Level of Photosynthetic Intermediates in Isolated Spinach Chloroplasts[1]

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Abstract. The level of intermediates of the photosynthetic carbon cycle was measured in intact spinach chloroplasts in an attempt to determine the cause of the induction lag in CO_2 assimilation. In addition, transient changes in the level of the intermediates were determined as affected by a light-dark period and by the addition of an excess amount of bicarbonate during a period of steady photosynthesis. Assayed enzymically were: ribulose 1,5-diphosphate, pentose monophosphates (mixture of ribose 5-phosphate, ribulose 5-phosphate and xylulose 5-phosphate, hexose monophosphates (mixture of glucose 6-phosphate, glucose 1-phosphate, and fructose 6-phosphate), glyceraldehyde 3-phosphate, dihydroxyacetone phosphate, glycerate acid 3-phosphate, a mixture of fructose 1,6-diphosphate and sedoheptulose 1,7-diphosphate, adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP).

The lag in CO_2 fixation appeared to be the result of low levels of pentose monophosphates. The level of ribulose 1,5-diphosphate was roughly equal in chloroplasts showing immediate linear kinetics with respect to CO_2 fixation and chloroplasts which exhibited an initial lag.

Following a light-dark transition, CO_2 fixation ceased immediately but the level of glycerate 3-phosphate increased while ribulose 1,5-diphosphate was only slightly effected. The increase in level of glycerate 3-phosphate was correlated with a decrease in triose phosphate. Within 3 to 5 min in the light, ATP reached a maximum concentration while in darkness, all was utilized in 30 to 60 sec. The rapid loss of ATP was ascribed to an ATPase rather than to its utilization in kinase reactions.

A rapid increase in CO_2 concentration enhanced the level of triose phosphate, but the level of glycerate 3-phosphate showed only a small overshoot and was considered as evidence that reducing power was not a rate limiting factor. Data were obtained indicating that triose phosphates similar to pentose monophosphates and in contrast to fructose 6-phosphate, glucose 6-phosphate and glucose 1-phosphate could be transported between chloroplast and suspending medium. Differential import and export of phosphorylated compounds may serve as routes alternative to starch and sucrose for the flow of carbon into biosynthetic pathways.

The fixation of $^{14}CO_2$ by isolated chloroplasts was first recorded by Allen *et al.* (2). There have been no reports, however, dealing with the total amounts or with transient changes of the intermediate compounds of the photosynthetic carbon reduction cycle in isolated chloroplasts. Hitherto changes in the level of photosynthetic intermediates have been reported solely with intact organisms or organs (4). It has now been recognized that compounds such as glycerate 3-P and sugar phosphates are sequestered not only in but also outside of the chloroplast. Furthermore, there is considerable traffic of intermediates of the photosynthetic cycle between the chloroplast and its environment. Hence certain assumptions must be made in arriving at conclusions about the pool sizes of the photosynthetic intermediates when analyzing intact cells.

We have undertaken a study of the total pool sizes of the photosynthetic intermediates of carbon metabolism by taking advantage of the fact that the isolated intact chloroplast is capable of achieving the overall process of photosynthesis (2, 9). In this study, we have determined the following compounds: ribulose 1,5-diP, pentose monophosphates (mixture of ribose 5-P, ribulose 5-P, and xylulose 5-P), hexose monophosphates (mixture of glucose 6-P, glucose 1-P, and fructose 6-P), glyceraldehyde 3-P, dihydroxyacetone-P, glycerate 3-P, fructose 1,6-diP with sedoheptulose 1,7-diP, ATP, ADP, and AMP. These compounds were measured in an attempt to determine the cause of the induction lag in CO_2 fixation (3, 6), and the effects on the transient levels of these intermediates during light-dark experiments and on addition of substantial amounts of bicarbonate during a period of steady-state photosynthesis.

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Materials and Methods

Preparation of Chloroplasts. Spinach (*Spinacia oleracea*) obtained from the Community Produce Company of Boston or from local farms was stored in the coldroom. Only whole mature leaves were selected, washed, placed in water at 20° and exposed to white light of roughly 1000 ft-c for 2 hr or kept in the dark for an equivalent period. Chloroplasts were prepared in 0.35 M NaCl and finally suspended in a fixation medium as described by Gibbs and Calo (8).

