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Light-Dark Regulation of Starch Metabolism in Chloroplasts

II. EFFECT OF CHLOROPLASTIC METABOLITE LEVELS ON THE FORMATION OF ADP-GLUCOSE BY CHLOROPLAST EXTRACTS[1]

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ABSTRACT

The rate of ADP-glucose formation from [^{14}C]glucose 6-phosphate and ATP by the soluble fraction of lysed chloroplasts is studied as a function of the levels of metabolites (3-phosphoglycerate, orthophosphate, hexose monophosphate, and ATP) as determined in whole chloroplasts of *Spinacia oleracea* in light and dark.

A change in 3-phosphoglycerate concentration (from 4 to 1.4 millimolar, as in whole chloroplasts during light-dark transition) decreases the rate of ADP-glucose formation 6- to 7-fold. An increase in hexose monophosphate concentration from 2 to 6 millimolar, which occurs at the same time in whole chloroplasts, stimulates ADP-glucose formation only slightly.

At constant levels of orthophosphate (4 millimolar) and 3-phosphoglycerate (4 millimolar), a change in ATP concentration from 0.2 to 1 millimolar causes an immediate 4- to 5-fold increase in the rate of ADP-glucose formation. Another significant stimulation of ADP-glucose formation (about 4- to 6-fold) is obtained after addition of dithiothreitol at high concentrations (50 millimolar). A simultaneous increase in the concentrations of 3-phosphoglycerate, ATP, and dithiothreitol, with orthophosphate and Mg^{2+} being constant at 4 and 5 millimolar, respectively, causes a 130-fold increase in the rate of ADP-glucose formation (from 0.042 to 5.49 microgram atoms carbon per milligram chlorophyll per hour).

The role of these and other factors is discussed with respect to light-dark regulation of starch formation in intact chloroplasts.

In the preceding paper (8) we investigated changes in metabolite levels in isolated intact chloroplasts in order to determine whether light-dark regulation of starch synthesis in chloroplasts does occur via the well known modulation of ADP-glucose formation by photosynthetic intermediates and Pi (4, 5, 14, 16). We found that after several min in the light, the level of Pi, which is a potent inhibitor of ADP-glucose pyrophosphorylase (4, 5, 14, 16), stayed constant throughout a following light-dark-light cycle (8). The level of PGA,[3] which stimulates ADP-glucose formation (4, 5, 14, 16), decreased dramatically in the dark, but the concentration of hexose-monoP, which also stimulate ADP-glucose pyrophosphorylase (4, 5, 14, 16), increased in the dark by a slightly larger factor (8). The concentrations of these metabolites changed somewhat slowly when compared with the fast light-dark kinetics of ADP-glucose levels in whole cells (2) or of starch formation in whole chloroplasts (8).

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[3] Abbreviations: PGA: 3-phosphoglycerate; G6P: glucose 6-phosphate; G1P: glucose 1-phosphate; F6P: fructose 6-phosphate.

These observations raise some questions with respect to the role of these metabolites in the fast light-dark regulation of starch formation. We have now investigated the formation of ADP-glucose from [^{14}C]G6P and ATP in the soluble fraction of lysed chloroplasts under various conditions. Because of different types of regulation of chloroplast metabolism involving ions, metabolites and small protein factors (for a review see 10), it is important to investigate regulation of starch synthesis in such a soluble system. These studies complement investigations with purified enzyme preparations previously reported elsewhere (4, 5, 14, 16). The rate of ADP-glucose formation was studied at the different levels of metabolites which we found in whole chloroplasts in light and dark (8). In addition we investigated the effects of several other factors which are known to regulate Calvin cycle enzymes such as different levels of ATP, Mg^{2+}, and pH under conditions which are now known to exist in whole chloroplasts, as well as the effects of dithiol compounds known to simulate some regulatory effects of analogous light-generated physiological reductants in chloroplasts (2, 10, 18, 19). Also, requirements for the formation of starch by the chloroplast extract were examined.

MATERIALS AND METHODS

Chemicals and Enzymes. [U-^{14}C]Glucose was obtained from New England Nuclear (NEN). Lyophilized hexokinase and P-glucomutase (EC 2.7.5.1.) were from Calbiochem, stocks 376 811 and 52 508, respectively.

