Plant Physiol. (1985) 78, 334–337
0032-0889/85/78/0334/04/$01.00/0

Role of Asparagine in the Photorespiratory Nitrogen Metabolism of Pea Leaves[1]

Received for publication December 13, 1984 and in revised form February 20, 1985

TRUNG CHANH TA[2], KENNETH W. JOY*, AND ROBERT J. IRELAND
Department of Biology and Institute of Biochemistry, Carleton University, Ottawa, Ontario K1S 5B6 Canada

ABSTRACT

In pea leaves, much of the metabolism of imported asparagine is by transamination. This activity was previously shown to be localized in the peroxisomes, suggesting a possible connection between asparagine and photorespiratory nitrogen metabolism. This was investigated by examination of the transfer of ^{15}N from the amino group of asparagine, supplied via the transpiration stream, in fully expanded pea leaves. Label was transferred to aspartate, glutamate, alanine, glycine, serine, ammonia, and glutamine (amide group). Under low oxygen (1.8%), or in the presence of α-hydroxy-2-pyridine methanesulfonic acid (an inhibitor of glycolate oxidase, a step in the photorespiratory formation of glyoxylate), there was a substantial (60–80%) decrease in transfer of label to glycine, serine, ammonia, and glutamine. Addition of isonicotinyl hydrazide (an inhibitor of formation of serine from glycine) caused a 70% decrease in transfer of asparagine amino nitrogen to serine, ammonia, and glutamine, while a 4-fold increase in labeling of glycine was observed. The results demonstrate the involvement of asparagine in photorespiration, and show that photorespiratory nitrogen metabolism is not a closed cyclic process.

In C-3 plants a portion of the carbon involved in photosynthetic reactions is diverted to the photorespiratory pathway via phosphoglycolate and glycolate (9). Oxidation of glycolate to glyoxylate and subsequent transamination to glycine occurs in the peroxisome (12). The involvement of nitrogen in photorespiration has been considered to be a cyclic process (6), with serine and glutamate as the amino donors for glycine synthesis. These donors are replenished as a result of condensation of two molecules of glycine to produce serine plus ammonia; the latter is thought to be reassimilated to give glutamate through the glutamine synthetase/glutamate synthase cycle. This is consistent with the presence of serine-glyoxylate and glutamate-glyoxylate aminotransferases in the peroxisome (12). ^{15}N studies have confirmed the participation of glutamate, but have also suggested that alanine may contribute nitrogen to glycine synthesis (2).

Pea shoots receive much of their nitrogen as asparagine, entering in the transpiration stream. As leaves expand, the metabolism of asparagine changes from predominantly deamidation to transamination (3, 10); in older leaves a substantial proportion of the incoming asparagine is reexported to the apex (13). The enzyme responsible for asparagine transamination is located in the peroxisomes (5) and is identical with the serine:glyoxylate aminotransferase (4). It is therefore of interest to investigate any connection between metabolism of asparagine and photorespiratory processes. This report describes labeling studies with [^{15}N-amino] asparagine supplied to mature pea leaves under conditions of normal and decreased photorespiration. The results show a flow of asparagine nitrogen into the photorespiratory pathway, and indicate that photorespiratory nitrogen metabolism cannot be regarded as a closed cycle.

MATERIALS AND METHODS

Pea plants (*Pisum sativum*, cv Little Marvel) were grown without nodulation in nutrient solution containing nitrate, with a 12-h photoperiod (1). At about 3 weeks, 5th leaves which had reached full expansion (stage 7; ref. 13) were detached, supplied through the petiole with inhibitor or water for 30 min, then transferred to 5 mM [^{15}N-amino]asparagine, with or without inhibitor. Asparagine solutions were passed through Dowex (acetate) at pH 6.5 to remove any aspartate.

For $^{14}CO_2$ feeding, detached leaves were transferred after 30-min pretreatment to a small Plexiglas chamber under normal growth chamber light conditions. $^{14}CO_2$ was released from $Na_2{}^{14}CO_3$ by addition of 0.5 N HCl producing a final concentration of approximately 320 μl/L, (3.7 mCi/mmol). To examine the effect of low O_2, intact plants were transferred, at the beginning of the light period, to a Plexiglas box which was continuously flushed with a mixture containing 1.8% O_2, 550 μl/L CO_2 in N_2. After 6 h, leaves were detached and maintained in this atmosphere during subsequent feeding.

