Plant Physiol. (1989) 89, 192–196
0032-0889/89/89/0192/05/$01.00/0

Received for publication May 11, 1988
and in revised form August 15, 1988

Effect of Methionine Sulfoximine on Asparaginase Activity and Ammonium Levels in Pea Leaves[1]

Konrad A. Sieciechowicz,[2] Kenneth W. Joy*, and Robert J. Ireland

Department of Biology, Carleton University, Ottawa, Ontario K1S 5B6, Canada (K.A.S., K.W.J.); *and Department of Biology, Mount Allison University, Sackville, New Brunswick E0A 3C0, Canada* (R.J.I.)

ABSTRACT

In developing leaves of *Pisum sativum* the levels of ammonium did not change during the light-dark photoperiod even though asparaginase (EC 3.5.1.1) did; asparaginase activity in detached leaves doubled during the first 2.5 hours in the light. When these leaves were supplied with 1 millimolar methionine sulfoximine (MSX, an inhibitor of glutamine synthetase, GS, activity) at the beginning of the photoperiod, levels of ammonium increased 8- to 10-fold, GS activity was inhibited 95%, and the light-stimulated increase in asparaginase activity was completely prevented, and declined to less than initial levels. When high concentrations of ammonium were supplied to leaves, the light-stimulated increase of asparaginase was partially prevented. However, it was also possible to prevent asparaginase increase, in the absence of ammonium accumulation, by the addition of MSX together with aminooxyacetate (AOA, which inhibits transamination and some other reactions of photorespiratory nitrogen cycling). AOA alone did not prevent light-stimulated asparaginase increase; neither MSX, AOA, or elevated ammonium levels inhibited the activity of asparaginase *in vitro*. These results suggest that the effect of MSX on asparaginase increase is not due solely to interference with photorespiratory cycling (since AOA also prevents cycling, but has no effect alone), nor to the production of high ammonium concentration or its subsequent effect on photosynthetic mechanisms. MSX must have further inhibitory effects on metabolism. It is concluded that accumulation of ammonium in the presence of MSX may underestimate rates of ammonium turnover, since liberation of ammonium from systems such as asparaginase is reduced by the effects of MSX.

The degradation of asparagine is important in supplying nitrogen for amino acid and protein synthesis in developing leaves, and ^{15}N-labeling studies have indicated that up to 75% of the nitrogen required for growth in half-expanded pea leaves was derived from the amide-nitrogen of asparagine (25). The amide-nitrogen of asparagine can be liberated directly by asparaginase (EC 3.5.1.1.) (8), producing aspartate and ammonium, or by deamidation of products (2-oxosuccinamic acid [14] or hydroxysuccinamic [25, 26]) resulting from asparagine transamination (asparagine: pyruvate transaminase, EC 2.6.1.14). In developing pea leaves, asparaginase activity undergoes diurnal variation, increasing in the light (20), a process dependent upon photosynthetic electron transport (21), and, asparaginase activity is also regulated by other factors (22). Increased asparaginase activity should result in increased production of aspartate and ammonium, which are quickly metabolized (25).

The oxidation of glycine during photorespiration produces appreciable amounts of ammonium in the light, and estimates based on treatments with MSX[3] (an irreversible inhibitor of GS [24]), have suggested that release of photorespiratory ammonium exceeds by 10-fold or more the rate of production from nitrate reduction (10). To demonstrate that nitrogen enters the photorespiratory pathway through the reaction catalyzed by serine-glyoxylate transaminase, AOA (an inhibitor of transamination reactions and glycine decarboxylation [5]) has been used in conjunction with MSX, since when supplied together, ammonium production resulting from photorespiration was assumed to be abolished (13, 29). Similar results were obtained in the presence of MSX together with other inhibitors of glycine decarboxylation, isonicotinyl hydrazide (12, 13, 27) or aminoacetonitrile (1), and these results supported the idea that most, if not all, of the ammonium detected *in vivo* was derived from photorespiration.

