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Effects of Carbon Dioxide and Oxygen on the Regulation of Photosynthetic Carbon Metabolism by Ammonia in Spinach Mesophyll Cells[1]

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ABSTRACT

Photosynthetic carbon metabolism of isolated spinach mesophyll cells was characterized under conditions favoring photorespiratory (PR; 0.04% CO_2 and 20% O_2) and nonphotorespiratory (NPR; 0.2% CO_2 and 2% O_2) metabolism, as well as intermediate conditions. Comparisons were made between the metabolic effects of extracellularly supplied NH_4^+ and intracellular NH_4^+, produced primarily via PR metabolism. The metabolic effects of $^{14}CO_2$ fixation under PR conditions were similar to perturbations of photosynthetic metabolism brought about by externally supplied NH_4^+; both increased labeling and intracellular concentrations of glutamine at the expense of glutamate and increased anaplerotic synthesis through α-ketoglutarate. The metabolic effects of added NH_4^+ during NPR fixation were greater than those during PR fixation, presumably due to lower initial NH_4^+ levels during NPR fixation. During PR fixation, addition of ammonia caused decreased pools and labeling of glutamate and serine and increased glycolate, glyoxylate, and glycine labeling. The glycolate pathway was thus affected by increased rates of carbon flow and decreased glutamate availability for glyoxylate transamination, resulting in increased usage of serine for transamination. Sucrose labeling decreased with NH_4^+ addition only during PR fixation, suggesting that higher photosynthetic rates under NPR conditions can accommodate the increased drain of carbon toward amino acid synthesis while maintaining sucrose synthesis.

High CO_2 and/or low O_2 concentrations increase net photosynthesis in C_3 plants (11, 22, 23, 25). Greater CO_2 pressure increases CO_2 fixation rates directly by supplying additional CO_2 and possibly by increasing the activity of ribulose-bis-P carboxylase through regulatory activation (5). At the same time, increased binding of CO_2 due to higher concentrations may decrease competitive binding of O_2. O_2 binding can also be decreased by lowering the O_2 concentration. Greater incorporation of photosynthetically fixed ^{14}C into glycolate, glycine, and serine is characteristic of photosynthesis under decreased partial pressures of CO_2 or increased O_2 levels (11, 22, 23).

Photorespiratory metabolism of glycolate results in both CO_2 release and production of NH_4^+ through the activity of glycine synthase associated with mitochondrial conversion of glycine to serine (24). The rate of NH_4^+ production via NO_3^- assimilation in C_3 plants is often insignificant compared to the rate of photorespiratory NH_4^+ release (8, 27). Plants with higher PR[4] rates might therefore be expected to have metabolic responses which are in part similar to the effects of adding extracellular NH_4^+ to cells. Spinach leaf discs, for example, were found to have higher NH_4^+ content during fixation at air levels of O_2 and CO_2 compared to conditions unfavorable to photorespiration (18). The pathway of carbon and nitrogen flow involving the release of NH_4^+ from glycine, its refixation back to glutamate via glutamine synthetase and glutamine:α-ketoglutarate aminotransferase activities, and transamination of glyoxylate with glutamate has been termed the PR nitrogen cycle (8).

The effects of NH_4^+ on photosynthetic carbon metabolism have been characterized in various systems including alfalfa leaf discs (21), isolated leaf cells of poppy (4, 17) and spinach (9, 28), and *Chlorella* (7). Ammonia's effects on higher plants have been recently reviewed (3).

This report describes the effects of NH_4^+ addition on the metabolism of isolated spinach mesophyll cells carrying out photosynthesis under differing concentrations of CO_2 and O_2. Internally produced NH_4^+ from photorespiration and externally supplied NH_4^+ are compared with respect to their effects on photosynthetic carbon metabolism and intracellular concentrations of amino acids.

MATERIALS AND METHODS

Plant Materials. Spinach (*Spinacia oleracea* L., Burpee Hybrid No. 7) was grown in a 17°C growth chamber in vermiculite and watered 3 times per week with Hoagland solution. Light (300–400 $\mu E\ m^{-2}\ s^{-1}$, 400–700 nm) was supplied 8 h per day by a combination of fluorescent tubes and incandescent bulbs.