The reaction tubes (1.5 × 20 cm) were illuminated at 2000 ft-c and gassed by slowly bubbling N_2 through the reaction mixture. The temperature was maintained at 15°.

For the determination of $^{14}CO_2$-incorporation, samples were pipetted into sufficient formic acid to yield a final concentration of 4 % formic acid. An aliquot was placed on a planchet which carried a piece of lens tissue, dried and assayed for radioactivity. The counting system was corrected for the efficiency of the counter and for self absorption. For the determination of intermediates of the cycle, 7.5 ml of the reaction mixture, containing approximately 2 mg chlorophyll were poured into 2.0 ml of 20 % (v/v) $HClO_4$, centrifuged for 3 min at 5000*g*. The supernatant fluid was decanted and titrated to pH 5.5 with 5 M K_2CO_3. The precipitated $KClO_4$ was filtered off and the clear filtrate was used for the determination of the photosynthetic intermediates. The assay of these substances was completed within 3 to 4 hr after addition of perchloric acid. According to their stability in the extraction medium the intermediates were assayed in the following sequence: dihydroxyacetone-P and glyceraldehyde 3-P, fructose 1,6-diP, and sedoheptulose 1,7-diP, ribulose 1,5-diP, pentose monophosphate, ADP, AMP, fructose 6-P, glucose 6-P, glucose 1-P, ATP, and glycerate 3-P.

The intermediates were determined at 25° in a recording Gilford spectrophotometer. All blank assays contained instead of chloroplast extract the same volume of CO_2 fixation medium to which $HClO_4$ and K_2CO_3 were added.

Glyceraldehyde 3-P, Dihydroxyacetone-P, Fructose 1,6-diP, and Sedoheptulose 1,7-diP. The following solutions were added per ml: initially 100 μmoles triethanolamine HCl buffer; 50 μmoles EDTA pH 7.6; 0.2 μmole NADH, 0.5 unit α-glycerol P-dehydrogenase (△ OD = dihydroxyacetone-P), then the addition of 0.5 unit of triose-P isomerase (△ OD = glyceraldehyde 3-P), and finally the addition of 0.5 unit of fructose 1,6 aldolase (△ OD = dihydroxyacetone-P + glyceraldehyde 3-P from fructose 1,6-diP + dihydroxyacetone-P from sedoheptulose 1,7-diP). Since the total amount of fructose 1,6 and sedoheptulose 1,7-diP in intact chloroplasts was found to be extremely low, no attempt was made to separate these diphosphates. Since the level of glyceraldehyde 3-P was low in contrast to dihydroxyacetone-P, the 2 compounds were assayed together and are referred to by the term, triose-P.

Glucose 6-P, Fructose 6-P, Glucose 1-P, and ATP. Initially, 100 μmoles tris pH 7.6; 0.2 μmole NADP; 5 μmoles $MgCl_2$ + 0.5 unit glucose 6-P dehydrogenase (△ OD = glucose 6-P), then the addition of 0.5 unit hexose-P isomerase (△ OD = fructose 6-P), and finally the addition of 0.5 unit phosphoglucomutase (△ OD = glucose 1-P) + 3 μmoles glucose and 0.5 unit hexokinase (△ OD = ATP).

Glycerate 3-P. 100 μmoles triethanolamine HCl buffer; 50 μmoles EDTA pH 7.6; 5 μmoles $MgCl_2$; 5 μmoles ATP; 3 units glyceraldehyde 3-P dehydrogenase; 0.2 unit glycerate 3-P kinase.

ADP, AMP. These compounds were assayed according to Adam (1).

Ribulose 1,5-diP. 0.2 ml extract + 0.2 ml 1 M HCl (to remove residual CO_2) + 0.075 ml (1 M triethanolamine HCl buffer containing 0.5 M EDTA pH 7.6) + 0.01 ml 0.5 M $MgCl_2$ + 0.05 ml NaH $^{14}CO_3$ + 0.05 ml ribulose 1,5-diP carboxylase (0.2 unit). The reaction was stopped after 30 min of incubation at 25° by addition of 0.1 ml 20 % $HClO_4$. To assess the ^{14}C fixed, 0.2 ml of the clear supernatant fluid was placed on a planchet with a piece of lens tissue, dried and counted. For the calculations of the ribulose 1,5-diP content a test curve was made using the same procedure with known amounts of ribulose 1,5-diP.

