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SITES OF SYNTHESIS AND TRANSPORT OF PHOTOSYNTHETIC PRODUCTS WITHIN THE LEAF CELL

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SUMMARY

1. After illumination of leaves in the presence of $^{14}CO_2$ for various times and subsequent freeze drying, chloroplasts were isolated using a nonaqueous procedure. The time-course of the distribution of a number of compounds between chloroplasts and the remainder of the cell was calculated from the ^{14}C-incorporation into the fractions obtained.

2. Labelled ribulose diphosphate, sedoheptulose diphosphate and sedoheptulose monophosphate occurred, at least during the first minutes of photosynthesis, solely in the chloroplasts. At the beginning of photosynthesis phosphoglyceric acid, fructose diphosphate, fructose monophosphate and glucose monophosphate appeared first in the chloroplasts, but were found later also in the non-chloroplastic part of the cell. The major part of glucose diphosphate, uridine diphosphoglucose, sucrose, malic acid and citric acid was always located in the non-chloroplastic part of the cell.

3. From the results it is concluded that the photosynthetic carbon cycle operates exclusively in the chloroplasts. Sugar phosphates, which are not needed in the cyclic regeneration of the CO_2-acceptor, are directly translocated into the cytoplasm. The synthesis of uridine diphosphoglucose takes place mainly in the cytoplasm. Glucose diphosphate and possibly also sucrose seem to be formed in the cytoplasm of the leaf cell.

INTRODUCTION

Isolated chloroplasts are capable of forming the same intermediates of photosynthesis as the intact cell[1–5]. Rates of photosynthesis in isolated chloroplasts are, however, at least one order of magnitude lower than in the intact leaf cell[6–9]. This suggests a possible cooperation of chloroplasts and cytoplasm as a prerequisite of high photosynthetic rates[10]. In the present study kinetic measurements have been made of the ^{14}C-incorporation into chloroplasts and cytoplasm of the intact leaf cell in order to obtain evidence concerning the sites of the reactions of the photosynthetic carbon

Abbreviations used: PGA, phosphoglyceric acid; DGPA, diphosphoglyceric acid; GlcMP, glucose monophosphate; GlcDP, glucose diphosphate; FruMP, fructose monophosphate; FruDP, fructose diphosphate; RuDP, ribulose diphosphate; SuMP, sedoheptulose monophosphate; SuDP, sedoheptulose diphosphate.

cycle. At the same time our experiments present information concerning the translocation of photosynthetic products within the leaf cell[11]. Results of similar experiments have been announced recently in a short communication of WILLIAMS, ONGUN AND STOCKING[12].

The investigation of the distribution of soluble compounds between chloroplasts and the remainder of the cell requires the isolation of chloroplasts as far as possible undisturbed by the isolation procedure.

Since water-soluble intermediates of photosynthesis are lost during the isolation procedure in aqueous media, we isolated chloroplasts from light petroleum–carbontetrachloride mixtures after exposure of the leaves to $^{14}CO_2$ in the light for different times and subsequent freeze drying of the vitrified material. This method has already been successfully applied in localization studies[13–19].

METHODS

Material

Leaves of young spinach plants (*Spinacia oleracea* L.) were obtained directly from the field. During the winter, leaves from young broad bean plants (*Vicia faba* L.), grown in the greenhouse and slightly etiolated, were also used.

Experiments

3–4 g of leaves were exposed for 4 min to $^{14}CO_2$ (from $Ba^{14}CO_3$, specific activity 25 mC/mmole) in the dark and were then illuminated with two 200-W incandescent lamps, 25 cm from the leaves. Care was taken to maintain room temperature. The experiments were terminated by immersing the leaves in liquid air and subsequent freeze drying at −25°.

Chloroplast isolation

A chloroplast fraction was isolated from the frozen dried leaves as described by THALACKER AND BEHRENS[20]. Two or three gradients were used. Further purification of the chloroplasts was achieved by repeated short time centrifugations in light petroleum–carbontetrachloride (50:50/v/v), which eliminated particles smaller than chloroplasts. 20–50 mg of chloroplasts and about 350 mg of residual material were thus obtained.

