

Effects of Light and Inhibitors on Glutamate Metabolism in Leaf Discs of *Vicia faba* L.

SOURCES OF ATP FOR GLUTAMINE SYNTHESIS AND PHOTOREGULATION OF TRICARBOXYLIC ACID CYCLE METABOLISM

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ABSTRACT

Metabolism of [¹⁴C]glutamate was studied in leaf discs of *Vicia faba* L. in light and in darkness. In white light glutamine was the main labeled product. In the dark label was principally in compounds closely associated with tricarboxylic acid cycle metabolism, predominantly aspartate. Entry of label from glutamate into tricarboxylic acid metabolism appeared to be at least partially by decarboxylation of glutamate to γ -amino butyric acid, followed by conversion to succinate. 3-(3,4-dichlorophenyl)-1,1-Dimethyl-urea inhibited light-enhanced synthesis of glutamine and caused reversion toward the dark pattern of metabolism. Methionine sulfoximine severely inhibited glutamine synthesis and caused accumulation of labeled malate.

Monochromatic 650 nanometer light gave similar results to white light. Monochromatic light of 710 nanometers had a much smaller effect on glutamine synthesis but did significantly raise the ratio of labeled malate to aspartate. γ -Amino [¹⁴C]butyric acid was metabolized entirely via tricarboxylic acid cycle metabolism in light or dark, and in the light the ratio of labeled malate to aspartate was raised.

These results suggest that illuminated leaves metabolize glutamate to glutamine mainly in the chloroplasts. When chloroplastic glutamine synthesis fails to take place, either in darkness or in the presence of inhibitors, glutamate is apparently metabolized outside the chloroplast. Light lowers the NAD⁺ to NADH ratio outside the chloroplast, consequently altering the equilibrium of the malate dehydrogenase reaction. Alteration of the malate to aspartate ratio by 710 nanometer light suggests that ATP generated by photosystem I-dependent cyclic photophosphorylation may affect extrachloroplastic NAD⁺ to NADH ratios.

Santarius and Stocking (20) first demonstrated that illuminated chloroplasts, isolated from *Spinacia* leaves, could synthesize glutamine from glutamate. Further studies on chloroplastic glutamine synthesis indicated that ATP required for the operation of GS³ was provided by photophosphorylation (1, 6, 14). GS has also been shown to be present in chloroplasts by means of direct enzyme assays on *Pisum* (16) and *Vicia* chloroplasts (11). Chloroplast GS activity may constitute one-half to two-thirds of the

total leaf GS (16, 29). There is evidence that GS plays a major role in nitrogen assimilation by plant tissues and that GS works in conjunction with glutamate synthase to produce glutamate (13, 23). GS is probably the sole enzyme-assimilating nitrogen in plants grown on nitrate (23, 26), but GS-catalyzed assimilation activity may be supplemented by a significant operation of glutamate dehydrogenase in ammonium-grown plants (*cf.* 7, 23).

Glutamate metabolism has been previously studied in leaf tissue (*e.g.* 15, 27). The central importance of glutamate and glutamine in assimilating nitrogen in photosynthetic tissues has led us to extend these earlier studies. We have employed monochromatic light and chemical inhibitors to help clarify the mechanism by which light influences the metabolic fate of glutamate in leaves.

MATERIALS AND METHODS

Broad bean plants (*Vicia faba* L. var. Midget) were grown in heat-sterilized soil for 3 weeks in a temperature-controlled growth room at 20 C, irradiance 2 w/m² at the leaf surface and under a light regime of 12 h light, 12 h dark.

Leaf laminae were removed from these plants and discs cut from the tissue using a 1-cm cork borer. For each experimental sample 10 discs were used, which gave a fresh weight of about 0.1 g. Discs were placed in 0.2 cm³ of solution containing 1 μ Ci of radioactive substrate, plus any other compound that was required for the experiment, and subjected to vacuum infiltration. Radioactive substrates entered the discs over the entire area and were not restricted to the cut margins.