However, photorespiratory ammonium production is not the only source of ammonium detected within leaf tissues in the presence of MSX. Increased levels of ^{15}N[ammonium] in the presence of MSX and 2% O_2 were observed after the addition of [^{15}N]KNO_3 indicating active nitrate and nitrite reductase activities (6). Furthermore, several reports have demonstrated that protein degradation leads to significant levels of ammonium production in the presence of MSX (4, 18), and similar levels of ammonium in the presence of MSX have been detected under conditions known either to support (21% O_2) or to limit (2% O_2,) photorespiration (17).

One alternate source of ammonium could be release through asparaginase activity. We therefore attempted to measure changes in the rate of ammonium production before and after a light-stimulated increase in asparaginase. MSX was supplied to arrest the assimilation of ammonium, and (in an attempt to reduce the background from photorespiratory ammonium) the effect of AOA was also studied. In the presence of MSX, the expected increase in asparaginase was not found, and it was concluded that MSX has unexplained inhibitory effects, and that measurements in the presence of MSX may underestimate the rate of ammonium production in a tissue.

[1] Supported by grants to K. W. J. and R. J. I. from Natural Sciences and Engineering Research Council of Canada.

[2] Current address: Department of Biological Sciences, Simon Fraser University, Burnaby, B. C. Canada, V6A 1S6.

[3] Abbreviations: AOA, aminooxyacetate; GDH, glutamate dehydrogenase; GOGAT, glutamate synthase; GS, glutamine synthetase; MSX, methionine sulfoximine.

MATERIALS AND METHODS

Nonnodulated *Pisum sativum* L. (cv Little Marvel No. 1; McKenzie Steele Briggs, Brandon, Manitoba, Canada) plants were grown with 16 h of light and 8 h of dark (18°C), as reported earlier (21). Half-expanded fifth leaves were used for all enzyme extractions and feeding studies (18–20 d after seed imbibition).

All biochemicals were obtained from Sigma Chemical Co., (St. Louis, MO.).

Enzyme Assays

Asparaginase and glutamine synthetase (biosynthetic assay) activities were assayed as reported earlier (20), except that 1 mM $CaCl_2$ and 10% glycerol were added to the asparaginase extraction and assay buffers.

The assay for NADH glutamate dehydrogenase was adapted from Pahlich and Joy (16). Leaf tissue was extracted and desalted (Sephadex G 25, equilibrated in the same buffer) in 50 mM Tris HCl (pH 7.8) containing 10 mM $MgSO_4$, 1 mM EDTA, and 1 mM DTT. For assaying reductive amination of oxoglutarate, 0.1 mL aliquots of the extract were added to mixtures to give final concentrations of 50 mM Tris HCl, 1 mM $CaCl_2$, 13 mM oxoglutarate, and 60 mM $(NH_4)_2SO_4$. Reactions were started by the addition of 0.3 mM NADH (total assay volume 1.2 mL), and the decreases in A_{340} were followed over a 5 min period at 25°C. Controls, without enzyme or substrates, were also run with both enzyme assays.

Analysis of Ammonium

The levels of ammonium in leaf extracts were analyzed by either microdiffusion followed with nesslerization (19), or with a Beckman 119BL amino acid analyzer (physiological mode).

Feeding Experiments

Just before the end of the dark period, or after 14 h of the light period, whole shoots were excized above the cotyledon and quickly transferred to distilled water. Petioles of half-expanded leaves were then cut under water just above the stipules and placed individually in small tubes containing 0.3 mL of distilled water or 8.0 mM asparagine, with 1 mM MSX and/or 4 mM AOA, or 10 to 100 mM NH_4Cl (all at pH 6.3).

Ten replicates were used per treatment. Control samples were examined for ammonium levels, and/or asparaginase and glutamine synthetase activities at the start of the feeding experiment, and the remaining leaves were placed in growth cabinets and illuminated with a combination of incandescent and fluorescent sources (285 μmol m^{-2} sec^{-1} PAR, see above) for 2.5 h.

RESULTS AND DISCUSSION

The deamidation of asparagine in developing pea leaves is catalyzed by asparaginase, in a reaction producing aspartate and ammonium. Other processes are also known to liberate ammonium in leaf tissues: the oxidation of glycine results in photorespiratory ammonium release; nitrate reductase and NiR activities reduce inorganic nitrogen to the level of ammonium; phenylalanine and tyrosine ammonia lyases, threonine dehydratase, other deamidases (*e.g.* glutaminase, and ω-amidase), and urease produce ammonium; ammonium is also liberated during protein turnover.