Fixation rate and radioactive products

Chloroplasts and residue were extracted first with 80% ethanol at 70° and then twice with water at room temperature. Hot alcoholic extraction was essential, contrary to the observations of BASSHAM AND KIRK[21], to avoid enzymic dephosphorylations of PGA and fructosephosphate which take place even in alcoholic suspensions of the leaf material at room temperatures (see also ref. 22). Aliquot portions of the combined acidified extracts were spread on metal plates, dried and counted with a flow counter[23] (Friesecke & Hoepfner FH 407, windowless). The radioactivity of the organic solvents used for the chloroplast isolation was determined in the same way. ^{14}C-labelled compounds were separated by two-dimensional paper chromatography (paper from Schleicher and Schüll 2043 b Mgl, prewashed in 0.5% oxalic acid and water) with butanol–propionic acid–water and phenol–water as solvent systems[24]. Radioactive spots were located by autoradiography (Agfa X-ray paper)

and counted directly on the chromatogram with a flow counter (Friesecke & Hoepfner, FH 407 A, with gold-sputtered mylar-window). The counting yield was 56 % windowless, 31 % with mylar-window and 6% on the chromatograms.

Spots on the chromatograms were eluted from the paper and identified by two-dimensional co-chromatography with authentic marker substances, if necessary, after enzymic dephosphorylation[25]. Sugar phosphates were routinely dephosphorylated and re-chromatographed. The individual compounds were counted.

Calculations

The chloroplast weight per total cell weight ratio (= z) of leaf cells and the yield (= w) of isolated chloroplasts are easily calculated (*cf.* ref. 19) from the chlorophyll content of chloroplasts and leaf tissue, which has been determined according to ARNON[26].

The fraction y of a particular compound present in the chloroplasts is

$$y = \frac{z \cdot a}{c} \tag{1}$$

where a and c are the amounts, on a unit weight basis, of this compound in chloroplasts and the total cell, respectively. Since c has not been determined, a substitute has to be found. An equivalent is

$$c = d + (a - d)\frac{z \cdot w}{100} \tag{2}$$

as has been derived elsewhere[19]; d, which is known, is the amount of the substance, of which the distribution within the cell is being investigated, per unit weight residue. If Eqn. 2 is inserted into Eqn. 1, the following equation results:

$$y = \frac{z \cdot a}{d + (a - d)\frac{z \cdot w}{100}} \tag{3}$$

The difference between the obtained value y and 1 represents the fraction of the particular compound present in the cytoplasm and the vacuole of a leaf cell.

RESULTS

Dependable information on the distribution *in vivo* of ^{14}C-labelled substances between chloroplasts and the remainder of the cell can be gained only if secondary diffusion within the cell after freezing of the leaves and leaching of soluble compounds during the chloroplast isolation can be ruled out. As will be shown at the end of this section, the following results are not influenced by such processes.

Under our experimental conditions we found fixation rates of 80–105 μmoles CO_2 per mg chlorophyll per hour, based on the ^{14}C-incorporation into stable products after 2–4 min photosynthesis. In Fig. 1 the ^{14}C-incorporation into chloroplasts is shown in a typical time experiment. After a lag phase of 1–2 min there is a rapid uptake of ^{14}C at about a constant rate.

The fraction of the total radioactivity of the cell present in the chloroplasts exhibited characteristic changes with time immediately after illumination of the leaves (Fig. 2); after 4 min dark fixation, 30–40% of the total radioactivity was located in the chloroplasts. Upon illumination there was a rapid increase to about

70%. This high percentage fell off slowly to a steady state of approx. 40–45%, probably because of the translocation of photosynthetic products from the chloroplasts to the cytoplasm (and the vacuole). In accordance with our results, WILLIAMS, ONGUN AND STOCKING[12] also found 45% of the total radioactivity of the cell in the chloroplasts after 5 min photosynthesis of leaves in the presence of $^{14}CO_2$.