Plant Material. Spinach (*Spinacia oleracea*) was grown in Vermiculite fertilized with Hoagland solution, under artificial light (3,000 ft-c) with an 8-hr light period and a 16-hr dark period at a temperature of 13 C. Chloroplasts were isolated from young leaves according to the method of Jensen and Bassham (7). Chl was measured following the procedure of Arnon (1).

Chloroplast Extract. For preparing a chloroplast extract, a suspension of freshly isolated whole chloroplasts in a volume of 2 ml and containing 2 mg of Chl was centrifuged for 1 min at 1,000 *g* (4 C) and the pellet was resuspended in 1.2 ml of a lysing solution containing HEPES-NaOH (pH 8.0) and 5 mM $MgCl_2$. After 10 min at 0 C, the suspension was centrifuged for 15 min at 27,000*g* (4 C). The resulting straw-colored supernatant contained the soluble components from the chloroplasts and had an average protein content of 5 mg of protein in 1.2 ml. It was stored on ice until it was used for the experiments (usually within 1 hr after isolation). The pellet was resuspended in 1.2 ml of lysing solution and used for Chl determination and for experiments, as indicated in the legends of the figures.

Preparation of [U-^{14}C]G6P. [^{14}C]G6P was prepared from [U-^{14}C]glucose. Two mg of hexokinase containing 10 IU and stoichiometric amounts of ATP were added to 0.5 ml of [^{14}C]glucose (1.25 mCi/4.1 μmol) in the lysing solution described above. The conversion of glucose into G6P was checked by two-dimensional

paper chromatography. Usually, more than 98% of the added [^{14}C]glucose was converted into G6P. After that, unlabeled G6P was added to give a specific radioactivity of 50 μCi/μmol, and the solution was stored at −15 C until needed.

Incubation Conditions. Three hundred μl of the chloroplast extract were added to the lysing solution containing 2 to 6 mM [^{14}C]G6P and different metabolites in a final volume of 500 μl. The solution was assayed in serum-stoppered flasks in a water bath at 21 C. At timed intervals 50-μl aliquot samples were removed with microsyringes and injected into 450 μl of methanol (final methanol concentration 90%).

Analysis of Products. Products of the metabolism of [^{14}C]G6P were separated by descending paper chromatography and radioautography (7). One hundred fifty-μl samples of the protein-methanol mixture were spotted on Whatman No. 1 paper and the chromatograms were developed in two dimensions as described earlier (13). The spots containing ADP-glucose were cut off the chromatograms and extracted for several hr with 5 ml of water. After addition of 15 ml scintillation liquid, the samples were counted by liquid scintillation. Rates of ADP-glucose formation were calculated based on the amount of Chl in the intact chloroplasts, from which the clear extract was prepared.

RESULTS AND DISCUSSION

Effect of Phosphoglucomutase on ADP-Glucose Formation. Since G6P is a natural intermediate in the biosynthesis of starch from F6P in the Calvin cycle, and ^{14}C-labeled G6P is readily available, we used this compound as the labeled substrate for studying ADP-glucose formation in the soluble fraction of lysed chloroplasts. However, this involves the conversion of G6P into G1P via P-glucomutase (EC 2.7.5.1.) as an additional reaction. In order to make sure that this step was not rate-limiting under our conditions, we measured ADP-glucose formation in the presence and absence of excess P-glucomutase (+ glucose-1,6-bisP, 0.02 mM). Addition of P-glucomutase stimulates ADP-glucose formation slightly, but only under conditions where ADP-glucose pyrophosphorylase is fully activated by high concentrations of PGA (and in the absence of Pi), so that ADP-glucose formation occurs at very high rates (Table I). Under more physiological conditions, *i.e.* at the levels of PGA and Pi as they exist in whole chloroplasts (8), the activity of endogenous P-glucomutase was not rate-limiting.

Effect of Chloroplastic Levels of PGA, Pi, and Hexose-MonoP on ADP-Glucose Formation. In our preceding work we found that the level of PGA in illuminated intact chloroplasts was about 3.6 to 4 mM (8). At this concentration of PGA, and in the absence of Pi, the rate of ADP-glucose formation by chloroplast extracts was high, reaching values between 13 to 50 μg atoms carbon/mg Chl·hr (compare Table I, Fig. 2A, and Fig. 3). These rates were at least as high, and usually higher than the rates of starch formation in whole chloroplasts.

