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A Possible Mechanism of Ammonium Ion Regulation of Photosynthetic Carbon Flow in Higher Plants[1]

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ABSTRACT

Addition of NH_4^+ to the photosynthesizing leaf cells of *Dolichos lab lab* L. var. *Lignosis* Prain and leaf discs of *Vigna sinensis* L. savi ex Hassk caused a significant increase in the flow of photosynthetic carbon toward amino acids with a concomitant decrease toward sugars without affecting the over-all photosynthetic rate. Similar diversion of photosynthetic carbon away from sugars was also observed in the photosynthesizing isolated chloroplasts of *V. sinensis*, but the latter differed in that they accumulated organic acids rather than amino acids. In an effort to understand the mechanism of NH_4^+-mediated regulation, the specific and total activities of NAD(P)-glutamate dehydrogenase, glutamine synthetase, pyruvate kinase, alkaline fructose 1,6-bisphosphatase, and NAD(P)-glyceraldehyde-3-phosphate dehydrogenase of the cells of *D. lab lab* were checked but none was affected by the added ammonium salts even after prolonged incubation. At certain concentrations, ammonium ions abolished the light activation of NADP-glyceraldehyde-3-phosphate dehydrogenase and alkaline fructose 1,6-bisphosphatase in isolated chloroplasts from dark-adapted *Vigna* leaves without interfering with the basal dark activity of these enzymes. Based on these observations, a possible mechanism of action of NH_4^+ in regulating the photosynthetic carbon flow is postulated.

Ammonium salts have been shown to regulate the photosynthetic carbon flow into various cellular components in *Chlorella* (16, 17), cotton leaf cells (28), bean leaf cells (23, 24), and alfalfa leaf discs (27). Similar regulation leading to the increased flow of photosynthetic carbon into amino acid fractions at the expense of sugars has been reported to be caused by blue light as well (22). Suggestions have been made that NH_4^+ brings about the increased amino acid synthesis in photosynthesizing cells mostly due to the stimulation of pyruvate kinase (16, 17), whereas the blue light effect is presumed to be due to the activation of PEP-case[2] (22). Although these reports emphasized the regulatory role of ammonia, very little substantive information is available to explain the mechanism of this regulation. This communication presents data from our attempts to understand the mechanism of action of NH_4^+ in regulating the carbon flow within the photosynthetic carbon flow pathways, in the isolated leaf cells of bean and in the leaf discs and chloroplasts of cowpea.

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[2] Abbreviations: PEP-case: phosphoenolpyruvate carboxylase; PEP: phosphoenolpyruvate; PGA: 3-phosphoglyceric acid; DCPIP: dichlorophenolindophenol; FCCP: carbonyl cyanide *p*-(trifluoromethoxy) phenyl hydrazone; GHA: γ-glutamyl hydroxamate; LDH: lactic dehydrogenase; FBP: fructose 1,6-bisphosphate; FBPase: fructose 1,6-bisphosphatase.

MATERIALS AND METHODS

Plant Materials. Bean (*Dolichos lab lab* L. var. *Lignosis* Prain) and cowpea (*Vigna sinensis* L. savi ex Hassk) were grown under field conditions. Fully expanded leaves were excised and kept in moist containers until either the leaf discs were cut out with a 5-mm-diameter cork borer or the mesophyll cells were prepared by mechanical grinding as described previously (13).

Chemicals. The chemicals used were of analytical grade obtained from BDH Chemicals, India. Finer chemicals were purchased from Sigma. The radioactive materials were from Bhabha Atomic Research Centre, Bombay, India.

Photosynthetic Carbon Fixation. The ^{14}C-bicarbonate fixation of the cell suspensions or the leaf discs (after vacuum infiltration of the reaction medium) was measured according to the modified procedure of Bassham and Calvin (3) using 20,000 lux of white light at 22 ± 1 C. The reaction media contained 50 mM phosphate buffer (pH 7.2), 35 mM NaCl, 5 mM $MgCl_2$, and 10 mM $NaH^{14}CO_3$ (1 μCi/10 μmol). The isolation and the fixation of ^{14}C-bicarbonate in the chloroplasts of young, freshly harvested leaves of *Vigna* were followed by the method of Kirk and Leech (18). Aliquots of samples of control and ammonium-treated leaf discs, cells, and chloroplasts were removed at the predetermined time intervals for analysis.