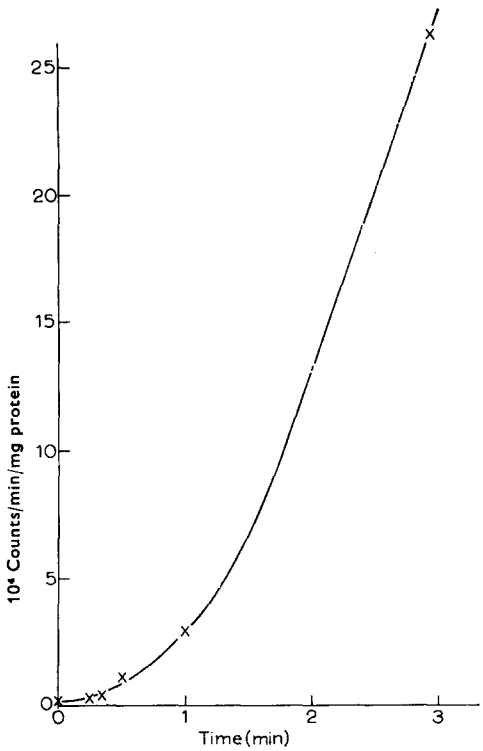

Fig. 1. Time course of the labelling of soluble compounds in the chloroplasts during photosynthesis of intact spinach leaves in the presence of $^{14}CO_2$.

The radioactivity of individual compounds in the chloroplast and residue fractions

The percentages of the total radioactivity in a few selected compounds are listed in Table I. The particular experiment shown in Table I had been performed in November. In the winter experiments phosphate esters contained the major part (about 70%) of the total radioactivity even after 3 min photosynthesis, while only a minor part (5–15%) was found in sucrose. On the other hand, plants grown during the summer incorporated, after 3 min photosynthesis, about 50% of the total ^{14}C into sucrose. Radioactivity in the phosphate esters was then accordingly lower (30%). Probably the reduction of PGA is a rate-limiting step of photosynthesis under short day conditions. In all experiments, however, the distribution of a given labelled substance between chloroplasts and the residual cell material was very similar.

From Table I it is apparent that the percentages of the phosphate esters in the chloroplasts, with the exception of GlcDP, were higher than those in the residue. The same also held true for other sugar phosphates not listed in Table I. On the other

hand, percentages higher in the residue than in the chloroplasts were found with GlcDP, sucrose, malic acid and (not listed in Table I) UDPG, glyceric acid, citric acid, aspartic acid, alanine and other compounds. Especially in the case of PGA and FruMP there were considerable changes with time in the ratio of the chloroplastic and the residual compounds.

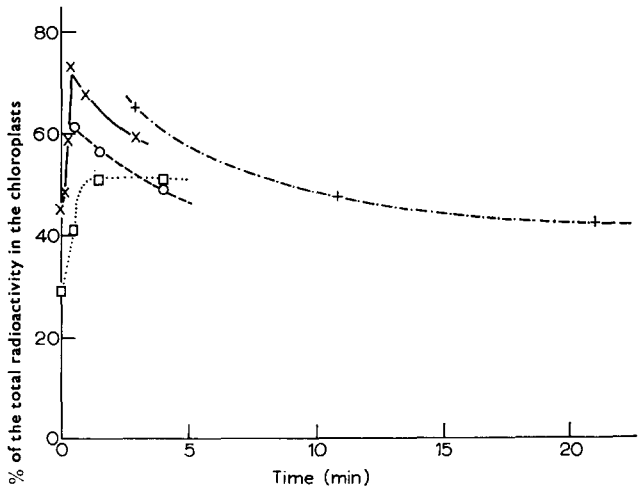

Fig. 2. Percentage of the total radioactivity of the cell present in the chloroplasts during photosynthesis of leaves in the presence of $^{14}CO_2$. Expt. 1 (spinach), O---O ; Expt. 2 (spinach), ×—× ; Expt. 3 (spinach), +·–·–·+ ; Expt. 4 (broad bean), □·····□.