After infiltration, discs were thoroughly washed in distilled H₂O and placed under the appropriate incubation conditions for 2 h (except where different time periods are specified) in a temperature-controlled water bath at 25 C. White light was provided by a bank of warm white fluorescent strip lights giving an irradiance of 14.0 w/m² at the level of the discs. In experiments involving monochromatic light, quartz-iodine projection lamps were fitted with interference filters to provide the light quality required. The transmission of the far red filter peaked at 711.4 nm and the red filter at 651 nm with bandwidths at 50% transmission of 11.0 and 10.4 nm, respectively. The irradiance of these two wavelengths at the level of the discs was 23 w/m² and 27 w/m², respectively. A YS1-Kettering model 65A radiometer was used to determine irradiance values.

In experiments where CO₂ fixation was measured discs were incubated in a bathing solution containing 10 μ Ci of sodium [¹⁴C]bicarbonate. Anaerobic conditions, where required, were obtained by flushing with O₂-free nitrogen gas. This gassing treatment caused a temperature drop of less than 1 C over the incubation period.

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³ Abbreviations: GS: glutamine synthetase (L-glutamate:ammonia ligase (ADP), EC 6.3.1.2). GABA: γ -amino butyric acid; MSO: methionine sulfoximine.

Incubations were stopped by placing the discs in boiling 70% (v/v) ethanol and extracting them by the method of Graham and Walker (10). Extracts were reduced in volume *in vacuo* at 35 C by a Büchi Rotavapor-E1 rotary evaporator. Aliquots of the ethanol-soluble extract (containing about 50×10^3 cpm) were then spotted onto Whatman No. 1 chromatography paper and the products separated by two-dimensional development. The solvent systems used were: 88% phenol-water-glacial acetic acid (84:16:1, first dimension); butanol-glacial acetic acid-water (12:3:1, second dimension). In some cases the latter system was replaced by butanol-propionic acid-water (47:23:30).

A radiochromatogram spark chamber supplied by Birchover Instruments Ltd., Letchworth, Herts., U.K., was used for the location of radioactive products on two-dimensional chromatograms. The radioactive products were identified by co-chromatography with authentic nonlabeled compounds which were located by ninhydrin for amino acids and amides, and by bromocresol purple for tricarboxylic acids. Confirmation of the identity of the labeled compounds was made by eluting them from the original paper and rechromatographing them (4, 17). Radioactivity was counted in a toluene-base scintillation fluid.

Radioactive substrates were purchased from the Radiochemical Centre, Amersham, Bucks., England. The radioactive compounds used were L-[U- 14 C]glutamic acid (10 mCi/mmol), 4-amino-*n*-[U- 14 C]butyric acid (224 mCi/mmol) and sodium [14 C]bicarbonate (40 mCi/mmol). All other chemicals were obtained from B.D.H. Chemicals, Poole, Dorset, U.K. or Sigma Chemical Co., London, except for twice recrystallized DCMU, which was a gift from E. I. du Pont de Nemours and Co., Industrial and Biochemical Department, Wilmington, Del.

RESULTS AND DISCUSSION

Table I shows data comparing the products of [14 C]glutamate metabolism in white light with those formed in the dark. The most marked difference was that [14 C]glutamine accumulation was approximately three times greater in the light than in the dark, confirming similar findings of Naylor and Tolbert (15) on barley leaves. From the ethanol-soluble extract (about 10% of the radioactivity supplied), average rates of glutamine formation were 15.5 nmol/g fresh weight·h when glutamate was supplied at 0.5 mM. Experiments carried out at a glutamate concentration of 5 mM showed the same labeling pattern with rates of glutamine formation of 140.5 nmol/g fresh weight·h. In the dark label largely passed to intermediates of tricarboxylic acid cycle metabolism and to aspartate. Aspartate is readily formed by transamination of oxaloacetate (*cf.* 30). One means by which carbon from glutamate can enter into tricarboxylic acid metabolism is by transamination to produce α -ketoglutarate. In the present investigation, no label was detected in α -ketoglutarate, which is in agreement with previous findings (10). α -Ketoglutarate will undoubtedly be formed

Table I. Influence of White Light on Metabolism of Glutamate

All data refer to cpm per labeled compound as a percentage of the total radioactivity recovered per chromatogram. The mean and standard error are calculated from 25 experiments in the light and 10 in the dark.