Pentosemonophosphates. Ribose 5-P, ribulose 5-P, and xylulose 5-P were assayed as described for ribulose 1,5-diP in presence of 0.01 ml 0.1 M cysteine + 0.01 ml ribulose 5-P kinase (0.3 unit) + 0.01 ml xylulose 5-P epimerase (0.2 unit) + 0.01 ml ribose 5-P isomerase (0.2 unit) and 0.01 ml 0.1 M ATP. For the test curve ribose 5-P was used. For calculations of the net amount of pentose monophosphates present in the extract, the amount of ribulose 1,5-diP was substracted.

The following enzymes were purified according to the published procedures: ribulose 1,5-diP carboxylase (19); ribulose 5-P kinase (11), xylulose 5-P epimerase (18); ribose 5-P isomerase (11). Other enzymes were obtained from C. F. Boehringer and Son or from Sigma Chemical Company.

Results

Changes in the Levels of the Photosynthetic Intermediates During the Initial Phase of CO_2 Fixation. The assimilation of CO_2 by chloroplasts isolated in a buffered NaCl medium showed an initial lag before achieving a maximal linear rate (Fig. 1D). The lag was eliminated when the leaves were preilluminated, the isolation procedure was performed in an extremely short time, and the chloroplasts were used immediately after isolation.

The chloroplasts filled up their ATP pools at the same velocity (Fig. 1D). In chloroplasts which did not show a lag, the level of glycerate 3-P increased

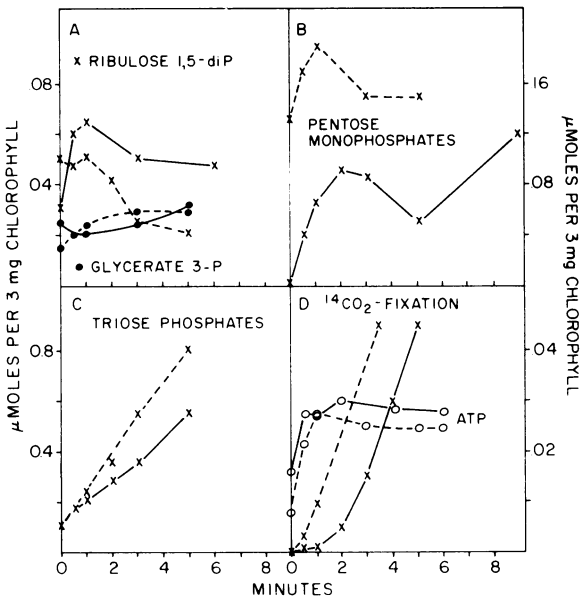

FIG. 1. Changes in the level of photosynthetic intermediates during the initial phase of CO_2 fixation in isolated chloroplasts. x———x, preparations showing an initial lag in CO_2 fixation; x------x, no initial lag. The reactions were run at 15°; illumination was 2000 ft-c; gas phase was N_2. CO_2 fixation was followed by taking aliquots from the same tube at the indicated intervals. For the assay of intermediates each symbol indicates the analysis of 1 tube. The reaction medium was the same as for CO_2 fixation, however, with unlabeled bicarbonate in place of radioactive bicarbonate. The maximum rate of CO_2 fixation was in both preparations 4.6 μmoles per mg chlorophyll per hr. The levels of intermediates are given as μmoles per 3 mg chlorophyll.

slightly during the first 2 min of illumination (Fig. 1A). In preparations with a lag in CO_2 fixation, the level of glycerate 3-P increased after the lag was finished. The overall increase in the level of glycerate 3-P was very low in both preparations indicating that the supply of ATP and NADPH was abundant.

During the first phase of illumination the increase of triose-P (dihydroxyacetone-P and glyceraldehyde 3-P) was lower in chloroplasts starting with a lag phase (Fig. 1C). Interesting transient changes in the level of ribulose 1,5-diP were noted. The largest change was a sharp increase during the lag and a decrease in both preparations as soon as linear CO_2 fixation started (Fig. 1A).

The only striking difference between the levels of intermediate in the 2 kinds of chloroplasts was observed with respect to the initial content of the pentose monophosphates (Fig. 1B). Chloroplasts without a lag in CO_2 assimilation contained relatively high levels of endogenous pentose monophosphates. In contrast, preparations with a lag contained little pentose monophosphates after isolation and showed a very intensive accumulation of these 5 carbon moieties during the lag phase. Eventually similar transient changes in the pentose monophosphates levels were observed in both preparations.