TABLE I

PERCENTAGES OF INDIVIDUAL COMPOUNDS IN THE TOTAL RADIOACTIVITY OF THE FRACTIONS CHLOROPLASTS AND RESIDUAL MATERIAL AT DIFFERENT TIMES OF PHOTOSYNTHESIS (MATERIAL: SPINACH LEAVES)

Compound	*Fraction*	*Dark 4 min*	*Photosynthesis Time (sec)*				
			15	*22*	*30*	*60*	*180*
RuDP	chloroplasts	0	2.2	6.2	14.8	14.5	8.7
	residue	0	1.6	3.7	9.8	8.8	4.5
GlcDP	chloroplasts	0	0.7	0.5	0.7	0.7	0.7
	residue	0	1.0	2.4	1.0	1.0	1.0
FruMP	chloroplasts	0	2.4	6.2	6.2	6.5	3.9
	residue	0	1.1	3.0	3.5	6.2	4.6
SuMP	chloroplasts	0	1.2	5.6	8.0	8.7	5.2
	residue	0	0.6	1.4	4.6	5.6	3.1
PGA	chloroplasts	26.1	29.0	40.8	47.5	46.6	45.8
	residue	8.9	9.0	27.1	42.0	42.0	34.4
Sucrose	chloroplasts	0	0	0	0.3	0.7	8.5
	residue	0	0	?	0.5	1.1	14.3
Malic acid	chloroplasts	3.1	2.3	1.3	2.0	0.8	0.2
	residue	7.8	6.6	4.0	4.2	1.8	0.5

The distribution of PGA and sugar phosphates between chloroplasts and the nonchloroplastic part of the cell

From the total fixation of $^{14}CO_2$ and the percentages of ^{14}C in a particular compound (as presented in Table I) the fraction of this compound present in the chloroplasts can be calculated by application of Eqn. 3. This has been done for PGA in

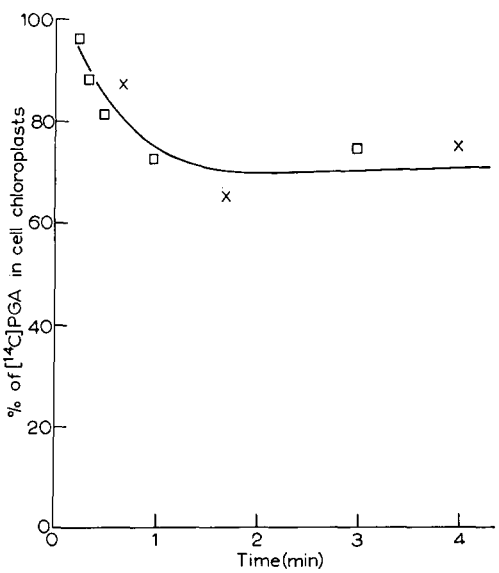

Fig. 3. Percentage of the total 3-[^{14}C]PGA of the cell present in the chloroplasts during photosynthesis of leaves in an atmosphere containing $^{14}CO_2$. Expt. 1 (spinach), □—□; Expt. 2 (spinach), ×—×.

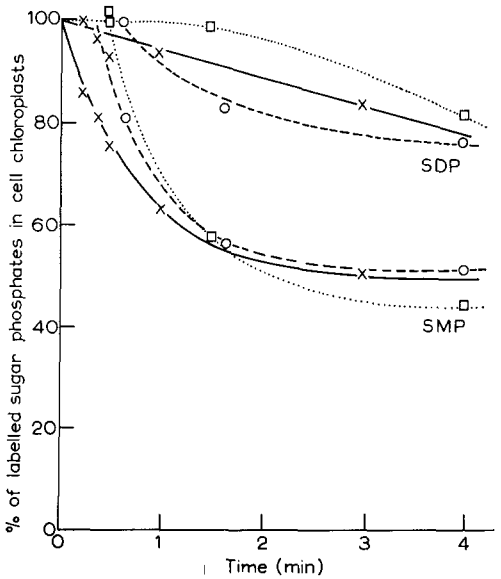

Fig. 4. Percentage of the labelled sugar phosphates of the cell present in the chloroplasts during photosynthesis of leaves in an atmosphere containing $^{14}CO_2$. Sugar diphosphates = SDP; sugar monophosphates = SMP. Expt. 1 (spinach), O---O; Expt. 2 (spinach), ×—×; Expt. 3 (broad bean), □·····□.