If Pi was present at the concentration found in intact chloroplasts in the light and dark (about 3.6–4 mM), the rate of ADP-glucose formation was much lower, reaching values between 1.5 to 8 μg atoms carbon/mg Chl·hr (Table II). Even these lower rates are well in the range of the rates of starch formation in isolated intact chloroplasts.

If Pi was kept constant at 4 mM (as it is in whole chloroplasts), and the concentration of PGA was reduced to the dark level of about 1.4 mM, the rate of ADP-glucose formation decreased dramatically by about 85% (Table II). These experiments were carried out at a concentration of added [^{14}C]G6P of 2 mM. We knew that the level of hexose-monoP increases in intact chloroplasts at light-dark transition from 2 to about 6 mM (8). Because about one-fourth of the total hexose-monoP is present as F6P, which is known to be an activator of ADP-glucose pyrophosphorylase (4, 5, 14, 15), the observed increase in hexose-monoP in the dark might partly balance the inactivation of ADP-glucose pyrophosphorylase caused by the decreased level of PGA. Changing the hexose-monoP concentration from 2 to 6 mM increased the rate of ADP-glucose formation only slightly (Table II).

The response of ADP-glucose pyrophosphorylase toward a change in [PGA] from 1.4 to 4 mM (with Pi and ATP being 4 mM and 1 mM, respectively) was found to be extremely fast (Fig. 1). We concluded that any change in the PGA level which occurs in intact chloroplasts during a light-dark-light transition (8) leads to an immediate and effective regulation of ADP-glucose pyrophosphorylase. This is despite the fact the Pi remains constantly high and that the hexose-monoP level undergoes change in the opposite direction.

The changes in the level of PGA during light-dark transition were found to be somewhat slower than the changes in the rate of starch formation in whole chloroplasts (8) or, even more significant, the changes in the rate of ADP-glucose formation *in vivo* (2). Therefore, it might be assumed that additional factors are involved in a fast light-dark regulation of ADP-glucose formation.

Effect of Changes in ATP Level on Formation of ADP-Glucose. It is known that the level of ATP in whole chloroplasts decreases from about 1 mM in the light to 0.2 mM in the dark within 5 to 15 sec, and in the light once again it increases as rapidly (9, 15, 17). These rapid concentration changes would make the ATP-level an excellent fast switch for a light-dark regulation of ADP-glucose formation. It was of interest to determine how the rates of ADP-

Table I. Effect of phosphoglucomutase on formation of ADP-glucose from ^{14}C-G6P.

Phosphoglucomutase (250 U/2 ml) was dialyzed over night at 4 C against 1 ℓ of a solution containing 0.025 M HEPES-NaOH pH 7.7, 5 mM $MgCl_2$, 2 mM DTT. 40 µl (5 U) of the enzyme solution was added to the reaction medium, which contained in a total volume of 0.5 ml: 0.025 M HEPES-NaOH pH 8.0, 5 mM $MgCl_2$, 4 mM PGA, 1 mM ATP, and Pi and phosphoglucomutase (+ glucose-1,6-bisphosphate 0.02 mM) as indicated. Rates were calculated from a linear time course (6 min). A and B represent separate experiments from different chloroplast preparations.

Conditions	ADP-glucose formed, µg-atoms/mg chl. x h	
	Exp. A	Exp. B
a) PGA 4 mM, no Pi		
Control	24.8	16.0
+ phosphoglucomutase	33.5	19.1
b) PGA 4 mM, Pi 4 mM		
Control	8.4	1.9
+ phosphoglucomutase	8.4	1.9

Table II. Formation of ADP-glucose at the levels of Pi, PGA and hexose monophosphate (added as G6P), found in intact chloroplasts in the light or dark (see ref. 8).

300 µl of chloroplast extract were added to a medium containing 0.025 M HEPES-NaOH pH 8.0, 5 mM $MgCl_2$, 50 mM DTT, and 1 mM ATP; U-^{14}C-G6P and other metabolites were present as indicated in the table. The expressions "light-conditions" or "dark-conditions" are used with respect to the metabolite levels found in intact chloroplasts in the light or dark. All rates were calculated from a linear time course (10 min). For further details see Materials and Methods.