Effect of Additional Bicarbonate on the Level of Intermediates. Fig. 2 shows the changes in level of glycerate 3-P, triose-P, pentose monophosphates and ribulose 1,5-diP in an experiment where "extra" bicarbonate was introduced during the linear phase of photosynthesis to increase its concentration from 1 mM to 20 mM. Following addition, CO_2 incorporation was enhanced slightly and triose-P accumulated (Fig. 2 A and C). The level of glycerate 3-P had only a small overshoot and provided further evidence that ATP and NADPH were not rate-limiting (Fig. 2D). From Fig. 2B, it is seen that the "extra" bicarbonate brought about a small drop in the level of ribulose 1,5-diP and a larger decrease in the level of pentose monophosphates.

Light-dark Transient Changes in the Level of Intermediates. In these experiments, chloroplasts were isolated from preilluminated leaves in order to eliminate the lag phase in CO_2 fixation (Fig. 3). Although the levels of intermediates of the cycle

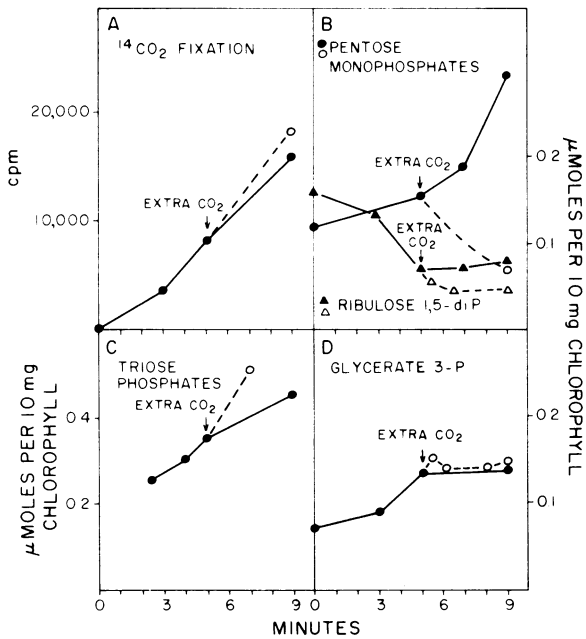

FIG. 2. Effect of additional bicarbonate on the level of intermediates in isolated chloroplasts: x———x, 1 mM bicarbonate; x-------x, 20 mM bicarbonate. CO_2 fixation was followed by taking aliquot samples from the same tube as indicated. For the assay of intermediates every point indicates the analysis of 1 tube. The reaction mixture was the same as for CO_2 fixation, however, with unlabeled bicarbonate in place of radioactive bicarbonate. Additional bicarbonate was added 5 min after the onset of photosynthesis. The incubations were carried out at 15° under N_2 and at 2000 ft-c. The maximum rate of CO_2 fixation in presence of 1 mM bicarbonate was 3.1 μmoles per mg chlorophyll per hr. The level of intermediates are given as μmoles per 10 mg chlorophyll.