Several of the above processes produce more ammonium during the light (*e.g.* asparaginase, photorespiration, phenylalanine ammonia lyase, nitrate reductase, nitrite reductase) than in the dark, yet when the levels of ammonium in half-expanded pea leaves were monitored over a 24 h photoperiod, they remained low (0.8–1.5 μmol NH_3 g fresh weight^{-1}), with only a slight increase in the light (Fig. 1). The importance of GS-GOGAT in the assimilation for ammonium into organic form in plant tissues has received much support over the past few years, and it has been suggested that preference of this pathway over the assimilation of ammonium by GDH depends on the relative affinities of GS and GDH for ammonium (24). The levels of GS and GDH were followed throughout the photoperiod. The activity of GS remained high, although it displayed an ultradian rhythm, similar to that found in sunflower roots (11), while GDH activity decreased to quite low levels in the light (Fig. 1) as found in some other tissues (23). These results support the role of GS as the primary scavenger of cellular ammonium, and suggest that GS has excess capacity for the maintenance of low ammonium levels within the cell.

Concentrations of MSX from 1 to 10 mM have been used to demonstrate the involvement of GS in the assimilation of ammonium derived from photorespiration in C_3 and C_4 leaf tissues (1, 10, 13), and 1 to 4 mM AOA (in the presence of MSX) to indicate that transamination is important in introducing nitrogen into the photorespiratory pathway (13, 29). Similar concentrations of MSX and AOA have been used to

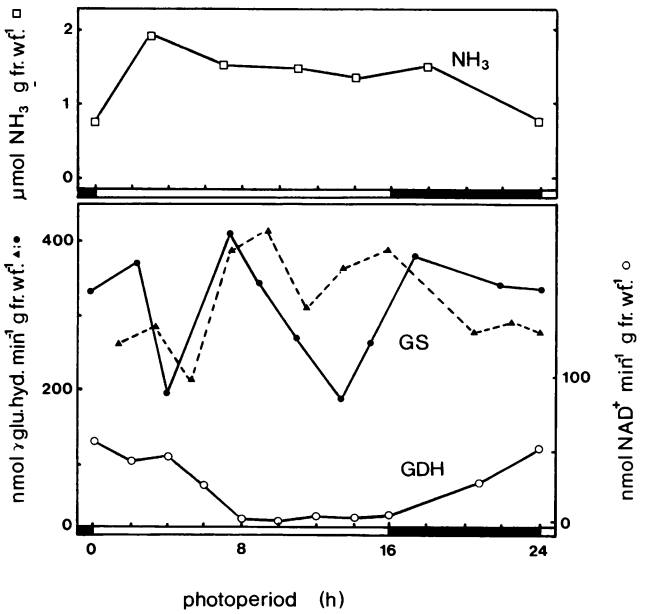

Figure 1. Variations in glutamine synthetase and glutamate dehydrogenase activities, and ammonium levels in pea leaves. Half-expanded leaves were obtained at the indicated times throughout the photoperiod and assayed for ammonium levels (□), and glutamine synthetase (●, ▲ from two separate experiments) and glutamate dehydrogenase activities (○).

indicate that transamination precedes deamidation in the liberation of ammonium from asparagine in pea leaves (8, 25).

Since asparaginase activity increased substantially in extracts of light-harvested leaves (20, 21), we wished to determine whether this resulted in a more rapid rate of deamidation, *in vivo*, of asparagine. Half-expanded leaves were fed with water or asparagine solutions containing MSX to inhibit GS activity and thus prevent the assimilation of asparaginase-derived ammonium. Other treatments included feeding AOA (with or without MSX) to reduce the production of photorespiratory ammonium.

