10	R5P	68.0	72.0	0.94
0–10	R5P	98.0	105.0	1.07
0–10	R5P + catalase	47.0	78.4	1.67
0–10	R5P + catalase	33.7	58.2	1.73
0–5	R5P	32.0	37.7	1.18
0–5	R5P + catalase	21.0	40.7	1.94
0–5	FBP	52.0	59.3	1.14
0–5	FBP + catalase	32.6	61.7	1.89

small amount of O_2 remaining in the reaction mixture following bubbling with N_2. When the reaction was run in a stoppered bottle, which was evacuated and refilled with O_2-free N_2 three times, there was no PGA formation beyond the initial burst due to pyridine nucleotide.

The optimum concentration of ferredoxin was 15 µM (Fig. 2) and the highest rate of PGA formation was 14.5 µmol/mg Chl·h which compares well with the values recorded in Table I. In the absence of added ferredoxin, there was a small but definite rate of PGA formation which varied with the chloroplast preparation. Ferredoxin-dependent PGA formation was specific for nitrate (Fig. 3).

Table II lists the stoichiometry of O_2 consumption to PGA formation in the absence and presence of catalase with substrate levels of triose-P generated from either R5P or FBP. With either sugar phosphate, and in the absence of catalase, the PGA:O_2 ratio was 0.94 to 1.18, whereas in the presence of catalase, the ratio was 1.67 to 2.22. These values are consistent with our postulated scheme and demonstrate that electrons generated during the oxidation of G3P to PGA can be transferred to O_2. The stoichiometric data also establish H_2O_2 rather than H_2O as the end product pointing to reduced ferredoxin as the electron donor to the terminal acceptor. The data in this table also indicate that the chloroplast particle contains transketolase, R5P isomerase, and ribulose-5-P-epimerase required to produce G3P from R5P and at a level equivalent to aldolase, the sole enzyme needed with FBP as substrate.

Nitrite and PGA Formation. While nitrate reductase is a cytoplasmic enzyme and requires NADPH as reductant, nitrite reductase is located within the chloroplast and uses reduced ferredoxin as electron donor (8, 30). In the presence of nitrite and ferredoxin, the rate of PGA formation was 4.2 µmol/mg Chl·h and was linear for at least 40 min (Fig. 3). In the absence of ferredoxin or nitrite, PGA only equivalent to the added NADP was found. KNO_3 and ferredoxin did not promote PGA formation which indicates the absence of nitrate reductase.

The reduction of 1 mol nitrite to 1 mol ammonia requires six electrons. Since 1 mol PGA formed is accompanied by 1 mol NADPH produced and NADPH is a two electron donor, a PGA: nitrite ratio of 3 would be expected. We observed a PGA:nitrite value of 2.62 in the 20 to 40 min and of 2.52 in the 40 to 60 min interval of the experiment recorded in Table III. Table IV shows the stoichiometry of nitrite consumed to ammonia formed. The ratio was in the range of 1.21 to 1.56. From the data in the two tables, we conclude that electrons derived from the oxidation of

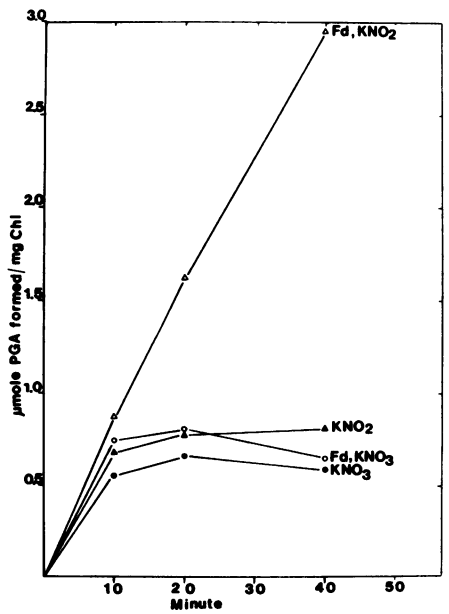


FIG. 3. Time course of PGA formation in the presence of nitrite. The reaction conditions are those of Table III. The concentration of Chl was 271.4 µg/4.0 ml reaction medium and nitrate was 0.5 mM.