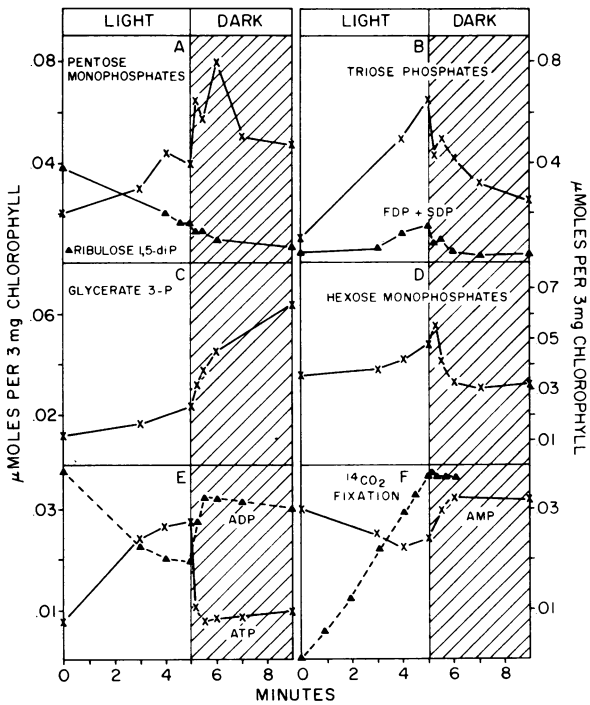

FIG. 3. Light-dark transient changes in the level of photosynthetic intermediates in spinach chloroplasts. The reaction was started by addition of chloroplasts to a light exposed reaction mixture. CO_2 fixation was followed by taking aliquots from the same tube at the indicated intervals. For the assay of intermediates each symbol indicates the analysis of 1 tube. The reaction mixture was similar to that used for $^{14}CO_2$ fixation, (see Methods), however, with unlabeled bicarbonate replacing the radioactive bicarbonate. The reactions were run at 15° and 2000 ft-c under N_2. The light was switched off 5 min after the onset of photosynthesis. The level of intermediates are given as μmoles per 3 mg chlorophyll. The maximum rate of $^{14}CO_2$ fixation was 4.3 μmoles per mg chlorophyll per hr. Abbreviations in B are FDP (fructose 1,6-diP) and SDP (sedoheptulose 1,7-diP).

were quite different than in the experiments depicted in Fig. 1 and 2, the general shape of changes was the same. As we demonstrated in previous experiments (Fig. 1 and 2), the level of ribulose 1,5-diP was found to decrease steadily during exposure of the chloroplasts to light and an additional drop of ribulose 1,5-diP was observed as soon as the light was turned off. The level did not fall to zero but instead remained, after the light was turned off at a nearly constant and easily measurable level (Fig. 3A). The level of glycerate 3-P increased rapidly throughout the dark period. The amount of glycerate 3-P formed during the dark period was roughly twice that expected if its sole precursor was ribulose 1,5-diP (Fig. 3C). Furthermore, CO_2 fixation ceased immediately when the light was turned off (Fig. 1F). These findings exclude ribulose 1,5-diP as the only or perhaps preferred source for glycerate 3-P formation during the darkness.

The level of pentose monophosphates increased slightly during the light phase but in the first minute of darkness there was a pronounced rise (Fig. 3A). This transient rise in the level of pentose monophosphates no doubt accounted for the drop in triose-P and hexose monophosphates (Fig. 3D) and would be the result of reactions catalyzed by transketolase. Fig. 3B shows that the level of triose-P increased linearly throughout the light phase. The rapid synthesis of triose-P in the light was approximately equivalent to its decrease during the dark period. No other intermediate analyzed reached the level of triose-P. In every experiment in this investigation, the rate of triose-P accumulation paralleled the rate of CO_2 fixation (Fig. 3B). Separate determinations of glyceraldehyde 3-P and dihydroxyacetone-P established that the ratio of aldehyde to ketone moiety was 4 to 96. Therefore, these preparations contained sufficient triose-P isomerase to maintain the 3-carbon compounds in equilibrium.

In Fig. 3B it can be seen that the level of diphosphates (sedoheptulose and fructose) increased slightly after the initial 2 min and this was followed by a sharper increase. In the dark they fell off approaching the endogenous level. A somewhat similar pattern was found for the hexose monophosphates (Fig. 3D). Since the activity of fructose 1,6-diP aldolase was found to exceed greatly the rate of CO_2 fixation (13), it was expected that the level of fructose 1,6-diP would be higher than that of triose-P. The unexpected ratio indicated that the triose-phosphates were not completely associated with aldolase. We have to conclude that the bulk of the triose-P was located in the medium rather than in the chloroplast.

The transient changes in the adenine nucleotides were striking. In the light as well as the dark period, the level of ATP was inverse to that of ADP and AMP. The sharp drop in the ATP level in darkness was usually finished after 30 to 60 sec. Decrease of AMP in the light and its increase in the dark followed the ADP curve but with a short delay (Fig. 3E and F).

Discussion

Lag Phase. A property of freshly isolated chloroplasts is an initial lag in CO_2 fixation (3, 6). The lag may be abolished either by illumination of the leaves prior to isolation of the organelle or by addition to the reaction mixture of a small amount of an intermediate of the carbon cycle such as ribose 5-P, fructose 1,6-diP, triose-P, or glycerate 3-P (3, 6). From the results presented in Fig. 1, we would conclude that the lag was not the result of low levels in the endogenous pools of triose-P, fructose 1,6-diP, glycerate 3-P, hexose monophosphates, ribulose 1,5-diP or of ATP. Rather the lag was associated with the level of pentose monophosphate. When this level approached 0.1 μmole per 3 mg chlorophyll, the rate of CO_2 fixation became equal to that of a chloroplast

isolated from preilluminated leaves. Our data lend support to the notion of others (3, 6) that a factor contributing to the initial lag in CO_2 fixation was a low level of an intermediate of the carbon cycle and extends it by singling out a pentose-P as the compound whose low level was responsible for the initial lag.