Cell Isolation and Storage. Cells were isolated as previously described (9) and stored in nitrate-free assay media. Cells used for the NPR experiments were stored in the dark at 6°C for 20.5 h, placed on a rotary shaker at 50 rpm in the light (200 $\mu E\ m^{-2}\ s^{-1}$) at 17°C for 4.5 h, and then placed in the dark at 6°C for 0.5 h before use. Cells used for the PR experiments were stored in the same manner except that the initial dark period was 23 h. The day-long storage of the cells was carried out to avoid assaying

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[4] Abbreviations: PR, photorespiratory; NPR, nonphotorespiratory; PEP, phosphoenolpyruvate; DHAP, dihydroxyacetone phosphate.

them during the period of metabolic stress following their isolation (16). The elimination of NO_3^- from the storage media, though intended to decrease the available nitrogen in the cells, had a relatively insignificant effect on their metabolism.

Photosynthetic $^{14}CO_2$ Incorporation. The experimental system consisted of a rotary shaker-water bath illuminated from below (650 μE m^{-2} s^{-1}) (6) with serum-stoppered 5-ml Fernbach flasks fitted with inlet and outlet ports. These flasks were connected to a closed steady-state gas circulation system as previously described (20).

Gas composition for the NPR experiment was 2.7% O_2 and 0.23% $^{14}CO_2$ (specific radioactivity, 9.3 μCi/μmol). The O_2 concentration was 20.4% for the PR experiment with the $^{14}CO_2$ level at 0.048% (12.8 μCi/μmol). Experiments with saturating [^{14}C]bicarbonate (8 mM, 16.36 μCi/μmol) were carried out simultaneously with both the NPR and PR experiments as controls and were not connected to the steady-state apparatus. The concentrations and specific radioactivities of the gases used in additional experiments are detailed in the legend to Fig. 1.

With the gas circulating, 680 μl assay medium (9) and 200 units carbonic anhydrase (Sigma) in 20 μl H_2O were added to the steady-state flasks. The closed-control flasks had 50 μl [^{14}C]bicarbonate and 650 μl assay medium added. After 10 min, 700 μl of 2-times concentrated cells (90 μg Chl/ml) were added to all flasks, initiating $^{14}CO_2$ fixation. Chl content was determined by the method of Arnon (1).

Samples of 200 μl were removed from the flasks and injected into methanol (80% v/v final concentration), 15 and 30 min after initiating $^{14}CO_2$ fixation. At 33 min, 50 μl $(NH_4)_2SO_4$ (final NH_4^+ concentration 1 mM) or 50 μl H_2O were added to the flasks and 200-μl samples were withdrawn and injected into methanol as above at 36, 45, and 65 min. Total $^{14}CO_2$ incorporation was determined as previously described (9).

Analysis of $^{14}CO_2$ metabolites. Samples were fractionated by cation exchange chromatography and 2-dimensional paper chromatography as described by Larsen *et al.* (9) with the following modifications. To every sample, 0.5 μmol of Ca glycolate and α-ketoglutarate were added. The order of the ethanol extractions was reversed and the ether extraction was omitted. Incorporation of $^{14}CO_2$ into glycolate was determined by 2-dimensional paper chromatography (19) using a portion of the effluent. Analysis of α-ketoacids was performed by conversion to their 2,4-dinitrophenylhydrazones using the method of Bachelard (2) following addition of 10 μg each of hydroxypyruvate and glyoxylate to samples amounting to 70% of the neutral and organic acid eluates. The derivatives were separated using two-dimensional paper chromatography (21). Incorporation of ^{14}C was determined by liquid scintillation counting.

Of the amino acids, ^{14}C incorporation into histidine, lysine, arginine, methionine, and cysteine was not determined. Specific radioactivities of [^{14}C]amino acids were determined by formation of their [^{3}H]dansyl chloride derivatives as described previously (9). For a few determinations, concentrated [^{3}H]dansyl chloride (24.4 mM, as opposed to 2 mM) of the same specific radioactivity (8.15 μCi/μmol) was used.

RESULTS

Effects of CO_2 and O_2 Concentrations on $^{14}CO_2$ Fixation. Photosynthetic incorporation of $^{14}CO_2$ into spinach cells was characterized under two concentrations of CO_2 (0.04 and 0.2%) and two concentrations of O_2 (20 and 2%) in addition to fixation under saturating bicarbonate (Fig. 1). Detailed analysis was carried out on the effects of adding NH_4^+ to spinach cells incorporating $^{14}CO_2$ under the two extreme combinations of CO_2 and O_2 gases (low CO_2 and high O_2, PR; high CO_2 and low O_2, NPR) (Figs. 2 and 3).