Metabolites added (mM)	ADP-glucose formed (µg-atoms/mg chl. x h)
a) PGA 4, no Pi, G6P 2	16.84
b) PGA 4, Pi 4, G6P 2 (= light conditions)	5.49
c) PGA 1.4, Pi 4, G6P 2	0.90
d) PGA 1.4, Pi 4, G6P 6 (= dark conditions)	0.99

glucose formation would respond to changes in ATP as they occur in whole chloroplasts (Fig. 2). With ADP-glucose pyrophosphorylase fully activates (PGA 4 mM, no Pi, Fig. 2A), an increase in ATP from 0.2 to 1.0 mM stimulates ADP-glucose formation only by a factor of 1.5 (Fig. 2A). In this experiment, the rates of ADP-glucose formation are linear for only a short time, especially at the low ATP concentration, since ATP is consumed very fast due to the high rates of ADP-glucose formation. At levels of PGA and Pi, as they exist in whole chloroplasts in light and dark, the rate of ADP-glucose formation is lower and therefore linear for a much longer time (Fig. 2B). Under these conditions, the rate of ADP-glucose formation is strongly stimulated (4- to 5-fold) by a change in ATP (from 0.2 to 1.0 mM) (Fig. 2B). In all cases, the response of ADP-glucose pyrophosphorylase toward a change in ATP was very fast. We concluded that a change in the level of ATP would give a very fast and effective additional regulation of ADP-glucose pyrophosphorylase, especially since it is usually coupled to an opposite change in ADP, which is an inhibitor of ADP-glucose pyrophosphorylase (5).

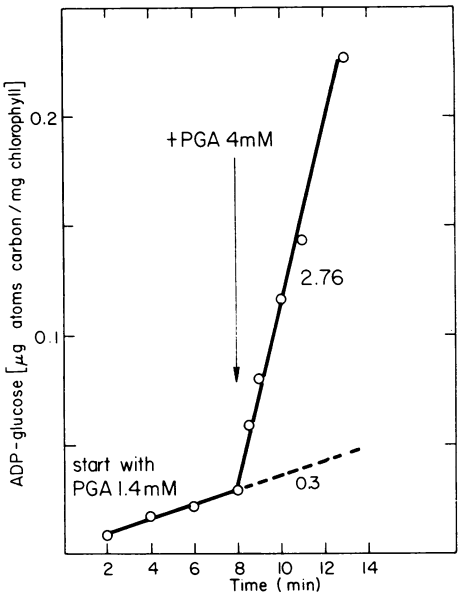

FIG. 1. Response of ADP-glucose formation with a change in PGA concentration. The change in PGA concentration from 1.4 to 4 mM was that found in whole chloroplasts during dark-light transition, with Pi being constant at 4 mM (8). The lysing medium and the basic reaction medium contained 0.025 M HEPES-NaOH (pH 8.0) and 5 mM $MgCl_2$. In addition, the reaction medium contained 2 mM [^{14}C]G6P, 1 mM ATP, 4 mM Pi (as KH_2PO_4), and 1.4 mM PGA at the beginning of the experiment. The concentration of PGA was increased by injecting 20 μl of a PGA solution into the reaction medium to give the final concentration of 4 mM PGA, as indicated in the figure.

Further Factors Affecting the Rate of ADP-Glucose Formation. Among other factors known to regulate the activity of several Calvin cycle enzymes during light-dark transition are changes in stromal pH in the range of pH 7.2 to pH 8.0 (18), changes in the Mg^{2+} concentration, activation by reduced —SH groups, or by the concentrations of $NADP^+$ and NADPH (10, 11). The studies of Preiss *et al.* (14) had already shown that the partially purified ADP-glucose pyrophosphorylase has a relatively broad pH optimum between pH 7 and pH 8, depending on the buffer used as well as on the activation state. In our experiments, the pH dependency of ADP-glucose formation was relatively minor and was inconsistent in the range from pH 7.3 to 8.0. A possible involvement of stromal pH changes in the regulation of ADP-glucose formation seems unlikely.