Labeled Compound	White Light Mean Percentage Incorporation	Dark Mean Percentage Incorporation
Glutamate	40.6 ± 1.9	33.1 ± 3.7
Glutamine	31.2 ± 2.3	11.1 ± 1.5
GABA	5.5 ± 0.8	10.3 ± 2.6
Succinate	3.3 ± 0.5	2.5 ± 0.7
Aspartate	6.0 ± 0.6	24.8 ± 3.5
Malate	7.5 ± 0.7	10.8 ± 2.0
Citrate	1.5 ± 0.3	1.7 ± 0.7
Alanine	2.4 ± 0.5	2.6 ± 0.5
Asparagine	2.9 ± 0.7	3.4 ± 1.1

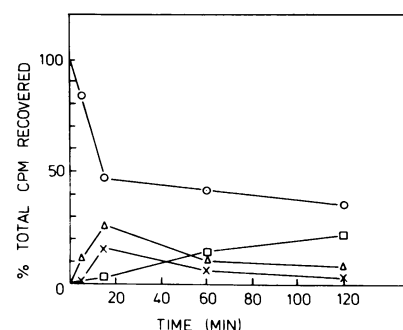


FIG. 1. Time course of glutamate metabolism illustrating the incorporation of radioactivity into GABA, aspartate, and succinate. Identical samples containing 0.1 g fresh weight of leaf discs were vacuum-infiltrated with 1.0 μ Ci [14 C]glutamate (0.5 mM) in 0.2 ml. The samples were allowed to incubate in the dark in a shaken water bath for the desired period. Data represent the mean from three experiments. Total cpm recovered is the cpm per chromatogram. (O—O): Glutamate; (Δ — Δ): GABA; (x—x): succinate; (\square — \square): aspartate.

from glutamate, but presumably does not accumulate because it is readily metabolized further.

A time course study of the metabolism of glutamate in the dark indicated rapid formation of GABA and succinate, followed by a gradual accumulation of labeled aspartate (Fig. 1) and loss of label from GABA and succinate. It is apparent that, as seen in other plants (*e.g.* 27), rapid decarboxylation of glutamate to GABA takes place mediated by glutamate decarboxylase. This enzyme is thought to be located in the cytosol (5) and in the present study, experiments on subcellular fractions showed no activity apart from that in the soluble phase. Chloroplast preparations that were capable of light-dependent glutamine synthesis from glutamate did not decarboxylate glutamate even under conditions that had proved favorable for the activity of the soluble enzyme. Present evidence suggests that the decarboxylation of glutamate to produce GABA takes place in the nonparticulate fraction of the cell. This decarboxylation step is followed by transamination, in the mitochondria, of GABA to succinic semialdehyde (5). The latter compound is then oxidized to succinate and consequently can enter into tricarboxylic acid cycle metabolism (5). Supporting this interpretation, Table II indicated a build-up of both GABA and succinate when tricarboxylic acid cycle metabolism was blocked by malonate at the succinic-dehydrogenase step. It seems that the carbon from glutamate can, at least partially, enter tricarboxylic acid cycle metabolism via reactions involving decarboxylation to GABA and subsequent conversion to succinate. Conversion of glutamate to glutamine in the dark never approached the levels found in white light. Conversely, incorporation of label from glutamate into aspartate was very much reduced in white light compared to dark samples.

These data indicate that in the light, tricarboxylic acid cycle reactions continue operating, but that the percentage of glutamate metabolized through them is much reduced. Thus, white light diverts glutamate toward glutamine synthesis and reduces formation of aspartate and intermediates of tricarboxylic acid metabolism. If the light-dependent glutamine synthesis takes place largely within the chloroplasts, as seems likely, then these organelles may provide a cellular compartment which spatially restricts the metabolism of glutamate via reactions associated with the mitochondrial tricarboxylic acid cycle. The possibility that increased uptake into the chloroplast may take place in the light is not ruled out by our data.