Distribution of ^{14}C Metabolites Under Photorespiratory and Nonphotorespiratory Conditions. Total $^{14}CO_2$ fixation (Fig. 2) and incorporation of ^{14}C into most compounds was greater under NPR conditions. Greater labeling occurred for sucrose (accounting for up to 40% of total incorporation), glucose, fructose, maltose, hexose-mono-P, pentose-mono-P, glycerate, citrate, dihydroxyacetone-P, pyruvate, glutamate, γ-aminobutyric acid, proline, threonine, isoleucine, alanine, valine, leucine, and phenylalanine. Compounds which were about equally labeled under PR and NPR conditions were α-ketoglutarate, fumarate, 3-P-glycerate, hydroxypyruvate, and aspartate. A few compounds were more labeled under PR conditions despite the slower total incorporation rate (Fig. 2). These include PR pathway intermediates (glycolate, glyoxylate, glycine, and serine), compounds directly affected by NH_4^+ (glutamine and asparagine), and compounds involved in the anaplerotic formation of C_5 amino acids (PEP and malate).

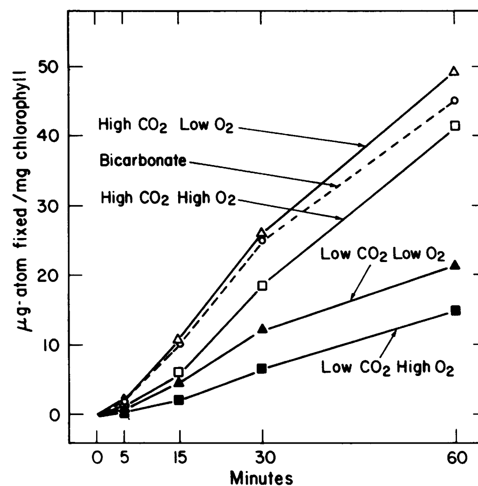

FIG. 1. Total $^{14}CO_2$ fixation by spinach cells under various atmospheres. Fixation was at 22° C and 650 μE m^{-2} s^{-1} under the following conditions: △——△, 0.21% CO_2 and 2.3% O_2; □——□, 0.20% CO_2 and 20.0% O_2; ▲——▲, 0.04% CO_2 and 2.4% O_2; ■——■, 0.04% CO_2 and 20.2% O_2; ○- - -○, 8.0 mM $H^{14}CO_2^-$. The cells were not pretreated in light. Each point represents duplicate determinations with a variation of less than 20%. Bicarbonate controls represent four separate assays, one carried out simultaneously with each of the four combinations of gas mixtures.

For some compounds, the metabolically active pool sizes could be estimated due to the saturation of labeling into the compounds during the experiment. Compounds for which metabolically active pool sizes increased under PR conditions were glycine, serine, and glyoxylate (Fig. 2). Glycerate, pyruvate, and DHAP had smaller active pool sizes under PR conditions.

The intracellular concentrations of most amino acids did not depend on whether CO_2 fixation was under PR and NPR conditions. The amino acids with concentrations which were affected are listed in Table I. Alanine and glutamate concentrations were lower in cells incorporating CO_2 under PR conditions. The concentrations of glutamine, glycine, and serine were higher.

Effects of NH_4^+ on $^{14}CO_2$ Fixation under Photorespiratory and Nonphotorespiratory Conditions. The labeling of a number of compounds was affected by the addition of NH_4^+ during $^{14}CO_2$ fixation under PR and NPR conditions (Figs. 2 and 3). Differences in labeling were observed for intermediates of PR carbon metabolism (glycolate, glyoxylate, glycine, and serine), PR nitrogen metabolism (glutamine, glutamate, and α-ketoglutarate) and other interrelated pathways (PEP, malate, fumarate, citrate, asparagine, aspartate, alanine, and valine).

DISCUSSION

Regulation of Photosynthetic $^{14}CO_2$ Incorporation by CO_2 and O_2 Concentrations. As expected, rates of photosynthetic $^{14}CO_2$