Enzyme activation may be another factor causing the lag. We have reported elsewhere that the activity of ribulose 5-P kinase was enhanced 3 to 4-fold during the initial phase of illumination (14). The fact that roughly equal amounts of ribulose 1,5-diP were present in chloroplast with and without a lag is considered as evidence that this pool was only partially associated with ribulose 1,5-diP carboxylase. It is also possible that the carboxylase was in an inactive form during the lag phase. We cannot exclude the possibility that the increasing level of pentose monophosphates might cause an allosteric activation of the carboxylase or of another enzyme of the cycle.

CO_2 Dependent Transients. Changing the partial pressure of CO_2 available to an intact alga or leaf during steady state photosynthesis has been a well known methodical aid to study the CO_2 dependent transients following or leading to the carboxylation step. Wilson and Calvin (23) used this technique to identify the reciprocal relationship between glycerate 3-P and ribulose 1,5-diP for intact *Chlorella*. A rapid increase in the partial pressure of CO_2 produced a large increase in glycerate 3-P and sucrose with a concomitant drop in ribulose 1,5-diP. In our experiments with the chloroplast, an increase in bicarbonate concentration from 1 mM to 20 mM resulted in a limited acceleration of isotope uptake. As seen in Fig. 2, this was accompanied by a striking increase in triose-P and was paralleled by a sharp decrease in the level of pentose monophosphates. In contrast, the change in level of glycerate 3-P was limited to a brief overshoot and the ribulose 1,5-diP level underwent a small decrease.

Considering the experimental data recorded in Fig. 2, the small overshoot of glycerate 3-P and a rapid increase in the level of triose-P implies that the formation of reducing power was not a rate limiting factor in our preparations. In experiments with the reconstituted broken chloroplast system, Kandler *et al.* (12) came to the same conclusion. The surge in the level of triose-P and the drop in the level of pentose monophosphates was observed in spite of an increase in its level in the control experiment (Fig. 2B, solid line). A steady increase in the level of pentose monophosphates and a steadily decreasing level of ribulose 1,5-diP were usually observed in each experiment. A rate limiting conversion of pentose monophosphates to ribulose 1,5-diP could be the result of insufficient ATP, ribulose 5-P kinase activity or pentose monophosphates supply. The rapid rise in ATP during the initial minutes of the light phase and the very small increase in glycerate 3-P was taken as evidence against a limited supply of ATP. With respect to ribulose 5-P kinase, Latzko and Gibbs (13) reported specific activities in the order of 200 μmoles per mg chlorophyll per hr for chloroplasts isolated in 0.35 M NaCl. This value is roughly 50 times the rate of CO_2 fixation. Since we observed an increase in the level of the pentose monophosphate concentration during the light phase we are tempted to exclude their pentose monophosphate as a factor. Therefore, we assume that the pentose monophosphates were not completely associated with the enzymes catalyzing its conversion to the CO_2 acceptor and that an intermediate of the carbon cycle had diffused from the chloroplast to the suspending medium. However, the experimental data recorded in Fig. 2B in which the rapid drop in the level of pentose monophosphates was observed on addition of CO_2 shows that pentose monophosphate can apparently re-enter the chloroplasts and be used metabolically. The rise in the level of triose-P can be interpreted either in terms of an export of this intermediate into the suspending medium without re-entry or as an impairment at the enzymic level in the conversion of the triose-P into hexose monophosphates. Triose-P can apparently diffuse into the intact chloroplast and come into contact with the enzymes of the photosynthetic cycle since the 3-carbon moieties like the pentose monophosphates but unlike the hexose monophosphates are known to eliminate the lag in CO_2 fixation (3, 6). The rate of triose-P to fructose and sedoheptulose diphosphates was found to be 5:1. The activity of aldolase in these preparations was approximately in the order of 80 μmoles per mg chlorophyll per hr and compared to the rate of CO_2 fixation should be sufficient to maintain an equilibrium favoring the diphosphates. On the other hand, the transient changes of the hexose monophosphates in contrast to the pentose monophosphates and triose-P showed a relatively high level (Fig. 3D) and only a small increase during the light. Although hexose-P isomerase and phosphoglucomatase were found to be low in comparison to aldolase (13), the ratio of glucose 6-P : fructose 6-P : glucose 1-P was close to equilibrium (19:8:1). Our data would imply a rate-limiting step in the conversion of fructose 1,6-P to hexose monophosphate and are in full agreement with the work of others that fructose 1,6-diphosphatase plays an important role in metabolic regulation of the photosynthetic carbon reduction cycle. The transient changes observed with the hexose phosphates contrast with those of the pentose-P and triose-P and indicate that the 6-carbon monophosphate do not diffuse into the medium. This conclusion is fully supported by Bassham, Kirk and Jensen (5) who directly measured the distribution of photosynthetic intermediate compounds between the chloroplasts themselves and their suspending medium and by the work mentioned earlier (3, 6) on the types of photosynthetic intermediate compounds which are capable of eliminating the lag in CO_2 fixation.