Light increased the labeled malate to aspartate ratio compared to the dark (Table I). Such a result has been explained previously in terms of the effect of photoreduction upon the equilibrium position of the malate dehydrogenase enzyme (10). Because the equilibrium for this enzyme does not favor oxidation (25) any

increase in reducing equivalents produced as a result of photosynthetic activity will favor malate accumulation and will retard aspartate synthesis via oxaloacetate.

The GS reaction mediating glutamine synthesis requires ATP. If ATP for the light-enhanced synthesis of glutamine were being provided by noncyclic or pseudocyclic photophosphorylation, then DCMU should block this source of ATP by suppression of PSII activity. Results shown in Table III illustrate decreased incorporation of radioactivity into glutamine from glutamate as the DCMU concentration was raised. The traditional interpretation of such results would be that a high proportion of ATP for the light-enhanced synthesis of glutamine is being provided by noncyclic or pseudocyclic photophosphorylation. Recent evidence, however, has indicated some DCMU sensitivity for native cyclic photophosphorylation dependent on PSI, *i.e.* that operating in the absence of artificial catalysts (*e.g.* 2, 3, 6). Table III also shows an increase in labeling of aspartate with increasing concentrations of DCMU. This would be expected if DCMU is inhibiting the formation of photosynthetically reduced pyridine nucleotides. With increasing DCMU concentration, intermediates of the tricarboxylic acid cycle also increased in labeling, but not to the same extent as aspartate.

Comparison of the inhibition of $^{14}\text{CO}_2$ fixation brought about by DCMU with its effect on glutamine synthesis (Table III) showed that CO_2 fixation was inhibited more severely than glutamine synthesis. This would suggest a source of ATP for glutamine synthesis additional to that provided by noncyclic photophosphorylation. This other ATP source could be another form of photosynthetic phosphorylation, *e.g.* cyclic, or, alternatively, ATP provided by dark respiratory processes. As some glutamine synthesis did take place in the dark, it would seem that ATP provided by respiratory processes could well account for the small percentage of glutamine synthesized in the presence of DCMU. The presence of a definite extrachloroplastic GS (29) could be oper-

ating to provide this glutamine synthesis.

To elucidate further the nature of the ATP used for glutamine synthesis a series of experiments was carried out using monochromatic light of wavelengths 650 and 710 nm. The rationale was that by using wavelengths of light absorbed preferentially by PSI or PSII, it should be possible to alter preferentially ATP formation due to noncyclic and cyclic phosphorylation, and to avoid the uncertainty concerning possible DCMU effects on cyclic phosphorylation. PSII would not be appreciably operative with actinic wavelengths above 700 nm. DCMU was also used at these wavelengths in some experiments (Table IV) to ensure further the suppression of ATP synthesis from noncyclic photophosphorylation and to prevent formation of NADPH by noncyclic electron transport. In other experiments (Table V) anaerobic conditions were employed in order to prevent ATP production from dark respiration.

Activation of both photosystems in 650 nm light produced a labeling pattern fairly similar to white light, *i.e.* considerable ^{14}C glutamine synthesis and a higher malate to aspartate ratio than in the dark. In 650 nm light DCMU was found to affect glutamate metabolism as it did in white light (Table IV). DCMU inhibited glutamine synthesis and increased the transfer of label toward tricarboxylic acid cycle compounds. Thus, 650 nm light provided factors similar to those in white light in respect to glutamate metabolism. In contrast, 710 nm light brought about a labeling pattern different from that in white light. Glutamine synthesis was comparatively low and was only slightly greater than in the dark under aerobic conditions (compare Tables IV and I). This synthesis of glutamine was completely insensitive to 100 μM DCMU, nor was there any significant effect of this DCMU concentration on any other aspect of glutamate metabolism in 710 nm light.

Table IV. *Effect of Monochromatic Light \pm DCMU on Metabolism of Glutamate*

Leaf discs were vacuum-infiltrated with 1.0 μCi ^{14}C glutamate (0.5 mM) \pm DCMU in 0.2 ml of phosphate buffer (pH 6.5). All data refer to cpm per labeled compound as a percentage of the total radioactivity recovered per chromatogram.