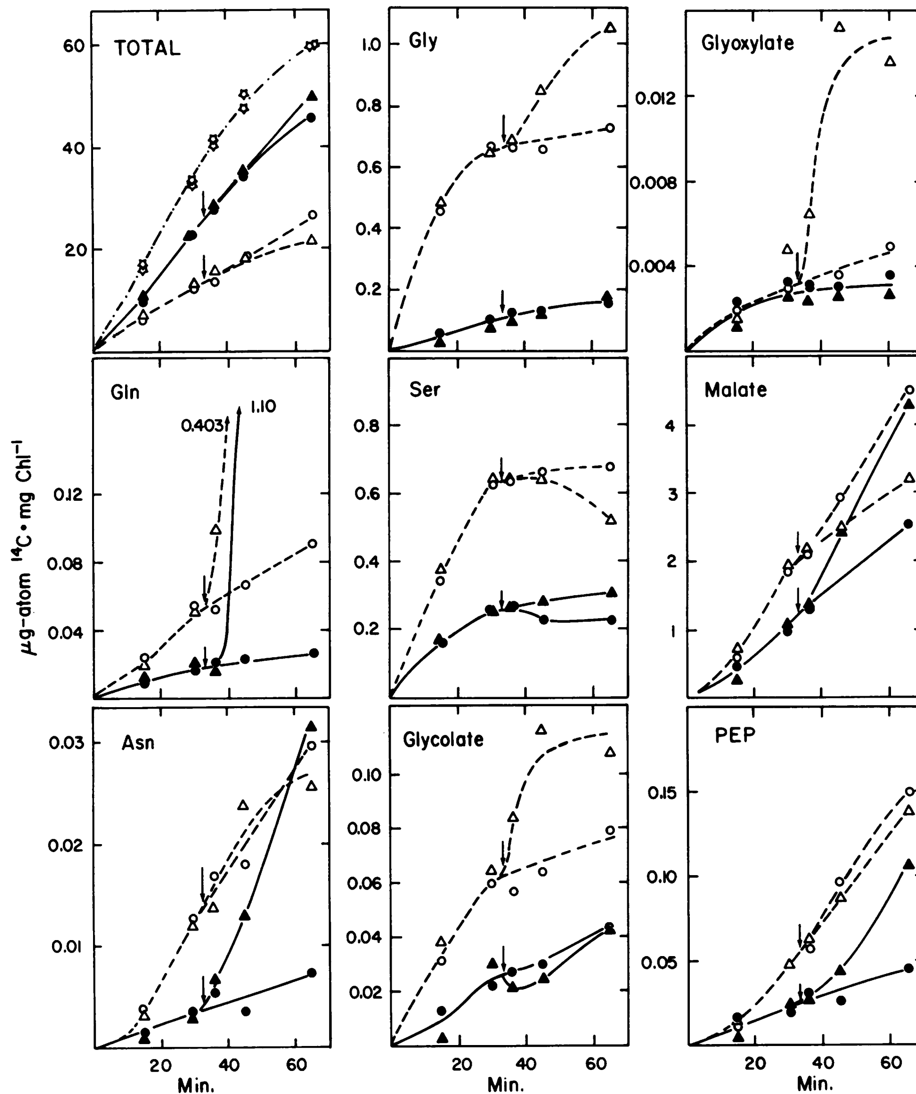

FIG. 2. Total $^{14}CO_2$ fixation and fixation into compounds under PR and NPR conditions. After isolation, cells were stored for about 22 h in the dark at 6°C in media lacking NO_3^-. The cells were then light-incubated for 4.5 h at 17°C and then dark-incubated for 30 min at 6°C until assayed for $^{14}CO_2$ incorporation for up to 65 min under the following conditions: O- - -O, PR control (PR, 0.048% CO_2 and 20.4% O_2); △- - -△, PR with NH_4^+ added at 33 min (final NH_4^+ concentration 1 mM); ●——● nonphotorespiratory control (NPR, 0.23% CO_2 and 2.7% O_2); ▲——▲, NPR with NH_4^+ added at 33 min; ☆-·-☆, $H^{14}CO_3^-$ control (8 mM).

fixation increased with higher CO_2 or lower O_2 levels (Fig. 1). The effect of changing CO_2 from 0.04 to 0.2% was greater than the effect of decreasing O_2 levels from about 20 to 2%. Increasing the O_2 levels decreased the initial fixation rates (before 30 min) but had little effect on incorporation rates after 30 min (Fig. 1).

The labeling of most compounds was proportional to the total $^{14}CO_2$ incorporation rates. The notable exceptions were compounds related to PR glycolate metabolism, compounds directly involved in the assimilation of NH_4^+, and intermediates in the anaplerotic reactions leading to synthesis of C_5 amino acids (Fig. 2).

Fixation under PR conditions increased the total labeling into the glycolate pathway intermediates: glycolate, glyoxylate, glycine, and serine (Fig. 2). Previous studies with leaves of tomato (11), beans (26), and alfalfa (22) have noted that fixation into glycolate and glycine increased with lowered CO_2 or raised O_2 levels. Serine labeling has often been unaffected or affected differently (22, 23, 25, 26). The intracellular concentrations of both glycine and serine were higher under PR conditions with our spinach cells (Table I), whereas Lee and Whittingham (11) found that the effect of CO_2 levels on glycine and serine pool sizes varied between experiments. The sensitivity of these pool sizes to experimental treatment might be due to the multiple pathways leading to glycine and serine synthesis in plants (12). Labeling of hydroxypyruvate and glycerate was greater under NPR conditions (data not shown) despite the fact that these two compounds are intermediates in the PR conversion of serine to 3-P-glycerate and might be expected to be labeled proportionately to PR flow. Both compounds are also intermediates in the alternative pathway for synthesis of serine via 3-P-glycerate (12). The labeling patterns of hydroxypyruvate and glycerate, therefore, indicate that serine synthesis via 3-P-glycerate in the reverse pathway of photorespiration may predominate over labeling via PR glycolate metabolism for these compounds.