Fig. 3. Immediately after illumination 100% of the newly formed PGA was located in the chloroplasts. There seems to have been a considerable translocation from the chloroplasts to the cytoplasm: after 90 sec illumination a steady state was reached of 70% of the labelled PGA in the chloroplasts and 30% in the remainder of the cell (= cytoplasm + vacuole). Similarly, by re-extrapolation of the obtained values to the beginning of the illumination (Fig. 4), 100% of the sugar phosphates was found in the chloroplasts. While sugar diphosphates appear only slowly in the cytoplasm, the percentage of sugar monophosphates present in the chloroplasts declined rapidly until a steady state distribution of about 50/50 was reached.

After phosphatase treatment of the sugar mono- and diphosphates and subsequent re-chromatography the individual sugar phosphates were determined (Table II). RuDP, SuDP, SuMP and possibly also DGPA (the activity of which was only about 5–10% that of the other diphosphates) were found exclusively or nearly exclusively in the chloroplasts. The values considerably in excess of 100% in the cases of SuDP and SuMP may be explained by the extensive manipulation of the material or by a partial destruction preferably in the residue. GlcDP resided, even

at short times of photosynthesis, mainly in the cytoplasm. As indicated by the 100 % localization in the chloroplasts after short time photosynthesis, FruDP was formed in the chloroplasts, but was found later also in the cytoplasm. The same holds true for FruMP and GlcMP. Thus the distribution kinetics of FruDP, FruMP and GlcMP suggest a translocation from the chloroplasts, where these compounds obviously are formed, to the cytoplasm, while other sugar phosphates, namely RuDP, SuDP and SuMP, are retained in the chloroplasts.

TABLE II

SUGAR PHOSPHATES PRESENT IN THE CHLOROPLASTS OF SPINACH LEAVES AT DIFFERENT TIMES OF PHOTOSYNTHESIS AS EXPRESSED IN PERCENTAGES OF THE TOTAL AMOUNT IN THE CELL

Compound	*Photosynthesis in sec*				
	15	*22*	*30*	*60*	*180*
Diphosphates					
GlcDP	17	14	52	48	19
FruDP	99	70	72	63	64
SuDP	130	110	158	112	120
RuDP	88	94	97	101	95
DPGA	110	110	82	100	75
Monophosphates					
GlcMP	70	80	50	44	42
FruMP	101	108	72	70	52
SuMP	93	165	108	98	89

These results have been confirmed by other experiments which yielded values very similar to those reported in Table II. Since, during the first few minutes of photosynthesis, FruDP, FruMP and GlcMP contain about 10–20 % of the total ^{14}C they may be of considerable importance in the translocation of carbon within the leaf cell.

The distribution of UDPG and sucrose between chloroplasts and the nonchloroplastic part of the cell

Only a small fraction of UDPG was located in the chloroplasts, while the major part resided in the cytoplasm (Fig. 5). At the beginning of one time experiment a relatively high percentage of UDPG was found in the chloroplasts. However, this can easily be explained by the fact that precursors of UDPG formation had not yet been made available in the cytoplasm to allow rapid synthesis. From a comparison of Fig. 5 with the distribution kinetics of the sugar diphosphates, whose molecular size is smaller than that of UDPG, it can be deduced that UDPG was not translocated from the chloroplasts to any large extent, but was synthesized preferably in the cytoplasm.

Since UDPG, besides taking part in the synthesis of starch, which is known to be formed in the chloroplasts, is one of the reactants in the synthesis of sucrose[27,28], the distribution kinetics of sucrose are of particular interest. Unfortunately in all experiments the initial labelling of sucrose was very slow (Table I) and could not be accurately determined in short time illumination experiments after a dark period.

In three kinetic experiments with spinach and broad bean leaves we found about 2/3 of the labelled sucrose in the nonchloroplastic part of the cell. This distribution did not change significantly with time between 1 and 4 min illumination and appeared closely correlated to that of UDPG, which, however, was labelled considerably earlier than sucrose (see also CALVIN AND BASSHAM[29], p. 54).