The extensive work of Ghosh and Preiss (4, 5) had also shown that ADP-glucose pyrophosphorylase has an absolute requirement for Mg^{2+}, the optimal Mg^{2+} concentration being at about 2.5 mM (4, 5). Our experiments essentially confirm these findings, despite the fact that our system is very different. The rate of ADP-glucose formation is very low in the complete absence of Mg^{2+} (with only some endogenous Mg^{2+} being present) (Table III). With increasing [Mg^{2+}] the rate of ADP-glucose formation also increases, reaching saturation at about 2 mM free Mg^{2+}. It is also evident (Table III) that a change in [Mg^{2+}] from 1 to 10 mM (as it might occur in whole chloroplasts at dark-light transition) would not change the rate of ADP-glucose formation. We assume that changes in stromal [Mg^{2+}] are not involved in light-dark regulation of ADP-glucose pyrophosphorylase.

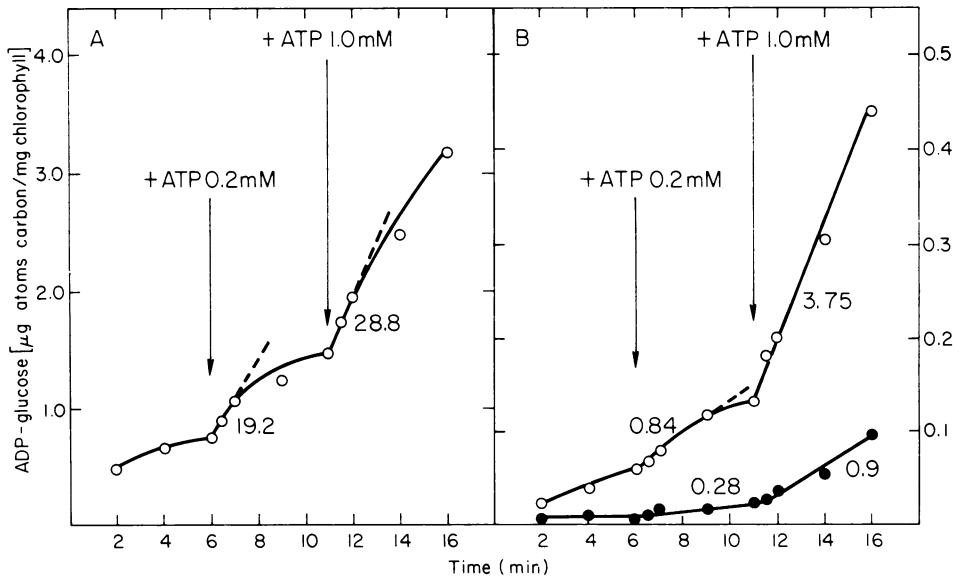

FIG. 2. Response of ADP-glucose formation toward a change in the ATP level at different PGA/Pi ratios. The lysing medium and the basic reaction medium consisted of 0.025 M HEPES-NaOH (pH 8.0) and 5 mM $MgCl_2$. In addition the reaction medium contained 2 mM [^{14}C]G6P, 4 mM Pi (A), 4 mM Pi + 4 mM PGA (B, ○), 4 mM Pi + 1.4 mM PGA (B, ●). Reaction was started without any ATP added (*i.e.* with only some endogenous ATP being present). ATP was added where indicated to give the final concentrations shown in the figure. Rates of ADP-glucose formation (μg atoms carbon/mg Chl·hr) are given by the numbers in the figure.

It is known that the light activation of certain enzymes by reduced —SH groups formed in the light can be simulated by adding dithiol-reagents such as DTT at high concentration (10, 19). Addition of DTT (10 or 50 mM) causes a very significant increase (3- to 6-fold) in the rate of ADP-glucose formation (Table IV). This stimulation was much less significant if ADP-glucose formation was limited by other factors, such as low Mg^{2+}, etc. From this experiment it appears that light activation of ADP-glucose pyrophosphorylase might also include modulation of enzyme activity by reducing —SH groups formed in the light, comparable to the well known regulation of the bisphosphatases or the P-ribulokinase (10).