Both the intracellular concentration and labeling rate of glutamine were higher under PR conditions (Table I, Fig. 2). Glutamate pool sizes and labeling were both lower (Table I, Fig. 3). It is likely that increases in the rate of PR NH_4^+ release is effective in two ways. First, increased ammonia levels coupled with the high affinity of glutamine synthetase for ammonia (15) leads to accelerated glutamine synthesis. Second, activities of PEP carboxylase

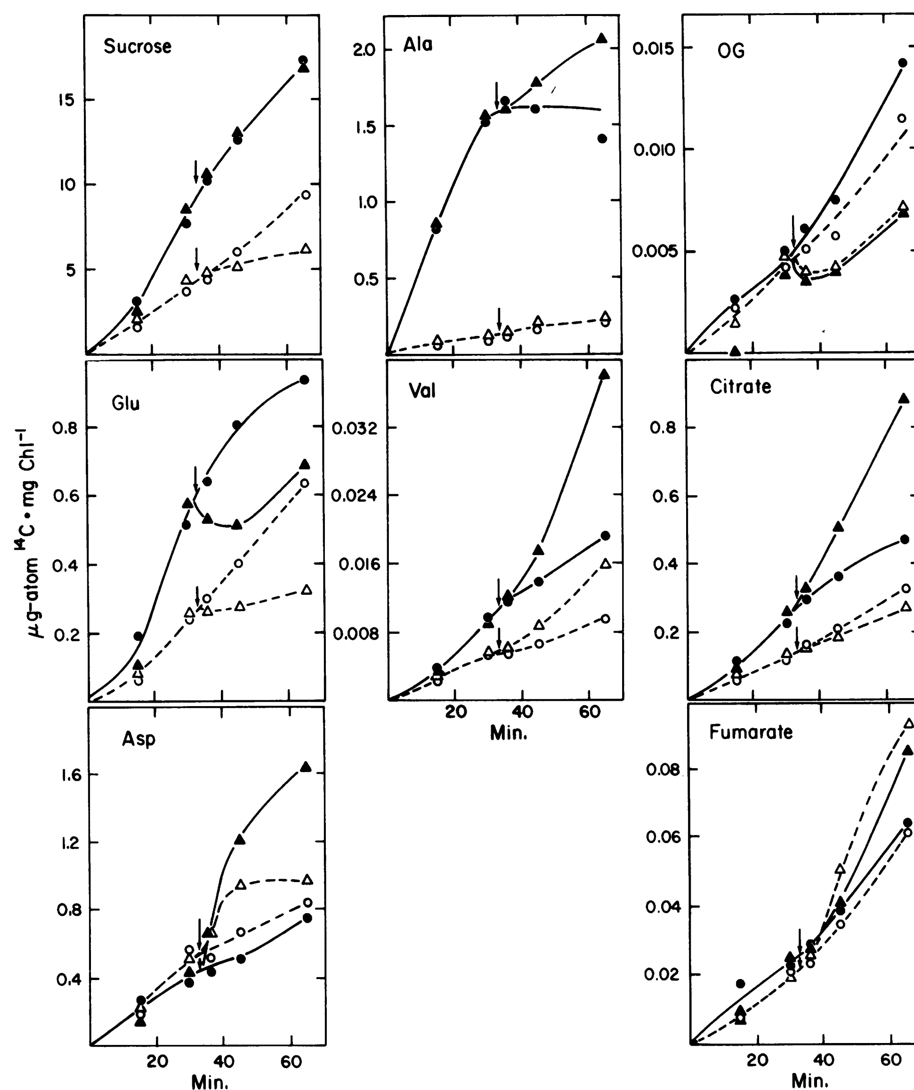

FIG. 3. Fixation from $^{14}CO_2$ into compounds under PR and NPR conditions. OG, α-Ketoglutarate. Conditions were as described in Figure 2.

Table I. *Effect of NH_4 on Free Amino Acid Concentrations Under Photorespiratory and Nonphotorespiratory Conditions*

The intracellular concentrations of [^{14}C]amino acids were determined by analyzing their [^{3}H]dansyl chloride derivatives as described under "Materials and Methods." The values shown are the final concentrations calculated after 65 min of $^{14}CO_2$ fixation; 35 min after either NH_4^+ or H_2O was added to the cell suspensions. Conditions for $^{14}CO_2$ fixation were the same as those described in Figure 2.