After treatment, leaf samples were frozen in liquid N_2, and extracted with cold 80% ethanol. Separation of nitrogenous components and estimation of ^{15}N by emission spectrometry was as described (11).

After $^{14}CO_2$ feeding, aliquots of tissue extract were also separated into basic (retained by Dowex 50) and acidic (retained by Dowex 1) fractions, together with an unabsorbed neutral fraction. Acidic fractions were further separated by differential elution according to the procedure of Zelitch (15). Amino acids were separated by amino acid analysis (Beckman 119BL), using a reduced flow of ninhydrin reagent, and peaks were collected and counted by liquid scintillation.

[^{15}N-amino]asparagine (95% atom excess) was obtained from Merck, Sharpe, and Dohm (Montreal). HPMS[3] was from Fluka Chemicals, Hauppauga, NY; other reagents were from Sigma.

RESULTS AND DISCUSSION

Changes in amino acid pool sizes, and flow of ^{15}N from the amino group of asparagine, were monitored under various con-

[1] Supported by a grant (to K.W.J.) from Natural Sciences and Engineering Research Council (of Canada), Canada.

[2] Present Address: Forage Crops Section, Canadian Department of Agriculture, Central Experimental Farm, Ottawa, Canada K1A OC6.

[3] Abbreviations; HPMS, α-hydroxy-2-pyridinemethanesulfonic acid; INH, isonicotinyl hydrazide.

ditions known to modify photorespiratory metabolism. In addition to the use of a low O_2 concentration, two inhibitors in particular were studied. HPMS inhibits glycolate oxidase, preventing flow of photorespiratory carbon through glyoxylate (14); ^{14}C labeling (discussed below) indicated that the inhibitor was active in the pea leaf tissue, although unexpected perturbations in some amino acid pools suggested that this inhibitor may have additional effects. INH inhibits glycine to serine conversion (8); under the conditions used here, it appeared to give about 65% inhibition of this reaction, as estimated by the effect on the formation of serine from [^{14}C]glycine supplied to the detached leaves after treatment with inhibitor (data not shown). The same concentration had very little effect (6% inhibition or less) on serine-glyoxylate or glutamate-glyoxylate transamination in extracts from the same leaves.

^{14}C-Labeling and Effect of Inhibitors. To confirm the effect of inhibitors on photorespiratory metabolism, their effect on flow of carbon (from $^{14}CO_2$) was examined under the same conditions that were used for ^{15}N studies (Table I). After 1 h, label was widely distributed; in the amino acid pool, labeling was particularly high in serine which together with alanine had the highest specific activity of those amino acids measured. HPMS decreased the overall fixation, and caused considerable changes in the distribution of label. The organic pool received increased label, which was found particularly in glycolate. Labeling of most amino acids was decreased, especially that of serine; labeling of alanine was increased, however, as was its overall pool size (see below). The effect of INH was principally to give an increase in labeling of glycine and decrease in serine. Labeling was also reduced in glutamine and glutamate. The results are consistent with the postulated action of the inhibitors outlined above (although the effects are not entirely specific), and also confirm a considerable flux of photosynthetically fixed carbon through photorespiratory compounds.

Amino Acid Pool Sizes. As reported previously (11), supply of asparagine at a concentration similar to that normally found in xylem sap had little effect on amino acid pool sizes. In the mature leaves used in this study, amino acid levels after a 1 h supply of 5 mM asparagine were slightly higher (by up to 12–17%) than at the time of detachment (results not shown). Table II shows the effects of inhibitors and low O_2 treatment on the major amino acid pools. HPMS caused considerable fluctuations. Pool sizes of glycine and serine were decreased, as would be expected if supply of glyoxylate was interrupted, but there was also a decrease in level of glutamate and increase of glutamine, suggesting an additional effect on glutamate synthase. Alanine and aspartate levels were also altered and the changes reflected the changes in labeling by CO_2 described above. In contrast, the changes caused by INH were much less widespread, showing an increase in glycine and decrease of serine, as expected. In low O_2, pool sizes of glycine, serine, ammonia, and glutamine were all decreased, as would occur if photorespiratory cycling was reduced. HPMS and INH still produced changes when supplied with low O_2, although the effects were greatly reduced.