Labeled Compound	650 nm +DCMU	650 nm -DCMU	710 nm +DCMU	710 nm -DCMU
Glutamate	28.7	36.9	29.1	28.5
Glutamine	21.4	35.7	13.5	14.2
GABA	7.6	3.2	7.6	4.7
Succinate	8.8	3.1	8.3	3.7
Aspartate	14.4	3.9	15.3	17.1
Malate	10.5	7.5	16.8	19.2
Citrate	1.5	2.0	4.8	4.4
Alanine	6.8	7.0	0.0	0.0
Asparagine	1.0	2.0	4.4	6.9
Total cpm per chromatogram	60,372	53,289	45,817	51,795

Table II. *Metabolism of ^{14}C Glutamate \pm Malonate in the Dark*

Leaf discs were vacuum-infiltrated with 1.0 μCi ^{14}C glutamate (0.5 mM) \pm malonate (0.1 M) in 0.2 ml phosphate buffer (pH 6.5). After infiltration the discs were incubated in the dark in a shaken water bath at 25 C for 2 h. Data represent cpm per labeled compound as a percentage of the total radioactivity recovered per chromatogram.

Labeled Compound	Control	+ Malonate
Glutamate	31.9	31.6
Glutamine	7.4	13.2
GABA	8.5	16.6
Succinate	4.1	31.1
Aspartate	19.6	2.9
Malate	16.0	2.1
Citrate	4.5	2.5
Alanine	4.7	0.0
Asparagine	3.2	0.0
Total cpm per chromatogram	46,305	52,158

Table III. *Comparison of Effect of Different DCMU Concentrations on Glutamine Synthesis, Aspartate Synthesis, and CO_2 Fixation*

To determine the incorporation of radioactivity into glutamine and aspartate, discs were vacuum-infiltrated with 1.0 μCi ^{14}C glutamate \pm DCMU in a volume of 0.2 ml. $^{14}\text{CO}_2$ fixation determinations were made on discs that had been vacuum-infiltrated with DCMU at the desired concentrations. These discs were then placed under white light in a shaken water bath at 25 C for 5 min preincubation. Ten μCi of sodium ^{14}C bicarbonate was then added to the bathing solution. All samples contained equivalent methanol concentrations of 2.5% (v/v). Irradiance was 14.0 w/m^2 at the level of the discs and the incubation time was 2 h. Data for glutamine and aspartate synthesis represent cpm per labeled compound as a percentage of the total radioactivity recovered per chromatogram. Data for $^{14}\text{CO}_2$ fixation represent total cpm of $^{14}\text{CO}_2$ fixed by the discs.

	Concentration of DCMU			
	None	10^{-6} M	10^{-5} M	10^{-4} M
Aspartate synthesis	6.9 (100) ^a	9.7 (141)	12.9 (212)	19.8 (287)
Glutamine synthesis	24.4 (100)	19.9 (82)	14.6 (60)	10.3 (42)
$^{14}\text{CO}_2$ fixation	7.2×10^5 (100)	6×10^5 (84)	3.9×10^5 (54)	1.4×10^5 (19)

^a Data in parentheses represent percentage of the control value (no addition of DCMU).

The relatively small role played by PSI-dependent cyclic photophosphorylation in driving glutamine synthesis is apparently at variance with results obtained in isolated *Pisum* and *Spinacia* chloroplasts (6, 14). We have no definite explanation for this discrepancy, but it is known that the operation of the cyclic phosphorylation process is highly variable according to various physiological factors (3).

A more striking effect of 710 nm light was that it did maintain an elevated malate to aspartate ratio compared to the dark (compare Tables I and IV). This effect may result from a rise in the extrachloroplastic NADH to NAD⁺ ratio caused by a small amount of ATP derived from cyclic photophosphorylation. Such an effect would be consistent with the findings of Sawhney *et al.* (21). These workers attributed photostimulation of nitrate reduction to a postulated effect of ATP, derived from photosynthetic processes, in increasing the NADH to NAD⁺ ratio outside the chloroplasts.