	Intracellular Concentrations			
	PR conditions		NPR conditions	
	H_2O	NH_4^+	H_2O	NH_4^+
	µmol/mg Chl			
Gln	0.253	0.754	0.134	1.02
Glu	1.74	0.898	2.00	1.16
Gly	1.06	1.29	0.584	0.488
Ser	0.537	0.399	0.350	0.379
Ala	0.503	0.542	1.04	1.32

and pyruvate kinase are somehow stimulated by the presumed increase in cytosolic ammonia, leading to a faster anaplerotic synthesis of C_5 skeletons (17). This anaplerotic response was indicated in our cells by the faster labeling rates of PEP and malate (Fig. 2) and the equivalent rates of labeling, under both PR and NPR conditions, of α-ketoglutarate, fumarate, and aspartate (Fig. 3). Increased PR metabolism, therefore, results in increased labeling provided via the anaplerotic synthesis of glutamate from α-ketoglutarate.

Fixation under conditions favorable to photorespiration also increased the labeling into asparagine (Fig. 2)—a compound not directly related to PR metabolism but directly involved in NH_4^+ assimilation. Increased asparagine labeling was probably the result of increased activity of glutamine-dependent and/or ammonia-dependent asparagine synthetase (10). Both glutamine and, presumably, NH_4^+ levels were higher under PR conditions. The intracellular concentration of asparagine, unlike that of glutamine, was unaffected by PR fixation conditions, due, possibly, to the small size of the metabolically active asparagine pools relative to the total intracellular aspargine level.

Effects of Ammonia Addition on $^{14}CO_2$ Incorporation Under Photorespiratory Conditions. The addition of NH_4^+ under PR conditions increased fixation of ^{14}C into some amino acids and decreased sucrose labeling; observations similar to earlier studies with isolated poppy (17) and spinach (9) mesophyll cells and alfalfa leaf discs (21). The primary effect of NH_4^+ on our spinach cells under PR conditions was to increase labeled glutamine (Fig. 2) and decrease labeled glutamate (Fig. 3) by equivalent amounts, largely due to the corresponding changes in the pools of these

amino acids (Table I), but not due to increased *de novo* synthesis of C_5 skeletons. Glutamine increases have been observed by others (4, 7, 9, 17, 21, 28). The effect of NH_4^+ on glutamine and glutamate is likely due to the high affinity of glutamine synthetase for NH_4^+ (15). The sensitivity of glutamine synthetase to added NH_4^+ was observed despite the presumably high internal levels of NH_4^+ present under PR conditions (18).

The temporary decrease in the amount of labeled α-ketoglutarate (Fig. 3) was probably the result of its increased reaction with glutamine mediated by glutamine:α-ketoglutarate aminotransferase, which has high affinities for both substrates. The increased flow of carbon through α-ketoglutarate into C_5 amino acids resulted in decreased labeling in malate (Fig. 2) and citrate (Fig. 3), possibly because the availability of fixed carbon was inadequate due to low rates of CO_2 fixation under PR conditions.

The addition of NH_4^+ under PR conditions also affected glycolate pathway metabolism. Increased PR activity, as indicated by the faster labeling into glycolate, glyoxylate, and glycine after addition of NH_4^+ (Fig. 2) could be the result of decreased glutamate concentrations (Table I). Glutamate has been shown to inhibit glycolate synthesis and photorespiration in tobacco (14) and, therefore, decreased glutamate concentrations could have stimulated glycolate pathway labeling under PR conditions. Glyoxylate has also been characterized as an inhibitor of glycolate synthesis (13) but at much higher concentrations than those observed upon addition of NH_4^+ (Fig. 2). Decreases in glutamate concentrations upon addition of NH_4^+ were probably also responsible for the observed decreases in serine concentrations (Table I) and labeling (indicative of the metabolically active pool size due to ^{14}C saturation) (Fig. 2). These observations suggest that serine was being increasingly used for glyoxylate transamination via serine: glyoxylate aminotransferase due to decreased availability of glutamate for glutamate: glyoxylate aminotransferase and increased rates of glycolate pathway metabolism.