Patterns of ammonium accumulation observed in MSX, AOA, or MSX+AOA feeding treatments were similar for leaves treated with or without addition of asparagine, although levels were higher in the presence of asparagine (Table I). Half-expanded leaves obtained at the beginning or end of the light period displayed increased ammonium levels when supplied with MSX and this result is consistent with the role of glutamine synthetase in the assimilation of ammonium in leaf tissues. The addition of AOA alone to feeding solutions did not greatly alter the levels of ammonium within leaves, yet when AOA was added together with MSX, the high values of ammonium observed in the presence of MSX were reduced and were only slightly higher than those of the control treatments (Table I). These results suggest that AOA-sensitive reactions (presumably transamination, involved in photorespiration) are responsible for production of most, if not all, of the ammonium which accumulates in the leaf when GS is inhibited. Yamaya and Matsumoto (29) and Martin *et al.* (13) have also observed similar trends in the levels of ammonium in the presence of MSX and AOA.

Since asparaginase levels are three- to fivefold higher after 14 h of light (21), much higher ammonium levels were expected in the leaves fed MSX or MSX+AOA at the end of the light period compared with those fed at the end of the dark. This was not observed (Table I) and suggested that either the production of ammonium by asparaginase is insignificant in comparison with the release from photorespiration, or that the inhibitor used interfered with asparaginase activity. Since increased asparaginase activity in the light requires photosynthetic electron transport (21), and MSX has been reported to inhibit photosynthesis (see below), the effect of MSX on asparaginase activity was investigated.

Neither MSX nor AOA had any direct inhibitory effect on asparaginase activity *in vitro* (Table II). The *in vivo* effect was investigated by supplying asparagine together with a range of MSX concentrations to detached half-expanded leaves for 2.5 h in light followed by the assay of GS and asparaginase activities, and ammonium levels. As expected, GS activity was reduced by over 90% and an eight- to ninefold increase in the levels of ammonium was also observed (Fig. 2a), confirming that GS was inhibited in the presence of MSX. Over the 2.5 h feeding period in the light, increased asparaginase activity (twofold) was observed in the leaves supplied with asparagine, but asparaginase activity in extracts obtained from the MSX treated leaves decreased below that of the 0 h control (Fig. 2a). The loss of asparaginase activity in detached leaves supplied with MSX was similar to the decrease in activity noted when detached leaves were fed asparagine alone in the dark for 2.5 h (21). Since MSX had no effect on asparaginase *in vitro*, and asparaginase is affected by photosynthetic electron transport (21, 22), decreased asparaginase levels in the light during MSX treatment may be a result of secondary effects of MSX on photosynthetic activity.

Increased ammonium levels caused by the presence of MSX could account for prevention of the increase in asparaginase activity, although asparaginase activity *in vitro* was not inhibited by the addition of high (up to 0.1 M) concentrations of NH_4Cl (Table II). When detached, half-expanded leaves were supplied with ammonium the increase in asparaginase was unaffected at low ammonium concentrations, with some progressive effect noted at higher concentrations (Fig. 2b). However, the increase in asparaginase was completely abolished only with the supply of 50 mM ammonium, producing internal concentrations much higher than that caused by MSX

Table I. Effects of MSX and AOA on Ammonium Accumulation in Half-Expanded Pea Leaves

Leaves were detached at end of dark (0 h) and light periods (14 h) and supplied with water or asparagine (8 mM) for 2.5 h in the light, after which the levels of ammonium were determined. Concentrations of MSX and AOA were 1 and 4 mM, respectively ($n = 4$).

Treatment	0 h ± SD	14 h ± SD
	μmol NH_3·g fresh wt^{-1}	
H_2O	1.61 ± 0.2	1.29 ± 0.3
+ MSX	8.44 ± 0.8	8.60 ± 1.0
+ AOA	1.04 ± 0.2	0.91 ± 0.1
+ MSX + AOA	1.78 ± 0.4	1.98 ± 0.2
Asn	0.97 ± 0.3	0.83 ± 0.2
+ MSX	14.09 ± 0.7	10.70 ± 0.3
+ AOA	1.33 ± 0.3	0.75 ± 0.5
+ MSX + AOA	1.76 ± 0.3	1.60 ± 0.2

Table II. Effects of MSX, AOA, and Ammonium on in Vitro and in Vivo Asparaginase Activity

Asparaginase activity in extracts obtained from half-expanded leaves was assayed in the presence of asparagine (20 mM), MSX, AOA, or ammonium for 30 min (*in vitro* activity). Asparaginase activity was also estimated in half-expanded leaves that were detached at the end of the dark period (0 h) and fed asparagine (8 mM), asparagine + MSX, or asparagine + AOA for 2.5 h in the light (*in vivo* activity). Where standard deviations are presented $n = 3$, otherwise the experiments were repeated with similar results.