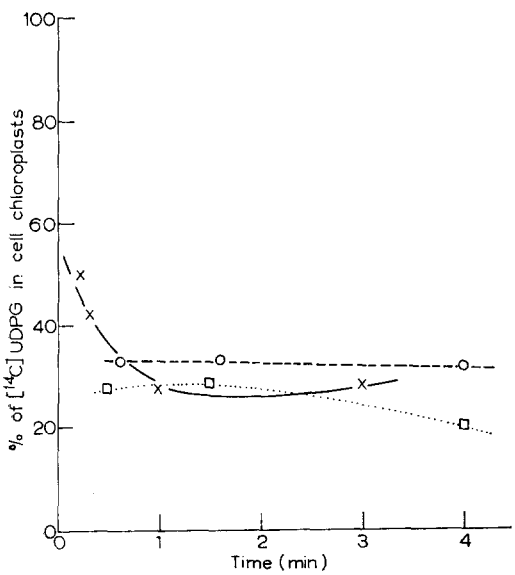

Fig. 5. Percentage of the total [^{14}C]UDPG of the cell present in the chloroplasts during photosynthesis of leaves in an atmosphere containing $^{14}CO_2$. Expt. 1 (spinach), O—O; Expt. 2 (spinach), ×—×; Expt. 3 (broad bean), □·····□.

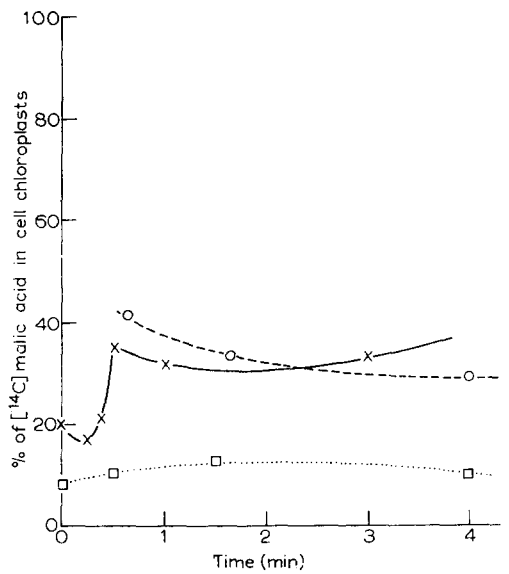

Fig. 6. Percentage of the total [^{14}C]malic acid of the cell present in the chloroplasts during photosynthesis of leaves in an atmosphere containing $^{14}CO_2$. Expt. 1 (spinach), O—O; Expt. 2 (spinach), ×—×; Expt. 3 (broad bean), □·····□.

The distribution of malic acid and citric acid between chloroplasts and the nonchloroplastic part of the cell

In the dark $^{14}CO_2$ was incorporated mainly into PGA, citric acid and malic acid. After illumination, the labelling of citric acid stopped completely, while malic acid was labelled more than ten times as quickly as in the dark. Enzymes of malic acid synthesis from PGA have been found in the chloroplasts by different workers (oxaloacetate carboxy-lyase (EC 4.1.1.3)[30], malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.1.37)[17,31]). However, as shown in Fig. 6, only the minor part of labelled malic acid resided in the chloroplasts. Citric acid had a very similar distribution pattern to that of malic acid. In all experiments 70–80 % of the citric acid was found in the nonchloroplastic part of the cell.

Possible diffusion artifacts and leaching of soluble substances during the chloroplast isolation

As has already been mentioned, diffusion which might take place during the freeze drying of the leaves, or in the frozen dried material, must be ruled out if the

results presented above are to resemble the situation *in vivo*. There are two main arguments against the possible occurrence of secondary diffusion processes in our material:

1. In various cases a labelled compound was found at the beginning of photosynthesis exclusively in the chloroplasts, while time-dependent variations in the distribution within the cell were observed later (Figs. 3 and 4, Table I). These results speak directly against the occurrence of secondary diffusion during the preparation of the material, since such diffusion would tend to reach a concentration balance between chloroplasts and the remainder of the cell, which should be about the same in different experiments and independent of time. Furthermore, different compounds were found to exhibit different distribution characteristics (sugar diphosphates, Fig. 4, and malic acid, Fig. 6), while a very similar pattern should be expected, if secondary diffusion leads to an equilibrium between chloroplasts and the remainder of the cell.