Table III. *Stoichiometry of PGA Formation and Nitrite Reduction*

The reaction mixture of 4 ml contained 50 mM Tricine-NaOH (pH 8.5), 4 mM DTT, 20 mM sodium arsenate, 5 mM KCl, 20 mM $MgCl_2$ 50 μM NADP, 14.5 μM ferredoxin, particles containing 210 μg Chl, 2 mM FBP, and 0.5 mM KNO_2. Aliquots at 0.2 ml were removed at various intervals for PGA and nitrite determination (24). To determine nitrite, aliquots of 0.2 ml were injected into 0.3 ml 0.2 N $ZnSO_4$ containing 0.2 ml 0.4 N NaOH, followed by 1.0 ml H_2O. After standing for 10 min, the tubes were centrifuged and 1.0 ml of the supernatant solution was added to 1.0 ml 1% sulfanilamide followed by addition of 1 ml 0.02% naphthylethylenediamine.

This experiment was conducted anaerobically by placing the FBP solution, chloroplast particulate suspension, and the remaining components of the reaction mixture in 160 ml stoppered serum bottles which were evacuated and refilled with O_2-free N_2. This procedure was repeated three times. To initiate the reaction, aliquots were combined in a bottle which had been rendered anaerobic. At the specified times, samples were removed from the reaction bottle using syringes which has been flushed with N_2.

Time	PGA Formed	Nitrite Concn	PGA to KNO_2
min	*mmol/ml*		*ratio*
0	0	505	
20	190	484	9.05
40	384	410	2.62
60	505	362	2.52

Table IV. *Stoichiometry of KNO_2 Consumed to NH_3 Formation*

The reaction mixture and conditions were similar to those in Table III except the Chl content was 185 μg/2 ml. Nitrite (see Table III) was determined by the method of Miflin (24) and NH_3 was determined by microdiffusion into 1 N H_2SO_4 followed by colorimetric assay with nitroprusside (6).

Time	KNO_2 Consumed	NH_3 Formed	NH_3 to KNO_2
min	*nmol/ml*		*ratio*
20	65.0	41.8	0.64
40	120.0	87.8	0.73
60	157.5	130.0	0.83

G3P can ultimately be transferred to nitrite with ammonia as the end product.

H_2 and PGA Formation. A number of green algae including *Chlamydomonas reinhardii* acquire a hydrogen metabolism when incubated under N_2. This metabolism includes both the evolution and uptake of H_2 in the dark (3). A hydrogenase which is readily inactivated by exposure to O_2 appears during anaerobic treatment. The enzyme has been shown to utilize reduced ferredoxin as the electron donor for H_2 evolution. It should be possible, therefore, to link the oxidation of G3P to PGA to H_2 production through ferredoxin and hydrogenase. To examine the potential for our reconstituted system to be coupled to these reactions, we measured PGA formation in the presence of partially purified *C. reinhardii* hydrogenase. As seen in Figure 4, PGA was formed at a rate of 1.13 μmol/mg Chl·h or roughly 10% of the rate when O_2 was the final electron acceptor. By omitting either hydrogenase or ferredoxin or using hydrogenase inactivated by exposure to air, PGA formation was substantially diminished.

CONCLUSIONS

Chloroplasts contain the sequential enzymes of the oxidative pentose-P cycle and of the glycolytic pathway between starch and PGA (16, 17). Experimental evidence for the existence of a chloroplastic mitochondrial-type electron transport chain linking NAD(P)H to O_2 has not been presented. The chloroplast equivalent of a Cyt *c*-Cyt *c* oxidase in the Z scheme of Hill and Bendall is Cyt *f*:P700 but the latter is involved in NAD(P)H formation rather than in its oxidation. A chloroplastic means of producing ATP in the dark as required in the glycolytic sequence has not been characterized. With the presumed absence of a mitochondrial-type ATP generating system, another source, presumable substrate level, must be available. Since the mitochondrion does have the capability of reoxidizing reduced pyridine nucleotide coupled to ATP formation, initially it seemed reasonable that this organelle accounted not only for sustaining a constant supply of oxidized pyridine nucleotide but also of ATP for the chloroplast. This concept had to be revised when convincing evidence accumulated that the chloroplast envelope had a low permeability towards the nicotinamide and the adenylate nucleotides.