Treatment		*In Vitro* Activity	*In Vivo* Activity
		% control activity	*% 2.5 h activity* ± SD
Control (no additions)		100	100
0 h			49 ± 5
+ MSX	0.5 mM	102	26
	1.0	96	23 ± 4
	5.0	98	21
+ AOA	1.0 mM	101	98
	4.0	99	85 ± 5
+ MSX + AOA[a]		100	30 ± 7
+ NH_3	0.01 M	102	99
	0.1	100	48

[a] MSX and AOA concentrations were 1.0 and 4.0 mM, respectively.

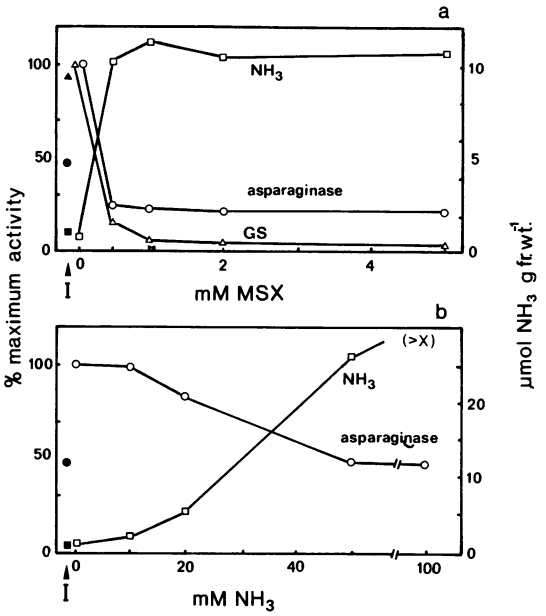

Figure 2. a, Responses of glutamine synthetase and asparaginase activities, as well as the levels of ammonium in pea leaves fed MSX. Half-expanded leaves were detached at the end of the dark period and fed asparagine (8 mM), or asparagine with varying concentrations of MSX, and placed into the light for 2.5 h. Activities of glutamine synthetase (▲, △) asparaginase (●, ○), and levels of ammonium (■, □) were determined before (closed symbols, I) and after the 2.5 h feeding (open symbols). b, Response of asparaginase activity and ammonium levels in half-expanded leaves supplied ammonium. Half-expanded leaves were detached at the end of the dark period and supplied asparagine (8 mM) together with increasing concentrations of ammonium for 2.5 h in the light. Asparaginase activity (●, ○) and ammonium levels (■, □, where '×' is maximum detectable limit for assay conditions) were determined before (initial values closed symbols,) and after the 2.5 h feeding (open symbols). Enzyme activities are expressed as a percent of values obtained in the control treatments. The experiments were repeated with similar results.

treatment (Fig. 2a). Even at these high concentrations, asparaginase did not decrease below the initial level, in contrast to the decrease seen in the presence of MSX (Fig. 2a), and this may reflect localized ammonium production during MSX feedings. A discrepancy between effects of ammonium resulting from MSX inhibition and from external supply has been noted elsewhere (7, 9, 27, 28, see below).

Initially then, it seemed that the effect of MSX on asparaginase might be due to the accumulation of ammonium, possibly through an effect on photosynthetic electron transport. It has been suggested that the effect of MSX on CO_2 fixation results from uncoupling of electron transport by increased ammonium (1, 15, 17). Since directly supplied ammonium has a lesser effect on photosynthesis (7, 27), an alternate mechanism explaining the effect of MSX inhibition might be the reduction of recycling of carbon skeletons from photorespiration to the Calvin cycle (2, 7, 27). Reduced rates of photosynthesis (measured as net CO_2 fixation) have also been observed in the presence of aminoacetonitrile (3), and AOA (9), supporting the idea that reduced carbon-cycling inhibits photosynthesis. Decreased rates of net CO_2 fixation are not a result of stomatal closure in the presence of MSX (17, 27), nor does MSX inhibit photosynthesis in isolated chloroplasts (15). However, other mechanisms for the inhibition of photosynthesis by MSX may exist.