2. Chloroplasts are surrounded by a distinct membrane, which can no longer be made visible under the electron microscope after chloroplasts have been isolated from nonaqueous media[8,14]. This points to a lipoidic nature of this membrane. It seems very unlikely that highly hydrophilic compounds such as the intermediates of photosynthesis can pass a membrane containing lipoidic material after water has been removed by freezing and lyophilization.

TABLE III

LOSS OF RADIOACTIVE MATERIALS INTO THE ORGANIC SOLVENT DURING CHLOROPLAST ISOLATION (IN % OF THE TOTAL RADIOACTIVITY OF THE LEAVES)

Material	*Dark 4 min*	*Photosynthesis in sec*				
		15	*30*	*90*	*180*	*240*
Broad bean	5.2	—	4.5	1.9	—	0.54
Spinach	1.1	0.6	0.4	0.3	0.3	—

Further, we must consider that, during the isolation procedure, leaching of labelled compounds from the leaf material into the medium may have occurred. However, the organic solvent used in the isolation procedure contained only a very low percentage of the total radioactivity of the leaves, after chloroplast isolation had been performed (Table III). Chromatographic examination revealed that more than 50% of the radioactivity found in the organic solvent was located in lipoidic compounds. Therefore, leaching of water-soluble compounds such as sugar phosphates or organic acids from leaf fractions during the isolation procedure can be neglected.

We conclude, for these reasons, that the foregoing results resemble the situation *in vivo*.

DISCUSSION

Sites of synthesis of photosynthetic intermediates

The distribution kinetics of a particular compound present, under suitable conditions, evidence about the site of its synthesis. A 100% localization in the chloroplasts at the beginning of photosynthesis may indicate exclusive synthesis in the chloroplasts. A possible exception of this rule is shown in Fig. 5, where, in one experi-

ment, high percentages of UDPG were found in the chloroplasts after short times of photosynthesis, most probably due to lack of precursors of UDPG formation in the cytoplasm; but there is little doubt that the main synthesis takes place in the cytoplasm. If, by re-extrapolation of the distribution curves to zero time photosynthesis, nothing is found in the chloroplasts, then synthesis occurs exclusively in the cytoplasm. Values ranging somewhere between these extremes suggest different sites of synthesis in chloroplasts and cytoplasm.

All of the PGA formed immediately upon illumination is located in the chloroplasts (Fig. 3). This is taken as evidence that photosynthetic formation of PGA occurs only in the chloroplasts, and is in accordance with the results of previous investigations on the localization of ribulose-1,5-diphosphate carboxy-lyase, which has been shown to be located exclusively in the chloroplasts[32,19]. Ribulose-1,5-diphosphate carboxy-lyase catalyzes the reaction:

$$\text{RuDP} + \text{CO}_2 \longrightarrow 2\ \text{PGA}$$

in which most of the carbon taken up in photosynthesis is incorporated into organic compounds[21].

RuDP, SuDP and SuMP were found during the first minutes of photosynthesis exclusively in the chloroplasts. This suggests that these compounds are synthesized only here. As indicated by the distribution kinetics, FruDP, FruMP and GlcMP are also formed in the chloroplasts, but are translocated to a certain extent into the cytoplasm. GlcDP and UDPG, finally, are not, or not only, synthesized in the chloroplasts, but rather in the cytoplasm.

In the INTRODUCTION the question was raised as to whether high rates of photosynthesis require a cooperation of chloroplasts and cytoplasm[10]. In the photosynthetic carbon cycle[33], CO_2 enters the cycle via RuDP. The resulting 3-PGA is phosphorylated to DPGA and subsequently reduced to triose phosphate. From two moles triose phosphate Fru-1,6-DP is formed, which is dephosphorylated to Fru-6-P. The latter is easily, via glucose-phosphate isomerase (EC 5.3.1.9), converted to Glc-6-P. In our experiments fractions of FruDP, FruMP and GlcMP leave the chloroplasts and seem then to be no longer available in the cyclic regeneration of the CO_2-acceptor. Remaining FruMP reacts with triose phosphate to form erythrose phosphate and xylulose phosphate. Only the latter has been found occasionally in our chromatograms. Erythrose phosphate plus triose phosphate gives SuDP, which is dephosphorylated to SuMP. The reaction of SuMP with triose phosphate leads then to the formation of ribose phosphate and xylulose phosphate which are isomerized to RuMP. The cycle is closed by the phosphorylation of RuMP to RuDP.

