Plant Physiol. (1983) 71, 780–784
0032-0889/83/71/0780/05/$00.50/0

Intracellular Localization of Aspartate Kinase and the Enzymes of Threonine and Methionine Biosynthesis in Green Leaves

Received for publication October 5, 1982 and in revised form December 6, 1982

ROGER M. WALLSGROVE, PETER J. LEA, AND BENJAMIN J. MIFLIN
Department of Biochemistry, Rothamsted Experimental Station, Harpenden, Hertfordshire, United Kingdom

ABSTRACT

The intracellular localization of several aspartate pathway enzymes has been studied in pea (*Pisum sativum* cv Feltham First) and barley (*Hordeum vulgare* cv Julia) leaves. Protoplast lysates were fractionated by differential or sucrose density gradient centrifugation, in media optimized for each enzyme. The results show that aspartate kinase, homoserine kinase, threonine synthase, and cystathionine γ-synthase are confined to the chloroplast. Cystathionine β-lyase appears to be present in several fractions, though more than 50% of the total activity is associated with the chloroplasts. In contrast, neither methionine synthase nor methionine adenosyltransferase were significantly associated with chloroplasts, and only a small proportion of the methionine synthase was associated with the mitochondrial fraction. Methionine adenosyltransferase, and hence *S*-adenosylmethionine synthesis, is not found in any organelle fraction. The conclusion is that whereas threonine, like lysine, is synthesized only in the chloroplast, the last step in methionine biosynthesis occurs largely in the cytoplasm.

The nutritionally essential amino acids, lysine, threonine, methionine, and isoleucine, are synthesized from aspartate. Efforts to improve the nutritional quality of cereal (deficient in lysine and threonine) and legume (deficient in methionine) seeds (20) have made it important to understand fully the aspartate pathway and its regulation *in vivo*. Part of this understanding involves the determination of where in plant cells these reactions occur.

Isolated, intact pea chloroplasts synthesize lysine, threonine, isoleucine, and possibly methionine from [^{14}C]aspartate (16), and this light-stimulated synthesis is regulated by exogenous amino acids in a manner consistent with the properties of the isolated enzymes. The first and last enzymes unique to lysine biosynthesis have been found wholly within the chloroplast (26, 28) and other enzymes of the pathway have been demonstrated in chloroplast extracts (see 8 for review). Some enzymes of the pathway have been reported to be outside the chloroplast, at least in part: homoserine dehydrogenase (EC 1.1.1.3) in the cytoplasm (18), methionine synthase (EC 2.1.1.13), and methionine adenosyltransferase (EC 2.5.1.6) in the mitochondria (9). This latter enzyme synthesizes *S*-adenosylmethionine, which acts as a feedback regulator of aspartate kinase (EC 2.7.2.4) (17) and threonine synthase (EC 4.2.99.2) (15) and which can be regarded as the end product of the methionine branch of the pathway.

The use of protoplasts as a source of organelles for localization studies has proved superior to mechanical homogenization techniques, leading to much higher recoveries of intact chloroplasts, mitochondria, and microbodies (27). We have previously used pea and barley leaf protoplasts to study the distribution of homoserine dehydrogenase (19); in this paper, we report the results of similar studies on the intracellular localization of aspartate kinase, homoserine kinase (EC 2.7.1.39), threonine synthase, cystathionine γ-synthase (unclassified), cystathionine β-lyase (EC 4.4.1.8), methionine synthase, and methionine adenosyltransferase. In every case, precautions have been taken to maximize recovery of each enzyme.

MATERIALS AND METHODS

Plant Material. Pea (*Pisum sativum* cv Feltham First) and barley (*Hordeum vulgare* cv Julia) seeds were soaked overnight in aerated tap water, planted in compost and grown in controlled environment cabinets as described earlier (27, 29).

Protoplast Isolation. Protoplasts from 5 to 12-d-old barley leaves and 15 to 25-d-old pea leaves were prepared as described previously (27, 29). Purification and wash media were made up in 25 mM Tricine, pH 7.5, 1 mM $MgSO_4$, 0.1% (w/v) BSA (buffer A) with the additions shown in Table I. The crude protoplast pellet was resuspensed in 0.5 M sucrose, 15% (v/v) Percoll in buffer A, and 5 ml of this suspension in a 12 × 1.5-cm centrifuge tube was overlaid with 2 ml 0.5 M sucrose, 10% (v/v) Percoll and 3 ml 0.5 M sorbitol, both in buffer A. After centrifugation at 300*g* for 5 min, intact protoplasts were collected from the upper interface, diluted with 0.5 M sorbitol, and collected by low speed centrifugation. The pellet was resuspended in a small volume of 0.5 M sorbitol and the protoplasts were ruptured by four passes through a 20-μm mesh nylon net on the tip of a syringe. Organelles were fractionated by differential centrifugation at 4,000*g* for 1 min and 10,000*g* for 30 min. Pellets were resuspended in buffer and aliquots were treated with Triton X-100 (0.05% [v/v] final concentration) before desalting on 6 × 0.8-cm columns of Sephadex G-25 equilibrated with the appropriate buffer (Table I). Alternatively, 5 ml of protoplast lysate were layered onto a sucrose density gradient and centrifuged, the gradient and centrifugation protocol being those described previously (27). Fractions, 2.5 ml, were collected by upward displacement.