Effects of Combined Changes in the Levels of PGA, ATP, and DTT. Our experiments showed that changes in the levels of PGA, ATP, and reduced thiols are the most effective modulators of ADP-glucose formation among all factors studied. Since it can be expected that *in vivo* all three factors would change simultaneously, it was of interest to see how the rate of ADP-glucose formation would respond to a combined change in the level of these compounds, as it is thought to occur during dark-light transition in whole chloroplasts. A change in the concentration of PGA (from 1.4 to 4 mM), of ATP (from 0.2 to 1 mM), of G6P (from 6 to 2 mM) together with the addition of DTT (50 mM) causes a dramatic 130-fold stimulation of the rate of ADP-glucose formation (from 0.042 to 5.49 μg atoms carbon/mg Chl·hr) (Table V). A combination of the described factors leads to an extremely effective regulation of ADP-glucose formation, even if the concentrations of Pi and Mg^{2+} are kept constant at 4 and 5 mM, respectively.

Starch Formation by Chloroplast Extracts. Despite the high rates of ADP-glucose formation in our experiments with chloroplast extracts, there was usually no starch formation at all. This is not surprising, since starch formation from ADP-glucose requires the presence of both a primer (*i.e.* an α-1,4-polyglucan) and starch synthetase. This enzyme exists either tightly attached to starch granules, or in a soluble form (3). In our experiments, the chloroplast starch granules usually spin down together with the chloroplast lamellae. If part of that pellet was resuspended and added to a chloroplast extract with fully activated ADP-glucose pyrophosphorylase (*i.e.* at high PGA and without Pi), starch was formed at a rate of 2 to 3 μg atoms carbon/mg Chl·hr (Fig. 3). If boiled starch was added instead, the rate of starch formation reached values of more than 7 μg atoms carbon/mg Chl·hr. This experiment does not necessarily show a coupling of ADP-glucose formation and starch formation inasmuch as a certain amount of starch was formed even in the complete absence of ADP-glucose formation (data not shown). This starch formation is presumably due to the action of the reversible starch phosphorylase, the enzyme that is usually thought to be responsible for the first step in starch degradation. Because in our experiments a high amount of unlabeled starch is added as primer, the actual rate of phosphorolytic starch degradation might well exceed the rate of starch synthesis mediated by starch phosphorylase.

The experiments demonstrate that our chloroplast extracts contain a high amount of soluble starch synthetase and that the rate

Table III. Effect of different Mg^{2+} concentrations on the rate of ADP-glucose formation.

For this experiment, the lysing medium and the basic reaction medium contained 0.025 M HEPES-NaOH, pH 8.0. In addition, the reaction medium contained 2 mM ^{14}C-G6P, 1 mM ATP, 4 mM PGA, 4 mM Pi, and $MgCl_2$ as indicated. Rates were calculated from a linear time course (12 min). For further details see Materials and Methods.

$MgCl_2$ (mM)	ADP-glucose formed (μg-atoms carbon/mg chl. x h)
--	0.37
1	1.35
2	1.70
5	1.69
10	1.18

Table IV. Effect of DTT (dithiothreitol) on ADP-glucose formation.

The lysing medium and the basic reaction medium contained 0.025 HEPES-NaOH pH 8.0, 5 mM $MgCl_2$; in addition, the reaction medium contained 2 mM ^{14}C-G6P, 4 mM PGA, 4 mM Pi, 1 mM ATP and DTT as indicated. Rates were calculated from a linear time course (15 min). For further details see Materials and Methods.

DTT (mM)	ADP-glucose formed (μg-atoms/mg chl. x h)
--	1.8
10	6.2
50	10.3

Table V. Effect of a simultaneous change in the concentration of PGA, G6P, ATP and DTT on ADP-glucose formation.

The lysing medium and the basic reaction medium contained 0.025 M HEPES-NaOH pH 8.0 and 5 mM MgCl; in addition, the reaction medium contained 4 mM Pi and ^{14}C-G6P, PGA, ATP and DTT as indicated in the table. The expressions "light-conditions" and "dark-conditions" refer to the concentrations of PGA, ATP and hexose monophosphates as well as to the presence or absence of light-generated SH-groups (here simulated by DTT), as they exist in whole chloroplasts in light and dark. Rates were calculated from a 6 min time course. For further details see Materials and Methods.