Analysis of ^{14}C-labeled Products. Each sample was macerated and successively extracted with 80, 40, and 20% ethanol (v/v) and then with water. The extracts of each sample were combined and evaporated to dryness under vacuum. The dried residue was redissolved in distilled H_2O and was separated into anionic (mainly organic acids), cationic (amino acids), and neutral fractions (sugars) following the procedure of Canvin and Beevers using ion exchange resins (8).

Enzyme Preparations. Three g of packed control and NH_4^+-treated leaf cells were homogenized in 10 ml of 20 mM Tris-HCl (pH 7.5), 1 mM 2-mercaptoethanol, and acid-washed sand in a prechilled mortar and pestle. The homogenate was strained through four layers of muslin and centrifuged at 23,000*g* for 30 min. All steps were carried out at 0 to 4 C. Enzyme activity was assayed in the dialyzed supernatant fraction.

Enzyme Assays. NAD and NADP-glutamate dehydrogenases (EC 1.4.1.3 and EC 1.4.1.4) were assayed spectrophotometrically following the oxidation of NADH or NADPH at 340 nm at 25 C in a 3-ml reaction mixture containing 220 μmol Tris-HCl (pH 8.0), 300 μmol ammonium chloride, 20 μmol α-ketoglutarate, 3 μmol NAD(P)H, and 100 to 150 μg protein of cell extract.

Pyruvate kinase (EC 2.7.1.40) was assayed according to the method developed by Nagelein, as described by Bucher and Pfleiderer (7). The reaction mixture contained, in a volume of 3 ml, 80 μmol Tris-HCl (pH 7.5), 220 μmol KCl, 2.4 μmol $MgSO_4$, 1.5 μmol ADP, 0.56 μmol NADH, 18 units purified LDH, 4.5 μmol PEP (freshly prepared), and 100 to 150 μg cell extract protein.

NAD(P)-glyceraldehyde-3-P dehydrogenases (EC 1.2.1.12 and EC 1.2.1.13) were assayed in the direction of reduction of 3-PGA. The reaction was assayed spectrophotometrically at 25 C. The reaction mixture contained, in a volume of 1.0 ml, 100 μmol Tris-HCl (pH 7.8), 1 μmol DTT, 15 μmol PGA sodium salt, 3.4 μmol ATP, 0.6 μmol NAD(P)H, and 100 to 150 μg cell extract protein.

Glutamine synthetase (EC 6.3.1.2) was assayed by the method described by Elliot (11). The ferric chelate of GHA produced is measured spectrophotometrically at 540 nm. An *A* of 0.034 for 15-min incubation time at 540 nm under the conditions specified was taken as 1 enzyme unit.

FBPase (EC 3.1.3.11) was assayed in a reaction mixture of 1.0 ml containing 100 μmol Tris-HCl (pH 8.4), 10 μmol $MgCl_2$, 10 μmol FBP (freshly prepared), 1 μmol sodium EDTA, and 100 to 150 μg cell extract protein. The reaction was stopped by adding 0.5 ml cold trichloroacetic acid and left at 4 C for 2 h. After centrifugation, the released Pi in the supernatant was assayed by the method of Fiske and Subbarow (12). The partial purification of the FBPase of bean leaves was followed by the method of Buchanan *et al.* (6). The protein, after acid precipitation, was extensively dialyzed before use.

Light Activation of NADP-Glyceraldehyde-3-P Dehydrogenase (EC 1.2.1.13). Chloroplasts were isolated from dark-adapted leaves of cowpea by the method of Cockburn *et al.* (10) as modified by Anderson and Avron (1). The chloroplast suspension (100 μg Chl/ml) was exposed to white light of 20,000 lux at 25 C, aliquots were removed and assayed immediately in a medium which was hypotonic to the chloroplasts. The effect of NH_4^+ and other inhibitors was studied by adding these substances at indicated concentrations to the chloroplast suspension just prior to illumination. The effect of dark activation of this enzyme by DTT, NADPH, and ATP was studied by preincubating the chloroplasts with these substances in 20 mM Tris-HCl (pH 7.8) at 25 C in the dark for 20 min.