AOA reduces the flux of photorespiratory intermediates (9), yet in the presence of AOA the increase of asparaginase was only slightly less than in the control (Table II). Furthermore, in the presence of AOA and MSX together, ammonium levels were not elevated (Table I), yet the light-stimulated increase of asparaginase was prevented (Table II). This suggests that neither inhibition of carbon recycling, nor the increased ammonium concentration are the principle cause of the MSX effect on asparaginase increase, and thus MSX must be suspected of an additional, unidentified inhibitory effect on metabolism.

Results obtained by using MSX + AOA (13, 29), MSX + isonicotinyl hydrazide (12, 13, 27), or MSX + aminoacetonitrile (1) have been presented as support for the idea that the major source of ammonium in leaves (an order of magnitude higher than primary assimilation [10]) is from photorespiration. Similar conclusions have also been reported from studies using only MSX (2, 7, 10, 17). However, decrease of asparaginase activity *in vivo* by MSX suggests that estimates of ammonium levels in leaves fed with MSX are not representative of ammonium production from all sources, although photorespiratory ammonium production appears to be unaffected by MSX (10; however, see Ref. 27). If there is a similar effect on other light-requiring ammonia-liberating enzymes, the discrepancy could even be greater.

The addition of MSX and AOA have also been used to demonstrate that flow of nitrogen from asparagine in half-expanded pea leaves is through the deamidation of 2-oxosuccinamic acid, the transamination product of asparagine, or its subsequent reduction product, hydroxysuccinamic acid, rather than through the deamidation of asparagine (8, 25, 26). In view of the decrease in asparaginase activity during MSX feeding experiments, these results may underestimate the contribution of asparaginase *in vivo*.

The results lend further support to the observations of others on the secondary effects of MSX on cellular metabolism (1, 7, 9, 17, 27), and indicate that MSX is not a reliable inhibitor for the estimation of ammonium production *in vivo* as had been widely assumed.

LITERATURE CITED

1. **Achhireddy NR, Vann DR, Fletcher JS, Beevers L** (1983) The influence of methionine sulfoximine on photosynthesis and nitrogen metabolism in excised pepper (*Capsicum annuum* L.) leaves. Plant Science Lett **32:** 73–78
2. **Berger MG, Fock HP** (1983) Effects of methionine sulfoximine and glycine on nitrogen metabolism of maize leaves in the light. Aust J Plant Physiol **10:** 187–194
3. **Creach E, Stewart CR** (1982) Effects of aminoacetonitrile on net photosynthesis, ribulose-1-5-bisphosphate levels, and glycolate pathway intermediates. Plant Physiol **70:** 1444–1448
4. **Cullimore JV, Sims AP** (1980) An association between photorespiration and protein catabolism: studies with *Chlamydomonas*. Planta **150:** 392–396
5. **Dry IB, Wiskich JT** (1986) Comparative aspects of aminooxyacetate inhibition of glycine oxidation and aminotransferase activity of pea leaf mitochondria. Plant Sci. **44:** 23–28
6. **Frantz TA, Peterson DM, Durbin RD** (1982) Sources of ammonium in oat leaves treated with tabtoxin or methionine sulfoximine. Plant Physiol **69:** 345–348