Effect of Ammonia Addition on $^{14}CO_2$ Incorporation Under Nonphotorespiratory Conditions. Sucrose labeling (Fig. 3), which was inhibited upon NH_4^+ addition during PR fixation, was unaffected during NPR fixation. This suggests that the partitioning of photosynthate away from sucrose upon the addition of NH_4^+ only occurs when fixation rates are inadequate to accommodate the increased synthesis toward glutamine and other amino acids, a hypothesis discussed by Larsen *et al.* (9). Woo and Canvin (28) also found no effect by NH_4^+ on sucrose labeling in isolated spinach cells assayed under saturating CO_2 concentrations (4 mM $NaH^{14}CO_3$) and high temperatures. Their high rates of $^{14}CO_2$ fixation were apparently adequate to accommodate increased carbon flow into amino acids due to the presence of NH_4^+, without decreasing sucrose synthesis.

Under NPR conditions, glutamine and glutamate were the compounds most affected by NH_4^+ addition. Intracellular concentrations of glutamate decreased and glutamine increased by about 0.80 μmol/mg Chl (Table I). NH_4^+ increased glutamine labeling by a greater amount than under PR conditions. In contrast to the PR experiment, increased glutamine labeling (1.0 μg-atom ^{14}C/mg Chl) could not be quantitatively accounted for by decreased glutamate labeling (0.25 μg-atom ^{14}C/mg Chl) under NPR conditions. The increased production of glutamine via glutamine synthetase was therefore carried out by net flow of ^{14}C into C_5 skeletons, presumably through anaplerotic formation of α-ketoglutarate. Under PR conditions, the rate of glutamate labeling decreased to less than half its original value in the presence of NH_4^+. α-Ketoglutarate labeling returned to its initial labeling rate after a 10-min lag. Under NPR conditions, however, the labeling of both α-ketoglutarate and glutamate returned to their initial rates after a short cessation of labeling (Fig. 3). This return to initial labeling rates of glutamate, despite the increased carbon drain into glutamine under NPR conditions with added NH_4^+, was probably aided by faster NPR photosynthetic rates. Also, there was a decreased use of glutamate to transaminate glyoxylate under NPR conditions. Transamination of glyoxylate uses up nearly half the glutamate produced by glutamine:α-ketoglutarate aminotransferase under PR conditions (8). The ability of anaplerotic reactions to accommodate the increased demand for carbon skeletons of C_5 amino acids under NPR conditions is further evidenced by the increased labeling of malate, citrate, fumarate, PEP, and asparate upon the addition of NH_4^+ (Figs. 2 and 3).

Upon addition of NH_4^+ during NPR fixation, asparagine labeling increased up to the levels present under PR conditions by 65 min (Fig. 2), whereas NH_4^+ had no effect under PR conditions. This suggests that maximal rates of asparagine synthesis had been obtained under these conditions of presumably high internal NH_4^+ content.

The total labeling of amino acids increased with NH_4^+ addition during NPR fixation, whereas with PR fixation, NH_4^+ addition caused no net change. In addition to glutamine and asparagine, the labeling of alanine, valine, and aspartate (Fig. 3), and threonine and proline were increased with NH_4^+ under NPR conditions. Only glutamate labeling decreased. Asparagine and glutamine labeling increases could be explained by the direct dependence of their formation on NH_4^+ and/or glutamine concentrations. The increased labeling of other amino acids, however, was unexpected in view of the lower glutamate concentrations available after NH_4^+ addition (Table I). Alanine labeling (Fig. 3) increased proportionally to increases in its intracellular concentration (Table I) after the addition of NH_4^+ under NPR conditions.

There was no effect of NH_4^+ on the labeling of photorespiration pathway intermediates during NPR fixation. This greatly contrasted the results under PR fixation and was indicative of the low rates of PR metabolism.

The perturbation of carbon metabolism during photosynthesis by added ammonia has been characterized in many biological systems (4, 7, 9, 17, 21, 28). The effects of PR NH_4^+ production on metabolism and amino acid concentrations were very similar to the effects of adding external NH_4^+ to the cells. The overall response of cells to externally supplied NH_4^+ was accentuated in cells whose internal NH_4^+ content was initially low presumably due to fixation under NPR conditions. Addition of NH_4^+ to cells during photosynthesis therefore just leads to an increased manifestation of a naturally occurring regulation carried out by plants.