Light Activation of Chloroplastic Alkaline Fructose-1,6-bisphosphatase (EC 3.1.3.11). The chloroplasts were suspended in a Warburg flask in a medium containing 50 mM Hepes-KOH (pH 7.6), 2 mM EDTA, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 400 mM sucrose, 10 mM sodium ascorbate, 0.1 mM DCPIP, and 0.2 mM benzyl viologen. The central well in the flask was filled with 0.2 ml of 7 N KOH. The air was flushed out with N_2 gas and closed air-tight before it was exposed to white light of 20,000 lux at 25 C. Aliquots of the chloroplast suspension were transferred directly to the assay medium for measuring the enzyme activity. Whenever the effect of NH_4^+ ions or other inhibitors was studied, selected concentrations of NH_4OH or other inhibitors were added to the activation medium prior to the illumination. Appropriate dark controls were routinely maintained simultaneously for all light activation studies. In these studies, NH_4^+ was added as NH_4OH and the amounts used had no effect whatsoever on the pH of the medium.

Total Chl was estimated according to the method of Arnon (2) and the protein was estimated by Lowry's method (19). The data presented here are the means of at least three experiments.

RESULTS

^{14}C-Bicarbonate Fixation. The bean cells in suspension incorporated ^{14}C-bicarbonate photosynthetically into acid-stable products almost linearly for 20 min at a rate of 12 to 60 μmol/mg Chl·h. The addition of 1 mM NH^+_4 did not change either the kinetics or the total fixation of ^{14}C-bicarbonate (Fig. 1). While the cowpea leaf discs fixed around 85 μmol CO_2/mg Chl·h, the isolated chloroplasts could fix 8.7 μmol CO_2/mg Chl·h.

Distribution of Photosynthetic Carbon. The water-soluble frac-

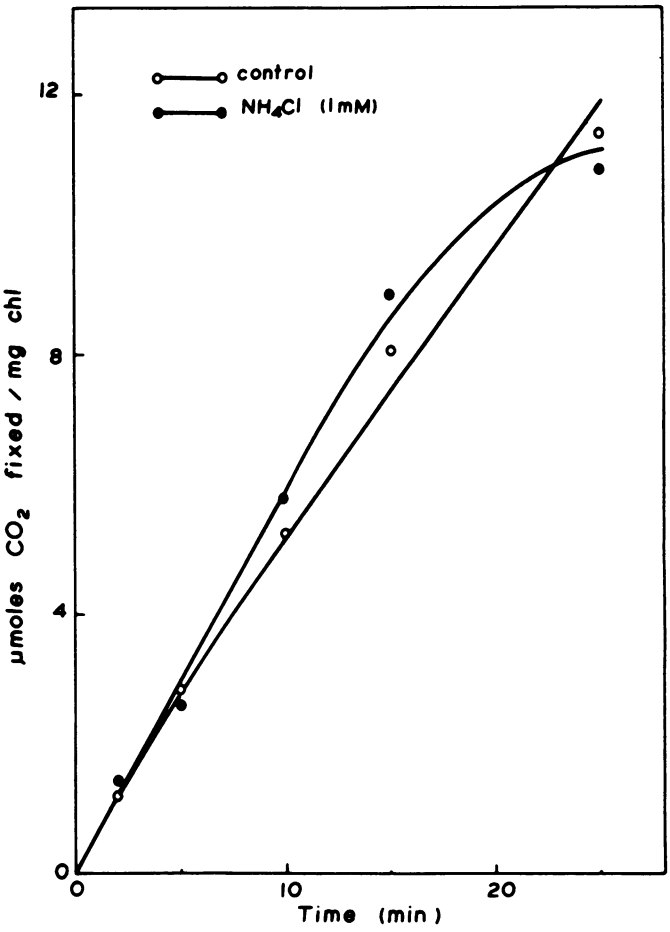

FIG. 1. Time course of ^{14}C-bicarbonate fixation in isolated leaf cells of *D. lab lab* in the presence and absence of ammonium chloride.

Table I. *Effect of Ammonium Ions on the Distribution of Labeled Carbon from ^{14}C-Bicarbonate into Various Fractions of the Primary Photosynthetic Products of Leaf Discs and Chloroplasts of V. sinensis*

The leaf discs were preincubated with ammonium salts for 1 h and the chloroplasts for 5 min at 20 C. ^{14}C-Bicarbonate fixation was done at saturating light intensity for 30 min.