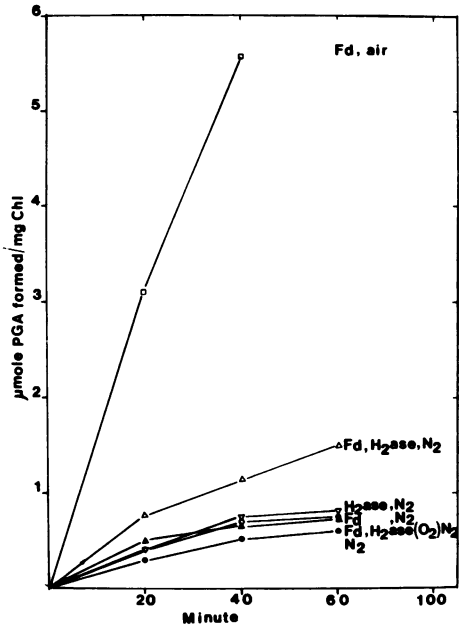


FIG. 4. Effect of hydrogenase on PGA formation. The reaction mixture with the exceptions that Chl was 427 μg/4.0 ml reaction medium and ferredoxin was 19.2 μM is described in Table I and the means of achieving an anaerobic environment is given in Table III.

In this report, we have documented that NADPH produced by the oxidation of G3P to PGA in a chloroplast particle fortified with ferredoxin, G3P and NADP can transfer its electrons to ferredoxin. Those electrons can be, in turn, used to reduce O_2 to H_2O_2 (Mehler reaction), nitrite to ammonia, or protons to H_2. Based on our observations, a model is presented in Figure 5 to account for a sustained regeneration of chloroplastic NADP necessary for the dissimilation of starch either by the glycolytic or oxidative pentose-P pathway. The overall stoichiometry takes into account the glycolytic need for ATP and for the counterexchange of Pi with triose-P or PGA promoted by the Pi translocator. In this model, only one of the two equivalents of triose-P derived from one glycosyl unit is oxidized to PGA. This oxidation of one triose-P yields two equivalents of reduced ferredoxin. Thus, 3 mol triose-P will be required to reduce 1 mol nitrite to ammonia. Two equivalents of Pi per glucosyl moiety are imported from the cytoplasm with one consumed in the phosphorylase reaction and the second is converted by substrate phosphorylation to ATP for utilization in the fructose-6-P kinase reaction.

We have not considered the further metabolism of glucose and maltose (15, 23) derived by amylolytic cleavage of starch since the level of hexokinase (or glucokinase) and the sequence of reactions dealing with the disaccharide remain unresolved. OAA is shown to be imported from the cytoplasm inasmuch as the enzymes linking starch with OAA have not been reported in the chloroplast.

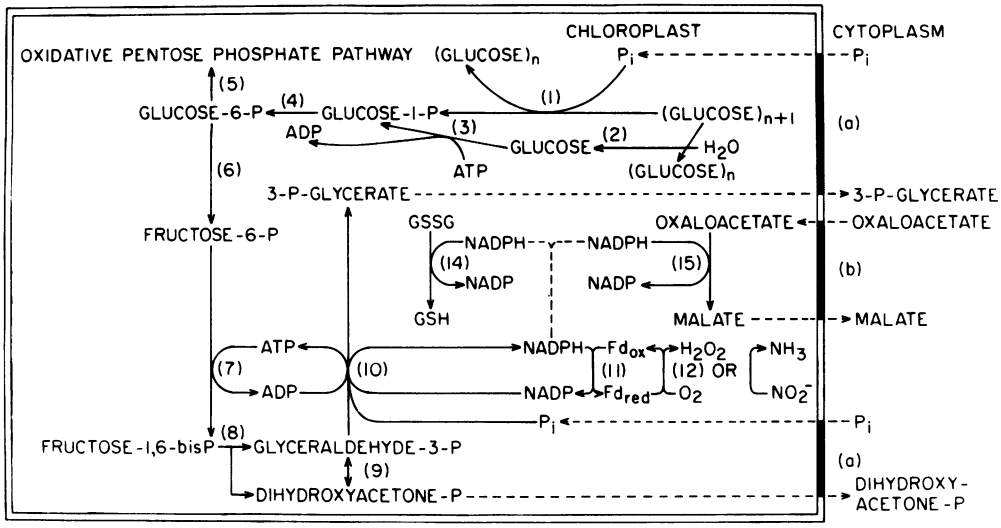


FIG. 5. Proposed scheme for starch respiration within the chloroplast (a), Pi translocator; (b) dicarboxylic acid translocator. (Fd), ferredoxin. Numbers indicate enzymic reactions. Stoichiometry between reactions 11, 12, or 13 is not indicated.