Transfer of ^{15}N from the Amino Group of Asparagine. ^{15}N was widely distributed after supply of [^{15}N-amino]asparagine to pea leaves, and was recovered in aspartate, glutamate, alanine, serine, glycine, glutamine (amide group), and ammonia (Table III). The changes in the latter four compounds, caused by inhibitors and low O_2, suggest a flow of amino nitrogen from asparagine into the nitrogenous components of the photorespiratory pathway. Labeling of all four compounds was substantially decreased by

Table I. *Incorporation of Label into Soluble Components of Detached Mature Pea Leaves Supplied for 60 Minutes with $^{14}CO_2$*

Detached leaves were pretreated for 30 min with water (control), 10 mM HPMS, or 10 mM INH, and supply of inhibitor continued during labeling. Labeling was expressed as percentage of total counts recovered in soluble fraction.

Soluble Components	Labeling in Solubles at Following CO_2 Fixation Rates (cpm × 10^{-6})		
	Control (9.78)	+HPMS (6.09)	+INH (10.46)
	%		
Phosphoglycolate	1.9	0.3	0.3
Glycolate	1.9	11.2	1.7
Malate	1.9	1.9	1.3
Other organic acids	8.6	20.2	9.9
Asp	1.2	0.5	0.5
Glu	3.0	2.1	0.3
Asn	0.6	0.8	0.4
Ala	5.7	13.1	7.7
Gly	1.0	0.5	3.9
Ser	8.3	4.7	4.9
Gln	2.9	2.9	0.8
Other amino acids	6.6	5.0	8.8
Neutral compounds	58.0	36.9	59.3

Table II. *Pool Sizes of Amino Acids in Mature Pea Leaves, and Effects of Inhibitors and Low Oxygen*

Detached, recently matured leaves were supplied with 5 mM asparagine alone (control), or together with 10 mM HPMS or 10 mM INH for 1 h, following a 30-min pretreatment with water or inhibitor only. For low O_2, leaf feeding was carried out under 1.8% O_2, 550 μl/L CO_2, and parent plants were pretreated for 6 h prior to leaf removal.

	Air			Low O_2		
	Control	+HPMS	+INH	Control	+HPMS	+INH
	μmol/g fresh wt					
Asp	2.55	0.92 (36)[a]	2.49 (97)	2.20 (86)	0.91	2.16
Glu	6.14	1.73 (28)	6.15 (100)	6.33 (103)	6.14	6.32
Asn	7.18	8.25 (115)	7.33 (102)	8.22 (114)	8.65	8.91
Ala	1.14	5.47 (477)	1.28 (112)	1.46 (127)	4.43	1.55
Gly	0.68	0.25 (37)	1.19 (175)	0.31 (46)	0.27	0.34
Ser	1.60	1.13 (71)	0.94 (59)	0.81 (51)	0.68	0.64
Gln	3.03	6.38 (211)	2.77 (92)	1.27 (42)	1.47	1.25
NH_3	1.16	0.85 (73)	0.81 (70)	0.65 (56)	0.65	0.61

[a] Values in parentheses are percentages of Asn only (air) control value.

Table III. *Flow of ^{15}N from [^{15}N-amino]Asparagine to Amino Acids in Mature Pea Leaves, and Changes Caused by Inhibitors and Low O_2*

Labeled asparagine was supplied for 60 min, under conditions as described in Table II. Actual ^{15}N contents of amino acids (ng ^{15}N/g fresh wt) were calculated from atom % excess values.

	^{15}N Content					
	Air			Low O_2		
	Control	+HPMS	+INH	Control	+HPMS	+INH
	ng/g fresh wt					
Asp	859	256 (30)[a]	802 (93)	611 (71)	254	606
Glu	1246	383 (31)	1189 (95)	1117 (90)	868	1123
Ala	234	443 (189)	174 (75)	267 (114)	374	211
Gly	78	12 (15)	272 (348)	28 (36)	10	41
Ser	225	63 (28)	76 (34)	81 (36)	37	54
Gln(amide)	84	35 (42)	36 (43)	34 (40)	10	21
NH_3	61	14 (23)	20 (33)	27 (45)	7	15

[a] Values in parentheses are percentages of Asn only (air) control value.

HPMS and low O_2. With INH, the amount of ^{15}N increased over 3-fold in glycine and decreased in serine, ammonia, and glutamine, normally derived from glycine. These changes were parallel to those observed for total pool sizes, but the changes in labeling were generally more pronounced than in pool size. For example, with INH, the pool size of glycine increased to 175% of the control, while serine decreased to 59%; the corresponding changes in ^{15}N content were 348 and 34%, respectively. This indicates that asparagine contributes nitrogen more specifically to the photorespiratory pool than to the nonphotorespiratory pool of glycine. The behavior of glutamine is also interesting, showing little change in pool size with INH and an actual increase with HPMS, yet the labeling is decreased to about 40% with both inhibitors.