Fractions	Leaf Discs				Chloroplasts			
	Control		+ 1 mM NH_4^+		Control		+ 0.8 mM NH_4^+	
	cpm	%	cpm	%	cpm	%	cpm	%
Neutral fraction (sugars)	39,000 ± 1,300	48	27,800 ± 3,000	34	11,500 ± 585	35	8,400 ± 493	25
Cationic fraction (amino acids)	9,200 ± 1,600	11	20,000 ± 1,400	24	3,400 ± 152	10	3,300 ± 152	10
Anionic fraction (organic acids)	33,900 ± 6,500	41	35,300 ± 7,913	42	18,000 ± 200	55	21,800 ± 400	65
	82,100	100	83,100	100	32,900	100	33,500	100

Table II. *Effect of Ammonium Ions on the Catalytic Activities of Certain Key Enzymes in Whole Cell Extracts*

The enzyme from the control cells was incubated with 1 mM NH_4Cl for 10 min at 15 C before the assay and the cells as such were incubated with 1 mM NH_4Cl for 1 h at 20 C. An equal quantity of cells and extraction buffer was taken for control and ammonium treatment there was no difference between their total enzyme activity. The units for glutamine synthetase are defined under "Materials and Methods."

Enzyme	Enzyme Source		
	Control	Control enzyme + 1 mM NH_4Cl	NH_4Cl-treated cell
	nmol NAD(P)H oxidized/mg protein·min		
NAD-glutamate dehydrogenase	21	21	22
NADP-glutamate dehydrogenase	138	137	140
NAD-glyceraldehyde-3-phosphate dehydrogenase	543	541	550
NADP-glyceraldehyde-3-phosphate dehydrogenase	155	153	156
Pyruvate kinase	44	44	42
	nmol Pi liberated/mg protein·min		
Fructose-1,6-bisphosphatase	45	46	48
	units/mg protein		
Glutamine synthetase	1.8	1.8	1.7

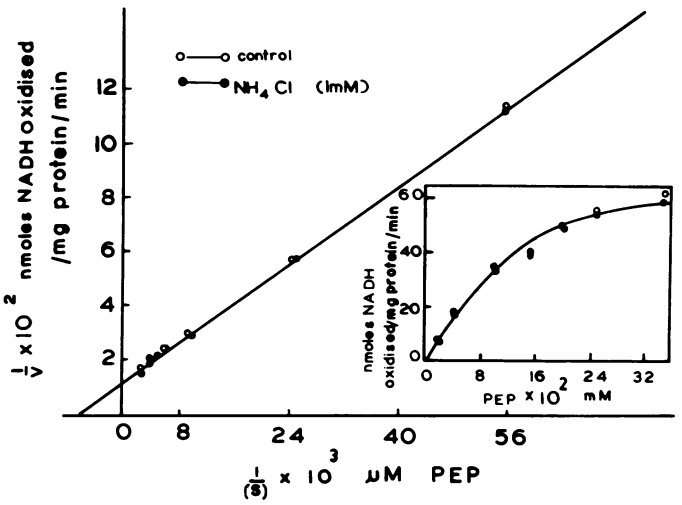

FIG. 2. Saturation curve of pyruvate kinase for PEP in dialyzed crude extract of bean leaf cells.

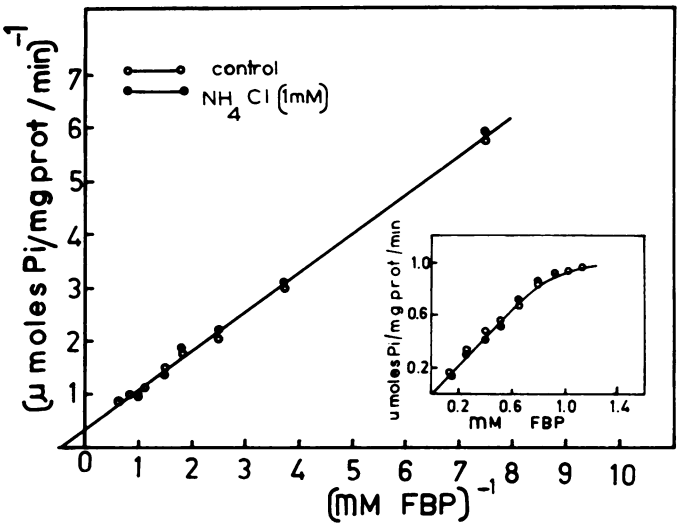

FIG. 3. Saturation curve of partially purified, alkaline FBPase for FBP in bean leaf cells.

tion of the ethanol extract of the *Vigna* leaf discs on fractionation using ion exchange resins showed that the addition of NH_4^+ caused a significant increase in the amount of label in the amino acid fraction with a concomitant decrease in sugar fraction. In the isolated, photosynthesizing *Vigna* chloroplasts, ammonium diverted the carbon flow more toward organic acids (Table I). Nitrate in the form of $Ca(NO_3)_2$ and Cl^- in the form of NaCl had no effect. Essentially the same pattern of results was observed in the leaf cells of bean as well (24).