7. **Ikeda M, Ogren WL, Hageman RH** (1984) Effect of methionine sulfoximine on photosynthetic carbon metabolism in wheat leaves. Plant Cell Physiol **25:** 447–452
8. **Ireland RJ, Joy KW** (1981) Two routes for asparagine metabolism in *Pisum sativum* L. Planta **151:** 289–292
9. **Johansson L, Larsson C-M** (1986) Relationship between inhibition of CO_2 fixation and glutamine synthetase inactivation in *Lemna gibba* L. treated with *L*-methionine-*D,L*-sulfoximine (MSO). J Exp Bot **37:** 221–229
10. **Kevs AJ, Bird IF, Cornelius MJ, Lea PJ, Wallsgrove RM, Miflin BJ** (1978) Photorespiratory nitrogen cycle. Nature **275:** 741–743
11. **Knight TJ, Weissman GS** (1982) Rhythms in glutamine synthetase activity, energy charge, and glutamine in sunflower roots. Plant Physiol **70:** 1683–1688
12. **Kumar PA, Nair TVR, Abrol YP** (1984) Effect of photorespiratory metabolites, inhibitors and methionine sulfoximine on the accumulation of ammonia in the leaves of mung bean and *Amaranthus.* Plant Sci Lett **33:** 303–307
13. **Martin F, Winspear MJ, Macfarlane JD, Oaks A** (1983) Effect of methionine sulfoximine on the accumulation of ammonia in C_3 and C_4 leaves. Plant Physiol **71:** 177–181
14. **Meister A** (1953) Preparation and enzymatic reactions of the keto analogues of asparagine and glutamine. J Biol Chem **200:** 571–590
15. **Mutich MJ, Fletcher JS** (1983) Influence of methionine sulfoximine on the photosynthesis of isolated chloroplasts. Photosynth Res **4:** 241–244
16. **Pahlich E, Joy KW** (1970) Glutamate dehydrogenase from pea roots: purification and properties of the enzyme. Can J Biochem **49:** 127–138
17. **Platt SG, Anthon GE** (1981) Ammonia accumulation and inhibition of photosynthesis in methionine sulfoximine treated spinach. Plant Physiol **67:** 509–513
18. **Rhodes D, Deal L, Haworth P, Jamieson GC, Reuter CC, Ericson MC** (1986) Amino acid metabolism of *Lemna minor* L. I. Responses to methionine sulfoximine. Plant Physiol **82:** 1057–1062
19. **Shelp BJ, Sieciechowicz KA, Ireland RJ, Joy KW** (1985) Determination of urea and ammonia in leaf extracts: applications to ureide metabolism. Can J Bot **63:** 1135–1140
20. **Sieciechowicz KA, Ireland RJ, Joy KW** (1985) Diurnal variation of asparaginase activity in developing pea leaves. Plant Physiol **77:** 506–508
21. **Sieciechowicz KA, Joy KW, Ireland RJ** (1988) Diurnal changes in asparaginase activity in pea leaves. I. The requirement for light for increased activity. J Exp Bot **39:** 695–706
22. **Sieciechowicz KA, Ireland RJ, Joy KW** (1988) Diurnal changes in asparaginase activity in pea leaves. II. Regulation of activity. J Exp Bot **39:** 707–721
23. **Srivastava HS, Singh RP** (1987) Role and regulation of *L*-glutamate dehydrogenase activity in higher plants. Phytochemistry **26:** 597–610
24. **Stewart GR, Mann AF, Fentem PA** (1980) Enzymes of glutamate formation: glutamate dehydrogenase, glutamine synthetase, and glutamine synthase. *In* BJ Miflin, ed, The Biochemistry of Plants, Vol 5. Academic Press, New York, pp 271–327
25. **Ta TC, Joy KW, Ireland RJ** (1984) Amino acid metabolism in pea leaves: utilization of nitrogen from amide and amino groups of [^{15}N]asparagine. Plant Physiol **74:** 822–826
26. **Ta TC, Joy KW, Ireland RJ** (1984) Utilization of the amide groups of asparagine and 2-hydroxysuccinamic acid by young pea leaves. Plant Physiol **75:** 527–530
27. **Walker KA, Keys AJ, Givan CV** (1984) Effect of *L*-methionine sulfoximine on the products of photosynthesis in wheat (*Triticum aestivum*) leaves. J Exp Bot **35:** 1800–1810
28. **Walker KA, Givan CV, Keys AJ** (1984) Glutamic acid metabolism and the photorespiratory nitrogen cycle in wheat leaves. Metabolic consequences of elevated ammonia concentrations and of blocking ammonia assimilation. Plant Physiol **75:** 60–66
29. **Yamaya T, Matsumoto H** (1985) Ammonia content in mitochondria isolated from detached pea shoots. Effect of light and inhibitors of photorespiratory nitrogen cycle. Plant Cell Physiol **26:** 1613–1616