A functional Mehler reaction, nitrite reduction, and H_2 formation coupled to starch breakdown within the darkened chloroplast, would solve a number of long-standing metabolic questions.

For example, it is well known that following a period of photosynthesis in intact cells and isolated intact chloroplasts, the level of pentose- and triose-phosphates decreases with a concomitant appearance of PGA (2, 22), and an uptake of O_2. While this sequence of reactions can be explained in the intact cell by coupling the oxidation of G3P to the reduction of OAA or GSSG, our results indicate that in the intact chloroplast and perhaps in the intact cell, the Mehler reaction could account for the turnover of NADPH. The resulting end product H_2O_2 can be eliminated either by ascorbate peroxidase (12) or by the recently reported NADH-ascorbate peroxidase (21). Both enzymes are located within the chloroplast. The latter enzyme can not only remove H_2O_2 but can also regenerate NAD(P) within the chloroplast.

The reduction of nitrate and nitrite via nitrate reductase and the photosynthetic electron transport chain in illuminated chlorophyll-containing tissue is well documented (18, 26). On the other hand, the routes by which green algae such as *Chlorella* and *Scenedesmus* can utilize nitrate for growth in the dark with glucose as the sole source of carbon have not been characterized (13, 25). Also, the coupling of nitrate and nitrite reduction to carbohydrate breakdown by green leaves in the dark is not fully understood (1). The nature of the nitrite reductant has not been identified, but by analogy to the light catalyzed sequence, it may be reduced ferredoxin and could be coupled to starch breakdown within the leaf or algal chloroplast.

Some 40 years ago, Gaffron and Rubin (11) demonstrated that the green alga *Scenedesmus* could evolve H_2 in darkness when incubated under an atmosphere of N_2. Since then the list of algae which can adapt to a hydrogen metabolism has grown and now includes members (18) of the Cyanophyceae, Chlorophyceae, as well as the Phaeophyceae and the Rhodophyceae. The responsible enzyme is hydrogenase. While its cellular location is unknown, several lines of evidence point to compartmentation within the chloroplast (3, 18). Gaffron suggested that a carbohydrate, either starch or glucose was the primary source of electrons for H_2 evolution. Our data given in Figure 4 lends support to this notion by linking carbohydrate breakdown to the evolution of H_2 through the NADP → ferredoxin pathway.

Finally, the reactions leading from sulfate to cysteine with the green cell are dependent upon a supply of ATP and reduced ferredoxin (29). Inasmuch as the chloroplast is the site of this sequence of reactions which can occur in darkness, the model presented in Figure 5 can also accomodate this reductive process.

The chloroplast, like the cytoplasm, has the enzymic capability of oxidizing glucose-6-P through the complete oxidative pentose-P pathway. In the chloroplast, hexose-P would be totally oxidized to CO_2 and H_2O_2

6 hexose-P + 12 $NADP^+$ + 6 H_2O	12 NADPH + 12 H^+ + 6 CO_2 + 6 pentose-P
4 pentose-P	2 hexose-P + 2 tetrose-P
2 pentose-P + 2 tetrose-P	2 hexose-P + 2 triose-P
2 triose-P + H_2O	hexose-P + Pi
12 NADPH + 12 H^+ + 12 O_2	12 H_2O_2 + 12 $NADP^+$
hexose-P + 7 H_2O + 12 O_2	12 H_2O_2 + 6 CO_2 + Pi

while production of CO_2 and H_2O would be characteristic of cytoplasmic and mitochondrial metabolism. Finally, the initial step of this pathway gives rise to gluconate-6-P which is known to regulate CO_2 fixation in the chloroplast by inhibiting ribulose-1 5-biP carboxylase (7).

We propose the broad term chloroplast respiration to account for those reactions in which reduced ferredoxin interacts with physiological electron acceptors other than NADP. Using the terminology adapted in the microbiol literature, it follows that the specific term nitrite, hydrogen, sulfate, or O_2 respiration be applied when nitrite, protons, sulfate, or O_2 is the ultimate electron acceptor.

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