The exclusive occurrence of labelled RuDP, SuDP and SuMP in the chloroplasts suggests that the enzymes transketolase (EC 2.2.1.1), ribose-phosphate isomerase (EC 5.3.1.6) and xylulosephosphate epimerase (D-xylulose-5-phosphate-3-epimerase), which catalyze their transformations, are located in the chloroplasts. Ribulose-1,5-diphosphate carboxy-lyase and NADP dependent triose phosphate dehydrogenase (D-glyceraldehyde-3-phosphate: NADP oxidoreductase, EC 1.2.1.9) have previously been found exclusively in the chloroplasts[19]. At least the majority of the enzymes of the photosynthetic carbon cycle may thus reside exclusively in the chloroplasts. From our results we conclude that the cyclic regeneration of the CO_2-acceptor takes place in the chloroplasts (see also ARNON[3]). There is as yet no evidence of a cooperation of

chloroplasts and cytoplasm in the cyclic regeneration of RuDP. Further, our results can be accounted for by CALVIN's scheme of photosynthesis[33] and do not require for their interpretation the existence of alternative pathways, which have been suggested recently[34].

Translocation of photosynthetic products from the chloroplasts into the cytoplasm

In our experiments we have observed considerable translocation of FruDP, FruMP, GlcMP and PGA. Since FruDP, FruMP and GlcMP contain about 10–20% or more of the carbon taken up during the first minutes of photosynthesis, they are considered as major, if not the prevailing, factors in the translocation of reduced carbon within the cell. Supposedly, translocation of PGA is of less importance, since reduction of PGA in the cytoplasm may contribute only a little to the total reduction of carbon in photosynthesis. Such a reduction would require one mole ATP and one mole $NADH_2$ per mole PGA reduced. However, labelling of citric acid is stopped completely after illumination, which may indicate an inhibition of the citric acid cycle of respiration[35–37]. But even if the citric acid cycle is operative in the cytoplasm during illumination[38], little reducing power seems to be available there as compared with the chloroplasts. Rather PGA is used in the cytoplasm in the synthesis of alanine and other compounds or is transferred back to the chloroplasts to be reduced there.

TOLBERT discusses a possible role of glycolic acid in the translocation of carbon within the cell[39–41]. However, we could not find remarkable quantities of either phosphoglycolic acid or glycolic acid in our chromatograms. We conclude, therefore, that *in vivo* glycolic acid is not involved to an appreciable extent in the transport of carbon.

Sugar phosphates thus transfer a considerable part of the carbon taken up in photosynthesis from the chloroplasts to the cytoplasm. It is interesting to note, in this connection, that transport of unphosphorylated intermediates of photosynthesis from the chloroplasts to the cytoplasm and subsequent transformations to storage products such as sucrose would result in a loss of free energy, since the formation of sucrose from unphosphorylated precursors would require "unnecessary" phosphorylation steps (see also CALVIN AND BASSHAM[29], p. 50).

Sugar phosphates cannot be considered, however, as final products of photosynthesis, since they are contained only in limited amounts in plant material. From our data it can be inferred that GlcMP, which is formed from FruMP by glucose-phosphate isomerase, reacts in the cytoplasm (and in chloroplasts) with UTP to form UDPG. From UDPG and FruMP or free fructose, sucrose may be synthesized[27,28]. Free fructose has been identified in small quantities in our fractions, contrary to results of CALVIN AND MASSINI[35]. Sucrose has always been labelled rather late in our experiments, later than UDPG. Therefore a dependable re-extrapolation of the distribution curves to the beginning of photosynthesis has not been possible. The prevailing occurrence of labelled sucrose in the nonchloroplastic part of the cell in all experiments may indicate, however, that at least the major part of the sucrose is synthesized in the cytoplasm.

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