The effect of inhibitors supplied under low O_2 conditions was also more pronounced for labeling results, relative to changes in pool sizes. It is clear that even below 2% O_2 and with somewhat elevated CO_2 levels, some flow through photorespiratory pathways continues. When similar ^{15}N experiments were performed with shorter (30 min) feeding times, or with low O_2 conditions imposed at the start of feeding, the trends were similar to all those noted above, although the changes were somewhat less pronounced (results not shown).

The results indicate that asparagine amino nitrogen enters the photorespiratory cycle, presumably through the transamination of glyoxylate in the peroxisome, where an asparagine aminotransferase has been localized (5). This appears to be an activity of the serine-glyoxylate aminotransferase, which shows higher activity with serine than with asparagine at saturating concentrations of these substrates. However, higher concentrations of asparagine, relative to serine, will favor the participation of asparagine as an amino donor. In recently matured leaves (Table II), the asparagine concentration may be about 5-fold higher than serine; in younger leaves, this excess can be 20-fold or greater (11).

An additional source of amino nitrogen in photorespiration is thought to be glutamate, and one interpretation of the labeling data above might be that asparagine nitrogen enters the photorespiratory path by way of glutamate, which is quite heavily labeled. This labeling of glutamate is derived by transamination of the aspartate which in turn results from deamidation of asparagine (10). When deamidation of [^{15}N-amino]asparagine was inhibited with 5-diazo-4-oxo-L-norvaline, labeling of aspartate and glutamate was inhibited by over 70%, while labeling of glycine and serine was affected less than any of the amino acids, with an inhibition less than 30% (10). Also, when [^{15}N]glutamate was supplied to detached pea leaves, the total ^{15}N (and atom % of excess) in the glutamate pool was over 3-fold higher than when asparagine was the source of label, yet the ^{15}N content (and atom % excess) of glycine was lower than with labeled asparagine (Ta and Joy, unpublished observations). Thus, the transfer via aspartate and glutamate represents only a minor route for the movement of amino nitrogen from asparagine to glycine.

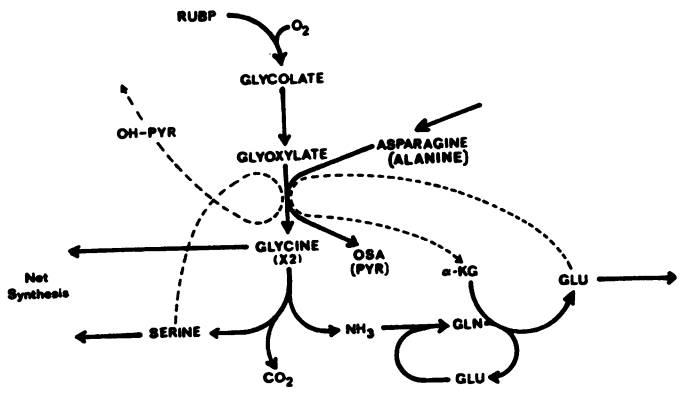

FIG. 1. Modified scheme for photorespiratory nitrogen metabolism, indicating the potential for amino acid synthesis. (OSA), 2-Oxo-succinamic acid; (PYR), pyruvate; (RUBP), ribulose 1,5-bisphosphate.

CONCLUSIONS

The results suggest that the view of photorespiratory nitrogen flow, as a closed cyclic process, may not be accurate under all conditions, and must be broadened to allow an exchange of nitrogen into and out of the cycle. It is clear that constituents of the pathway can be utilized in other reactions since the levels of nitrogenous components run down when supply of glyoxylate is curtailed by HPMS or low O_2 (Table II). The work of Betsche (2) indicates that nitrogen from alanine may enter the pathway. The data presented here show that there is also a contribution from asparagine, which in many plants is a major transport compound present in high concentrations. Entry of external nitrogen to photorespiratory processes will balance any removal of glycine and/or serine (Fig. 1).