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LITERATURE CITED

1. Arnon DJ 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris.* Plant Physiol 24: 1–15
2. Bachelard HS 1965 Glucose metabolism and α-ketoacids in rat brain and liver *in vivo.* Nature 205: 903–904
3. Givan CV 1979 Metabolic detoxification of ammonia in tissues of higher plants. Phytochemistry 18: 375–382
4. Hammel KE, KL Cornwell, JA Bassham 1979 Stimulation of dark CO_2 fixation by ammonia in isolated mesophyll cells of *Papaver somniferum* L. Plant Cell Physiol 20: 1523–1529
5. Jensen RG, JT Bahr 1977 Ribulose 1,5-bisphosphate carboxylase-oxygenase. Annu Rev Plant Physiol 28: 379–478
6. Jensen RG, JA Bassham 1966 Photosynthesis by isolated chloroplasts. Proc Natl Acad Sci USA 56: 1095–1101
7. Kanazawa T, MR Kirk, JA Bassham 1970 Regulatory effects of ammonia on carbon metabolism in photosynthesizing *Chlorella pyrenoidosa.* Biochim Biophys Acta 205: 401–408
8. Keys AJ, IF Bird, MJ Cornelius, PJ Lea, RM Wallsgrove, BJ Miflin 1978 Photorespiratory nitrogen cycle. Nature 275: 741–743
9. Larsen PO, KL Cornwell, SL Gee, JA Bassham 1981 Amino acid synthesis in photosynthesizing spinach cells. Effects of ammonia on pool sizes and rates of labeling from $^{14}CO_2$. Plant Physiol 68: 292–299
10. Lea PJ, L Fowden 1975 Asparagine metabolism in higher plants. Biochem Physiol Pflanzen 168: 3–14
11. Lee RB, CP Whittingham 1975 The influence of partial pressure of carbon dioxide upon carbon metabolism in the tomato leaf. J Expt Bot 25: 277–287

12. Miflin BJ, PJ Lea 1977 Amino acid metabolism. Annu Rev Plant Physiol 28: 299–329
13. Oliver DJ, I Zelitch 1977 Increasing photosynthesis by inhibiting photorespiration with glyoxylate. Science 196: 1450–1451
14. Oliver DJ, I Zelitch 1977 Metabolic regulation of glycolate synthesis, photorespiration, and net phtosynthesis in tobacco by L-glutamate. Plant Physiol 59: 688–694
15. O'Neal D, KW Joy 1974 Glutamine synthetase of pea leaves, divalent cation effects, substrate specificity, and other properties. Plant Physiol 54: 773–779
16. Paul JS, JA Bassham 1977 Maintenance of high photosynthetic rates in mesophyll cells isolated from *Papaver somniferum*. Plant Physiol 60: 775–778
17. Paul JS, KL Cornwell, JA Bassham 1978 Effects of ammonia on carbon metabolism in photosynthesizing isolated mesophyll cells from *Papaver somniferum* L. Planta 142: 49–54
18. Platt SG, GE Anthon 1981 Ammonia accumulation and inhibition of photosynthesis in methionine sulfoximine treated spinach. Plant Physiol 67: 509–513
19. Platt SG, JA Bassham 1977 Separation of ^{14}C-labeled glycolate pathway metabolites from higher plant photosynthate. J Chromatography 133: 396–401
20. Platt SG, Z Plaut, JA Bassham 1976 Analysis of steady state photosynthesis in alfalfa leaves. Plant Physiol 57: 69–73
21. Platt SG, Z Plaut, JA Bassham 1977 Ammonia regulation of carbon metabolism in photosynthesizing leaf discs. Plant Physiol 60: 739–742
22. Platt SG, Z Plaut, JA Bassham 1977 Steady-state photosynthesis in alfalfa leaflets. Effects of carbon dioxide concentrations. Plant Physiol 60: 230–234
23. Snyder FW, NE Tolbert 1974 Effect of CO_2 concentration on glycine and serine formation during photorespiration. Plant Physiol 53: 514–515
24. Tolbert NE 1979 Glycolate metabolism by higher plants and algae. *In* M Gibbs, E Latzko, eds, Photosynthesis II. Encyclopedia of Plant Physiology, Vol 6. Springer-Verlag, New York, pp 338–352
25. Viil J, T Pärnik 1974 Influence of oxygen upon photosynthetic carbon metabolism at high CO_2 concentrations and saturating irradiance. Photosynthetica 8: 208–215
26. Voskresenskaya NP, YA Wiil, GS Grishina, TR Parnik 1970 Effect of oxygen concentration and light intensity on the distribution of labelled carbon in photosynthesis products in bean plants. Photosynthetica 4: 1–8
27. Wallsgrove RM, AJ Keys, IF Bird, MJ Cornelius, PJ Lea, BJ Miflin 1980 The location of glutamine synthetase in leaf cells and its role in the reassimilation of ammonia released in photorespiration. J Exp Bot 31: 1005–1007
28. Woo KC, DT Canvin 1980 Effect of ammonia on photosynthetic carbon fixation in isolated spinach leaf cells. Can J Bot 58: 505–510