Under anaerobic conditions (Table V) the principal product accumulating with a 2-h dark incubation was GABA, as seen by previous investigators (15, 27). White light had essentially the same effect typically seen in aerobic experiments, namely a strong promotion of glutamine synthesis. Photosynthetic O₂ production was presumably initiated under these conditions. Monochromatic light (710 nm) in the presence of DCMU, caused a 2-fold increase in glutamine accumulation, compared to the dark control. The ratio of labeled malate to aspartate, while it could not be meaningfully compared with the ratio in darkness under anaerobic conditions (since both values were zero), was substantially greater than that seen in the dark under aerobic conditions (Table I). Although the metabolism of glutamate in 710 nm light under anaerobic conditions was significantly different from that found in white light, it clearly did not revert to a dark pattern of glutamate metabolism. Indications are that 710 nm light may allow the provision of ATP in sufficient amounts to alter the aspartate to malate:ratio, even though the effect on glutamine synthesis is relatively small. The latter effect appeared somewhat more pronounced under anaerobic conditions.

MSO, an inhibitor of GS (19), was employed in order to see how glutamate would be metabolized in the light in the absence of glutamine formation (Table VI). As expected, MSO caused a very severe inhibition of glutamine synthesis, confirming that GS was the enzyme responsible for synthesis of glutamine in the leaves of *V. faba*. This inhibition differed from that found with DCMU in several ways. First, it almost completely prevented glutamine formation and did not allow the residual level equivalent to that found in the dark. Also, in the presence of MSO, label entering tricarboxylic acid metabolism from [¹⁴C]glutamate accu-

Table V. Effect of Light on Glutamate Metabolism under Anaerobic Conditions

Anaerobic conditions were maintained by flushing with nitrogen gas (oxygen-free) for the 2-h incubation period. In this period the temperature of the bathing solution fell by <1 C. All data refer to cpm per labeled compound as a percentage of the total radioactivity recovered per chromatogram.

Labeled Compound	Dark	White Light	710 nm Light +10 DCMU
Glutamate	23.5	40.0	30.4
Glutamine	8.0	44.2	17.2
GABA	42.7	0.0	5.2
Succinate	14.7	3.6	8.3
Aspartate	0.0	3.8	14.4
Malate	0.0	6.0	12.1
Citrate	0.0	2.4	12.7
Alanine	11.0	0.0	0.0
Total cpm per chromatogram	39,985	48,457	45,704

Table VI. Effect of MSO on Metabolism of Glutamate in Light

The MSO concentration was 1 mM in a volume of 0.2 ml vacuum infiltration solution. Data represent cpm per labeled compound as a percentage of the total radioactivity recovered per chromatogram.

Labeled Compound	-MSO	+MSO
Glutamate	44.6	19.3
Glutamine	32.0	3.0
GABA	5.3	3.8
Succinate	3.4	26.0
Aspartate	2.1	0.0
Malate	7.6	42.0
Citrate	1.3	3.4
Alanine	4.0	2.1
Total cpm per chromatogram	33,188	36,641

Table VII. Metabolism of [¹⁴C]GABA in the Light and in the Dark

One μ Ci of [¹⁴C]GABA (0.5 mM) \pm malonate (0.1 M) in 0.2 ml phosphate buffer (pH 6.5) was supplied to the discs. Data represent cpm per labeled compound as a percentage of the total radioactivity recovered per chromatogram.