The present data do not allow an accurate calculation of the extent of asparagine contribution to the photorespiratory pathway. Values given in Table III underestimate the flow, since they represent actual ^{15}N (*i.e.* atom % of 100), whereas the precursor asparagine was considerably more dilute. Minimum values can be calculated from the atom % excess value for glycine, compared to that of asparagine recovered from the same treatment. For example, the value for glycine (control treatment; table III) was 0.82 atom % excess, while that of the amino group of asparagine was 16.35% (data not shown), thus asparagine supplied at least 5.02% of the nitrogen in glycine. A similar calculation for young leaves (data from Ref. 10) gives a minimum value of 8%. These are minimum values, since the atom % content is averaged over the whole glycine pool, while it appears that asparagine contributes much more to the photorespiratory glycine than to the nonphotorespiratory glycine pool. Changes, caused by inhibitor treatment, in labeling of glycine and serine are much more pronounced than the changes in total pool size. For glycine, treatment with INH increased the total content by 0.51 μmol (Table II), while ^{15}N content increased by 194 ng (Table III). Thus, it can be calculated that the atom % value for the additional accumulated glycine is 2.72% (194/510 × 100/14). Asparagine in the INH-treated leaves had 16.4 atom % excess, and therefore contributed 16.5% of the nitrogen in the accumulating glycine, and this may indicate more closely the value for the photorespiratory glycine. Although approximations, these values indicate that the contribution of asparagine to the photorespiratory pathway in pea leaves is not insignificant. One factor that is likely to regulate this contribution is the relative concentrations of asparagine and serine, which will compete for the same aminotransferase site. The concentration of asparagine is highest in young leaves and decreases in older leaves. Thus, in the growing leaves, the high availability of asparagine nitrogen for entry into photorespiration will allow a net synthesis of glycine and serine, which would be withdrawn for synthetic reactions in the cell. It is also possible that asparagine could contribute to serine synthesis by transamination of hydroxy-pyruvate (7). Glycine and serine make up about 20% of the residues in pea leaf protein, and serine is also required in cysteine synthesis. In this way, the photorespiratory pathway may have a useful synthetic function in growing leaves, allowing a direct utilization of imported asparagine in amino acid synthesis.

LITERATURE CITED

1. BAUER A, AA URQUHART, KW JOY 1977 Amino acid metabolism of pea leaves: diurnal changes and amino acid synthesis from ^{15}N-nitrate. Plant Physiol 59: 915–919
2. BETSCHE T 1983 Aminotransfer from alanine and glutamate to glycine and

serine during photorespiration in oat leaves. Plant Physiol 71: 961–965
3. IRELAND RJ, KW JOY 1981 Two routes of asparagine metabolism in *Pisum sativum* L. Planta 15: 289–292
4. IRELAND RJ, KW JOY 1983 Purification and properties of an asparagine aminotransferase from *Pisum sativum* leaves. Arch Biochem Biophys 223: 291–296
5. IRELAND RJ, KW JOY 1983 The subcellular localization of asparaginase and asparagine aminotransferase in *Pisum sativum* leaves. Plant Physiol 72: 1127–1129
6. KEYS AJ, IF BIRD, MJ CORNELIUS, PJ LEA, RM WALLSGROVE, BJ MIFLIN 1978 The photorespiratory nitrogen cycle. Nature 275: 741–743
7. LIANG Z, C YU, AHC HUANG 1984 Conversion of glycerate to serine in intact spinach leaf peroxisomes. Arch Biochem Biophys 233: 393–401
8. MIFLIN BJ, AFH MARKER, CP WHITTINGHAM 1966 The metabolism of glycine and glycollate by pea leaves in relation to photosynthesis. Biochim Biophys Acta 120: 266–273
9. OGREN WL 1984 Photorespiration: pathways, regulation and modification. Annu Rev Plant Physiol 35: 415–442
10. TA TC, KW JOY 1985 Transamination, deamidation and the utilisation of asparagine amino-nitrogen in pea leaves. Can J Bot. In press
11. TA TC, KW JOY, RJ IRELAND 1984 Amino acid metabolism in pea leaves. Utilization of nitrogen from amide and amino groups of [^{15}N]asparagine. Plant Physiol 74: 822–826
12. TOLBERT NE 1981 Metabolic pathways in peroxisomes and glyoxysomes. Annu Rev Biochem 50: 133–157
13. URQUHART AA, KW JOY 1982 Transport, metabolism, and redistribution of xylem-borne amino acids in developing pea shoots. Plant Physiol 69: 1226–1232
14. ZELITCH I 1959 The relationship of glycolic acid to respiration and photosynthesis in tobacco leaves. J Biol Chem 234: 3077–3081
15. ZELITCH I 1965 The relation of glycolic acid synthesis to the primary photosynthetic carboxylation reaction in leaves. J Biol Chem 240: 1869–1875