Assay of Key Enzymes. When enzyme activities in control and NH_4^+-treated bean leaf cell extracts were compared, no significant difference either in specific or total activity that could account for the observed diversion of photosynthetic carbon into cationic fractions was observed. The addition of NH_4^+ to the extracted enzymes from control cells also did not show any effect on their activities (Table II). The saturation curve of pyruvate kinase, the suspected regulatory enzyme (17) of the photosynthetic carbon flow pathways, was hyperbolic with respect to PEP and hence the double reciprocal plot was linear and the kinetics was same even after ammonium treatment (Fig. 2); likewise, the kinetics of the partially purified FBPase was not altered in the presence of NH_4^+ (Fig. 3).

Light Activation of Chloroplastic Enzyme. Since none of the measured enzymes of the photosynthetic carbon flow pathways showed any altered response to added ammonium ions, and as ammonium ions were found to modulate only the product proportions and not the fixation itself, the possibility of NH_4^+ interfering in some way in the light activation of some of the chloroplast enzymes was suspected. As the leaves of beans yield only intact cells readily but not intact chloroplasts, the light activation studies were confined to cowpea chloroplasts.

The kinetics of the light activation of the chloroplastic NADP-glyceraldehyde-3-P dehydrogenase is shown in Figure 4. The activity of the enzyme in the chloroplasts of dark-adapted leaves did not show any decay in storage at 25 C for at least 30 min. When the chloroplasts were illuminated the activity nearly doubled within 10 min of illumination and declined slowly after 20 min. The observed dark activity of the enzyme and the extent of light activation are comparable to the reports in pea chloroplasts (1), although activation kinetics different from that reported here has been shown in spinach chloroplasts (9). The light activation was abolished by DCMU and sodium sulfite. At the concentration levels used, these inhibitors did not affect the basal dark activity of the enzyme much, indicating the involvement of photoelectron transport and disulfides (1) in the light activation process. Preincubation of the chloroplast extracts with DTT and NADPH in the dark also activated the enzyme but only together, to the same extent as did light. Addition of ATP in the dark failed to elicit any marked change in the activity of this enzyme (Table III). Perhaps the photoproduced NADPH or the vicinal dithiols (1, 25, 29) might activate the otherwise not very active enzyme proteins by some unknown mechanism. The effect of different concentrations of NH_4^+ on the light activation of NADP-glyceraldehyde-3-

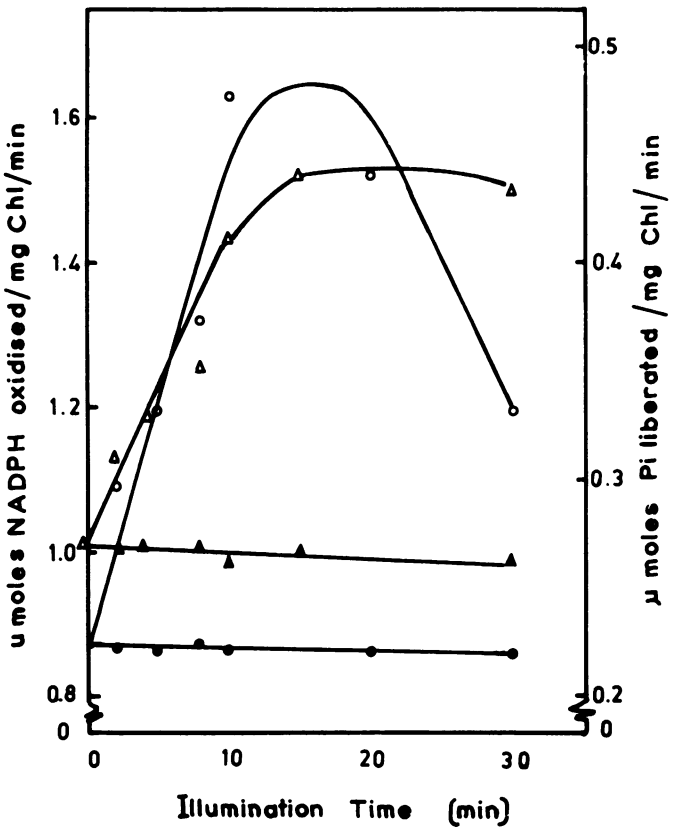

FIG. 4. Time course of light activation of NADP-glyceraldehyde-3-P dehydrogenase (○, ●) and alkaline FBPase (△, ▲) from chloroplasts of *V. sinensis*. (●, ▲): Enzyme activity in dark; (○, △): activity in light.