Labeled Compound	Light	Light + Malonate	Dark
Glutamate	8.9	1.1	12.0
Glutamine	3.0	0.0	3.6
GABA	27.8	31.8	35.4
Succinate	9.8	54.7	4.1
Aspartate	14.3	6.9	30.0
Malate	25.2	3.4	10.3
Citrate	2.2	1.1	1.5
Alanine	1.5	1.1	1.0
Asparagine	6.5	0.0	3.0
Total cpm per chromatogram	59,581	68,976	57,329

mulated very strikingly in malate and to a lesser degree in succinate, therefore showing again the influence of increased reducing equivalents. When the preferential conversion of glutamate to glutamine was inhibited, label from glutamate rapidly entered intermediates of tricarboxylic acid metabolism under light conditions, confirming the normal operation of tricarboxylic acid cycle reactions during periods of photosynthesis in the light (4, 8). Experiments carried out with [¹⁴C]GABA (Table VII) showed that label passed rapidly into intermediates of the tricarboxylic acid cycle both in the light and in the dark and can be blocked by the presence of malonate. Moreover, light was found to increase the incorporation into malate, which is expected if light has an influence on the malate dehydrogenase equilibrium. These results confirm GABA as a fairly direct precursor of tricarboxylic acid cycle metabolites. All available evidence here (Fig. 1 and Tables II and VII) accords with the view (5) that label from GABA enters tricarboxylic acid cycle type intermediates at the succinate level. The present results do not support the view that GABA is a close precursor of glutamine (12).

CONCLUSIONS

Several conclusions emerge in respect to the influence that light has upon the metabolism of glutamate and on tricarboxylic acid cycle metabolism in leaf tissue. In the dark the synthesis of glutamine is low, and label supplied as [¹⁴C]glutamate enters into tricarboxylic acid cycle type reactions, accumulating principally in aspartate by transamination of oxaloacetate. The ratio of labeled aspartate to malate is high in the dark, in agreement with other workers (10, 27). If the synthesis of glutamine is inhibited in the light, label from glutamate rapidly enters intermediates of the tricarboxylic acid cycle, which are to a large degree mitochondrial in location. However, the amount of accumulation in each intermediate is dependent upon the means through which diversion to

glutamine is prevented. MSO leads to an accumulation mainly in malate, whereas DCMU causes aspartate to become predominantly labeled. The most logical explanation of this (in line with the concepts developed by Graham and Walker, [10]), is that in the light, in the absence of DCMU, there is an increase in reducing equivalents provided by photosynthetic activity. Because chloroplast envelopes have been reported as being largely impermeable to pyridine nucleotides, the transfer of reducing equivalents from the chloroplast to the cytosol is probably mediated by P-glycerate/dihydroxyacetone-P malate/oxaloacetate shuttle systems (28). This increase in reducing equivalents can then affect the equilibrium of the malate dehydrogenase reaction, which is normally strongly in favor of the formation of malate (25). Consequently, the inhibition of glutamine synthesis by DCMU, which concurrently inhibits photosynthetic NADPH and ATP formation, leads to the predominant labeling of aspartate, as is the case in the dark.

Alterations in the aspartate to malate ratio are also found in 710 nm light (\pm DCMU) and can possibly be explained in terms of ATP synthesized by cyclic photophosphorylation. The increased chloroplast adenylate charge may then be transported to the cytosol and/or mitochondria and cause an increase in reduced pyridine nucleotides, possibly by the mechanism of Sawhney *et al.* (21). They have suggested that increased cytoplasmic adenylate charge due to photosynthesis inhibits the mitochondrial electron transfer chain between NADH and O₂, causing an accumulation of NADH. There is further the possibility of an ATP-mediated removal of NAD⁺ by conversion to NADP⁺ (9). Experimental evidence showing slow or even absent transfer of adenylates across envelopes of mature spinach chloroplasts *in vitro* might seem to contradict the above explanation, but there is recent evidence that the situation in mature spinach may not be representative of younger leaves or of other species (18, 24). A direct transfer of adenylates, independent of shuttle systems based on phosphorylated carbon compounds, may therefore take place to a greater extent than previously supposed, even though the mechanism is not yet fully understood. Certainly it is well documented that ATP derived from cyclic photophosphorylation can stimulate energy-dependent processes outside the chloroplast, even in the absence of CO₂ assimilation and in the absence of PSII activity (cf. 22). Although a number of questions remain unanswered, the present results highlight the important role of light both in regulating chloroplast glutamine synthesis in leaf tissues and in controlling the redox ratio of extrachloroplastic pyridine nucleotides.

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