Conditions	ADP-glucose formed (μg-atoms carbon/mg chl. x h)
a) Light-conditions: ^{14}C-G6P 2 mM; PGA 4 mM; ATP 1 mM; DTT 50 mM	5.490
b) Dark-conditions ^{14}C-G6P 6 mM; PGA 1.4 mM; ATP 0.2 mM; no DTT	0.042

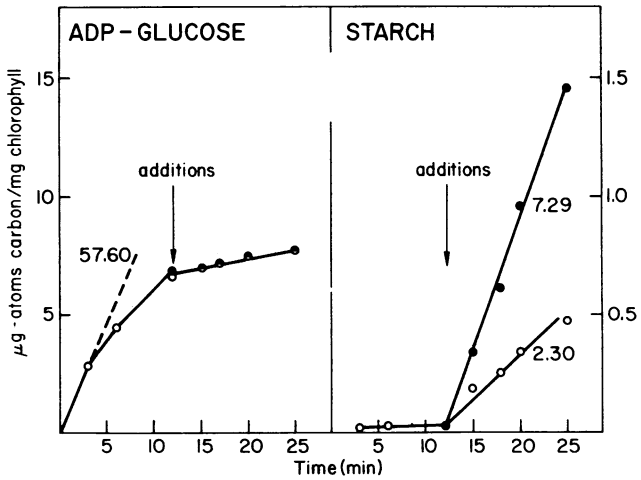

FIG. 3. Formation of ADP-glucose and starch from [U-^{14}C]G6P and ATP by chloroplast extract. The reaction mixture contained 300 μl of chloroplast extract, 0.025 M HEPES-NaOH (pH 7.6); 5 mM $MgCl_2$, 1 mM EDTA; 1 mM ATP; 2 mM [^{14}C]G6P and 3.6 mM PGA in a final volume of 500 μl. Where indicated, 30 μl of chloroplast pellet (○) or 30 μl of boiled starch (1.5 mg, ●) was added. The chloroplast pellet was obtained by resuspending the sediment of the high speed centrifugation step in 0.3 ml of the lysing solution. The pellet added to the reaction medium contained 275 μg of Chl and was equivalent to the amount of soluble chloroplast material in the reaction flask.

of starch formation in this system is limited by the availability (and concentration) of primer. Such a lack of primer might well be a physiological situation in plants, *e.g.* after long periods of low light intensity or darkness. One might speculate that the main purpose of an amylolytic starch breakdown in chloroplasts (12) would be to maintain a certain level of primer inside the chloroplast without keeping too much carbon in the form of starch. This would guarantee a fast onset of starch formation in the light under all conditions.

CONCLUSIONS

Similar to other enzymes of the Calvin cycle, ADP-glucose pyrophosphorylase seems to be subject to regulation by several factors. The most effective modulator of enzyme activity was found to be the change in the concentration of PGA, as it occurs in whole chloroplasts during light-dark transition (8), and this was seen even though the level of Pi remained practically constant. This does of course not mean that the Pi level plays no role in the over-all regulation of starch formation or degradation, since the stromal [Pi] will necessarily change inside the chloroplasts due to the reactions catalyzed by the phosphate translocator with every variation of the cytoplasmic levels of Pi, PGA or triose-P (for a review see Ref. 6). Also, the extensive work of Preiss and coworkers (4, 5, 14, 16) has clearly demonstrated that it is not only the absolute level of PGA or Pi that regulates the enzyme activity, but the ratio of inhibitor (Pi) *versus* effector (PGA).

Somewhat less dramatic than the effect of PGA, but still very significant, is the modulation of ADP-glucose formation caused by a change in the ATP level. We did not investigate the effect of ADP. It was shown earlier (5) that ADP is a very potent inhibitor of ADP-glucose pyrophosphorylase. Since ATP and ADP levels in chloroplasts are usually inversely related, a combined effect in opposite changes of the ATP and ADP levels might be even more effective.

The strong increase in the rate of ADP-glucose formation induced by addition of DTT at high concentrations might indicate an involvement of light-mediated —SH groups in the regulation of ADP-glucose pyrophosphorylase, as was described for other Calvin cycle enzymes (for a review see Ref. 10).

Our experiments demonstrated that the described changes in PGA and ATP levels together with the presence and absence of DTT are sufficient to switch ADP-glucose pyrophosphorylase almost completely on and off, even if Pi and Mg^{2+} are kept constant.

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