Table III. *Effect of Inhibitors and Activators on the Light Activation of NADP-Glyceraldehyde-3-P Dehydrogenase (EC 1.2.1.13) in Chloroplasts of V. sinensis*

Figures in parentheses indicate percentage of dark control values.

Inhibitors	Dark	Light
	µmol NADPH oxidized/mg Chl-h	
Control	38.6 (100)	72.8 (189)
+ Na_2SO_3 (100 µM)	35.7 (93)	23.6 (61)
+ DCMU (10 µM)	34.4 (89)	36.1 (94)
Control	56.6 (100)	80.2 (142)
+ DTT (10 mM)	76.4 (135)	
+ ATP (8 mM)	53.2 (94)	
+ NADPH (2 mM)	71.2 (126)	
Control	40.8 (100)	80.0 (196)
+ DTT (10 mM)	70.3 (172)	
+ NADPH (2 mM)	53.2 (130)	
+ DTT (10 mM) + NADPH (2 mM)	79.3 (194)	

P dehydrogenase showed a biphasic pattern (Fig. 5). When dark-adapted chloroplasts were incubated with increasing concentrations of NH_4^+ and exposed to light for 10 min, the extent of light activation declined at low NH_4^+, but increased back to normal with further addition of NH_4^+, until at 5.0 mM NH_4^+ the enzyme activity was back to the no-NH_4^+ level. This could be due to the uncoupling of photophosphorylation at this concentration and the consequent increased production of reductants. The chloroplasts incubated with corresponding concentrations of NH_4^+ in the dark showed very little change in enzyme activity.

Light activation of the FBPase was observed when the dark-adapted chloroplasts were illuminated anaerobically in a special activation medium containing ascorbate and DCPIP as electron donor and benzyl viologen as acceptor. Omission of any one of the components from the activation medium decreased the magnitude of light activation, but none of the additives affected the enzyme activity in the dark (Table IV). The time course of light activation of the enzymes shows a linear increase for 10 min and a leveling off thereafter (Fig. 4). The basal level of activity and the extent of light activation of the FBPase observed in the plant species are close to those reported in spinach (6). The light activation was abolished when either 1 mM mercuric chloride, 5 mM KCN, the known inhibitors of PSI (5, 14), or 0.5 mM NH_4OH was added to the chloroplasts during illumination (Table IV). The dark activity of this enzyme was not affected by these compounds. Illumination of the chloroplasts with different concentrations of NH_4^+ for 10 min also showed a biphasic trend in the activation of this enzyme very much like that of glyceraldehyde-3-P dehydrogenase (Fig. 5). As in the case of light-activated dehydrogenase, the dark basal activity of the biphosphatase was not affected by the added NH_4^+.

In order to understand the nature of action of NH_4^+ on the light activation of FBPase at a concentration which uncouples photo-

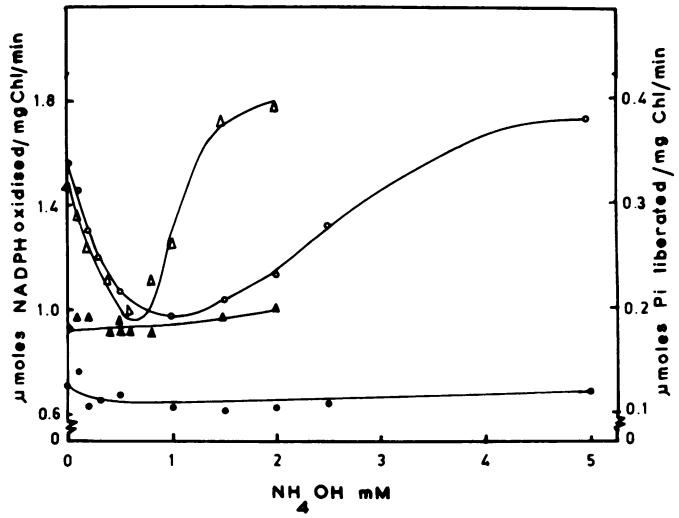

FIG. 5. Effect of different concentrations of NH_4^+ on light activation of NADP-glyceraldehyde-3-P dehydrogenase (○, ●) and alkaline FBPase (△, ▲) from chloroplasts of *V. sinensis*. (●, ▲): Enzyme activity in dark; (○, △): activity in light.