Light-Dark Regulation. Transient changes in the photosynthetic carbon cycle which occur as a result of altering the partial pressure of CO_2 are primarily those involving the intermediates of the carboxylation step whereas the light-dark switch immediately stops the formation of ATP and reduced pyridine nucleotide and consequently the reductive part of the cycle and the formation of ribulose 1,5-diP. As a result of the light-dark transition in our study (Fig. 3), the levels of glycerate 3-P and pentose monophosphate increased sharply. In the dark phase the bulk of the ATP was consumed within 15 sec (the shortest time in which the first sample was taken in the dark phase). Increase of ADP was reciprocal to the oscillation of ATP whereas the level of AMP apparently as a result of ADP kinase (myokinase) activity followed with a short delay. Assuming an active ribulose 1,5-diP carboxylase during the dark period, it is clear that the sharp drop in the level of ATP was not the result of its metabolism in the ribulose 5-P kinase reaction since CO_2 fixation ceased immediately after turning off the light. It would appear that a light dependent ATPase as described first by Petrack and Lipmann (16) may be responsible for the rapid loss of ATP.

It is clear from the small drop in ribulose 1,5-diP and the immediate stoppage of CO_2 uptake during the light-dark switch that the enormous increase in the level of glycerate 3-P cannot be accounted for in terms of the carboxylation of ribulose 1,5-diP. Since our chloroplast preparations do not contain fructose 6-P kinase (13), hexose monophosphates can be eliminated as a source of glycerate 3-P. One possible explanation is an oxidation of the triose-P. A flow of substrate reverse to that of the photosynthetic carbon reduction cycle implies a source of oxidized pyridine nucleotide. Perhaps the reoxidation is catalyzed by glyoxylic acid reductase found in chloroplast preparations prepared by our procedure (13).

In summary, our results are in general agreement with those reported earlier from *in vivo* studies with *Chlorella pyrenoidosa* (15) and with leaf tissue (21) of the kinetics of the transients changes in the levels of intermediate compounds of photosynthesis following a light-dark transition and with spinach chloroplasts on the diffusion of certain photosynthetic intermediates into the surrounding medium (5). Unresolved is the question of regulating the carboxylation reaction which stops in the dark as evidenced by immediate cessation of isotope uptake even though there apparently remained adequate amounts of ribulose 1,5-diP and CO_2. Another is the apparent block in the regeneration of the CO_2 acceptor between triose-P and pentose monophosphate. Enzymic profiles of the enzymes catalyzing the photosynthetic carbon reduction cycle provided initial evidence that fructose 1,6-diphosphatase may be a pace-maker (12). More recent evidence has strengthened this notion (7, 15). Finally, the concept concerning the flow of carbon from the chloroplast into biosynthetic pathways first stated by Sachs (20) appears to require modification. Classically, it has been held that the autotrophic phase of carbon nutrition was terminated with the carbohydrates starch and sucrose and that the heterotrophic phase began with their breakdown and subsequent formation of other carbon containing compounds in the plant cell. The results presented here and elsewhere (6, 10, 22) have provided evidence for the hypothesis that the differential import and export of phosphorylated compounds of the photosynthetic carbon reduction cycle may function *in vivo* as transport metabolites during photosynthesis and as routes alternative to the so-called nutritional carbohydrates for the flow of carbon into biosynthetic pathways.

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