Table IV. *Effect of Light, Inhibitors, and Uncouplers on the Activity of FBPase (EC 3.1.3.11) in Chloroplasts of V. sinensis*

Figures in parentheses indicate percentage of dark control values.

Reaction Mixture	Dark	Light
	µmol Pi liberated/mg Chl·min	
Complete (control)	0.450 (100)	0.698 (155)
− Benzyl viologen	0.468 (104)	0.544 (121)
− Benzyl viologen, ascorbate and DCPIP	0.453 (100)	0.528 (117)
Gas phase: air, − KOH	0.456 (101)	0.494 (109)
Control	0.302 (100)	0.498 (165)
+ 0.5 mM NH_4OH	0.312 (103)	0.396 (131)
+ 5.0 mM KCN	0.309 (102)	0.327 (108)
+ 1.0 mM $HgCl_2$	0.304 (100)	0.326 (107)
Control	0.341 (100)	0.555 (163)
+ 0.5 mM NH_4Cl	0.341 (100)	0.369 (108)
+ 1.0 mM NH_4Cl	0.375 (110)	0.600 (176)
+ 10.0 µM FCCP	0.335 (98)	0.631 (185)

phosphorylation, the effect of FCCP was studied. The uncoupler FCCP increased the extent of light activation of the enzyme comparable to that caused by NH_4^+. At the concentration level used, FCCP did not show any inhibitory action over the enzyme activity of chloroplasts kept in the dark (Table IV).

DISCUSSION

The rate of CO_2 fixation observed with bean leaf cells and cowpea leaf discs is comparable to the values reported by others for cells separated by mechanical grinding or by enzymic means (13, 15), even though much higher rates have also been reported recently (27). The addition of NH_4^+ to photosynthesizing cells and leaf discs caused a significant increase in the flow of photosynthetic carbon toward amino acids with a concomitant decrease toward the sugar fractions without affecting the over-all photosynthetic rates; the level of photosynthetic carbon associated with the organic acids was not altered much. The observations reported here are in agreement with the reports of Kanazawa *et al.* (16, 17), Blackwood and Miflin (4), Mohamed and Gnanam (23), Rehfeld and Jensen (28), and Platt *et al.* (27).

In the presence of NH_4^+ the flow of photosynthetic carbon in *Vigna* chloroplasts was diverted away from sugars and found to accumulate into organic acids, rather than amino acids. This is understandable since although the chloroplasts are known to have the enzyme complements for the synthesis of most of the amino acids, the keto acids required for amination are to come from outside the chloroplasts (18).

One possible way for NH_4^+ to increase the production of amino acids is by simple mass action in the reductive amination of intermediate organic acids, thus drawing the photosynthate away from the production of sugars. Alternatively, it might act as a true regulator, besides a substrate, and modulate the rate of flow of carbon within photosynthetic carbon flow pathways defined as to a set of limited intermediates into which photosynthetic carbon finds its way (Fig. 6). As the level of photosynthetic carbon flowing into the organic acid pool was not altered much by ammonia in systems with complete photosynthetic carbon flow pathways, the second alternative, *viz.* NH_4^+ acting as a regulator besides being a substrate, is a distinct possibility.

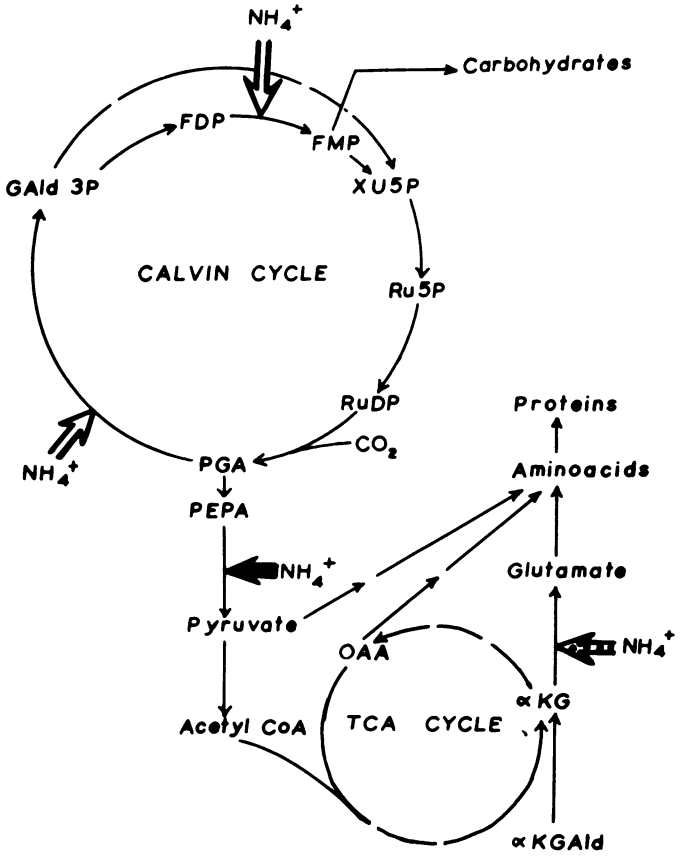

FIG. 6. Photosynthetic carbon flow pathways. Closed arrow indicates speculated site of regulatory function of ammonium ions. Open arrows indicate established sites of regulatory functions of ammonium ions. Dotted arrow indicates site of action of ammonium ions as substrate for aminating reactions.

The possibility of NH_4^+-induced *de novo* synthesis of some enzymes of carbon reductive pathways or aminating and transaminating enzymes appears to be remote since the observed effects occurred in short term experiments, while 1 h of preincubation of cells with NH_4^+ did not reveal any substantial increase in the [^{14}C]leucine incorporation into cellular proteins as compared to the untreated cells (data not included).

The absence of any significant change in the specific activity or total activity per unit number of cells of any of the measured enzymes rules out the possibility of NH_4^+ regulating the carbon flow directly through activation or inactivation of the enzymes. Although the data do not explore the possible effects of NH_4^+ on the enzyme-substrate affinity since the assays were done with maximal substrate concentration, the studies with two crucial enzymes, *viz.* pyruvate kinase and FBPase, did not reveal any significant difference in either V_{max} or K_m upon the addition of NH_4^+. Pyruvate kinase, which failed to show any significant alteration in activity due to the added NH_4^+ in bean leaf cells, has been suggested by Kanazawa *et al.* (16, 17) as the key enzyme involved in the diversion of photosynthetic carbon toward amino acids in *Chlorella*. As the enzyme from bean leaf cells showed hyperbolic kinetics toward PEP, as do the enzymes from many higher plants (20, 21, 26), it may not be regulatory in function. One cannot, however, exclude the possibility that full activation of the enzyme is an artifact by-product of enzyme extraction. It has been reported that although the partially purified enzyme from *Euglena gracilis* is unaffected by NH_4^+, it could still be regulatory in function (31).

The ammonium-mediated regulation of carbon metabolism is observed only in the photosynthetic carbon flow. The distribution of [^{14}C]acetate fed to the cells is unaffected by NH_4^+ (data not shown). It is probable that ammonium effect is through some light-dependent activation steps of the photosynthetic carbon pathways, as evidenced by the observations that NADP-glyceraldehyde-3-P-dehydrogenase and FBPase are affected by NH_4^+ only in the light-activated portion of their total activity without affecting the basal level. As seen from the preferential diversion of carbon flow by NH_4^+ without affecting the over-all rate of CO_2 fixation, it is likely that the FBPase involved in the regeneration of the intermediates of Calvin cycle for carboxylation and the FBPase involved in the synthesis of sugars are highly compartmentalized within the chloroplast and NH_4^+ interacts only with the latter. Alternatively, the light activation of the FBPase would be lowered by NH_4^+ *in vivo* to that extent only to retard the rate of flow of photosynthetic carbon to the synthesis of sugars, but not to the level of affecting the rate of regeneration of the intermediates for the carboxylation to proceed. This may be true of the NADP-glyceraldehyde-3-P dehydrogenase also.

The simultaneous lowering of activities of FBPase and NADP-glyceraldehyde-3-P dehydrogenase by NH_4^+ will result in the increased level of 3-carbon pieces leaving the Calvin cycle for diversion into the direction of PEP rather than glucose, thus eventually increasing the pool of the acid (30) intermediates in amino acid synthesis. This is corroborated by the finding from the photosynthetic carbon flow in isolated chloroplasts. Thus, NH_4^+ can serve as a regulator in photosynthetic carbon metabolism.

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