Enzyme Assays. Aspartate kinase was assayed by the method of Aarnes and Rognes (4). Homoserine kinase was assayed by a modification of the method of Aarnes (1). The reaction mix contained, in a total of 100 μl, 0.1 M K-phosphate, pH 7.1, 4 mM L-[^{14}C]homoserine (4.6 GBq mol^{-1}), 5 mM ATP, 8 mM $MgSO_4$, and up to 50 μl enzyme. After incubation at 30°C for 1 h, the reaction was stopped by the addition of 25 μl 15% (w/v) TCA. Threonine synthase was assayed by a modification of the method of Thoen *et al.* (22). In a total of 100 μl, the reaction mix contained 30 mM Hepes, pH 8.5, 30 mM K-phosphate, 0.1 mM pyridoxal-P, 0.7 mM SAM,[1] 3 mM L-[^{14}C]-*O*-P-homoserine (prepared by the method of [22]) and up to 50 μl enzyme. The reaction was stopped after 20 to 40 min at 30° C by adding 20 μl 15% TCA containing 2 mM

[1] Abbreviations: SAM, *S*-adenosylmethionine; DHDP, dihydrodipicolinate.

Table I. *Medium Used for Each Enzyme*

Enzyme	Additions to Buffer A	Resuspension and Desalting Buffer
Aspartate kinase	10 mM KCl, 2 mM DTT, 0.5 mM L-lysine, 0.5 mM L-threonine	50 mM K-phosphate, pH 7.5, 100 mM KCl, 2 mM $MgSO_4$, 10% glycerol, 0.2% β-mercaptoethanol
Homoserine kinase	10 mM KCl, 1 mM L-homoserine, 2 mM DTT	50 mM K-phosphate, pH 7.2, 100 mM KCl, 1 mM $MgSO_4$, 1 mM L-homoserine, 10% glycerol, 0.2% β-mercaptoethanol
Threonine synthase (pea)	None	50 mM K-phosphate, pH 7.4, 5 mM EDTA, 10% glycerol, 0.2% β-mercaptoethanol
Threonine synthase (barley)	None	50 mM Hepes, pH 8.5, 100 mM K-phosphate, 2 mM EDTA, 10% glycerol, 0.1% β-mercaptoethanol
Cystathionine synthase	2 mM DTT	50 mM K-phosphate, pH 7.0, 5 mM DTT, 5 mM EDTA, 10% glycerol
Cystathionine β-lyase	2 mM DTT	50 mM Tris, pH 8.5, 5 mM DTT, 5 mM EDTA
Methionine synthase	None	100 mM K-phosphate, pH 7.0
Methionine adenosyltransferase	2 mM DTT, 0.1 mM L-methionine	(Not desalted, enzyme unstable). Fractions suspended in buffer A

threonine and 2 mM homoserine. Methionine adenosyltransferase was assayed by a modification of the method of Aarnes (2). Reaction mixtures contained in a volume of 100 μl 0.1 M Tris/HCl, pH 8.5, 0.1 M KCl, 4 mM L-[^{35}S]methionine, 10 mM ATP, 30 mM $MgSO_4$, and up to 50 μl enzyme. After incubation at 30°C for 30 to 60 min, the reaction was stopped by the addition of 20 μl 15% TCA containing 2 mM SAM. For all these assays, reaction products were separated by high voltage paper electrophoresis on Whatman No. 3MM paper in 45 mM Na acetate, pH 5.1, at 3 kv. After drying, areas of the paper containing the product were cut out and radioactivity was measured in a liquid scintillation counter. For threonine synthase, products were alternatively separated by paper chromatography in *n*-butanol/acetone/diethylamine/H_2O (10:10:2:5). This system separates homoserine from threonine; under the reaction conditions used, insignificant amounts of homoserine were produced from labeled P-homoserine. Cystathionine synthase was assayed by the method of Aarnes (3) using [^{14}C]-P-homoserine. Products were separated by electrophoresis in 0.4 M formic aid containing 2 ml L^{-1} β-mercaptoethanol. Cystathionine lyase was assayed after $(NH_4)_2SO_4$ precipitation (0–50% saturation) of the fractions, and desalting the redissolved pellet on Sephadex G-25. The coupled assay of Giovanelli and Mudd (13) was used. Methionine synthase was assayed by the method of Clandinin and Cossins (9). Fumarase, Cyt *c* oxidase, catalase, and Chl were assayed as described previously (27). Nitrite reductase was assayed by the method of Vega *et al.* (25) and Cyt *c* reductase by the method of Tolbert (24).

RESULTS

The distribution of aspartate kinase activity between the lysed protoplast fractions is shown in Table II and compared with marker enzymes for chloroplasts (nitrite reductase), microbodies (catalase), and mitochondria (Cyt *c* oxidase). In both pea and barley leaf protoplasts, the proportion of aspartate kinase in the 4000*g* pellet is very similar to the proportion of nitrite reductase, which has been shown to be wholly in the chloroplast (27), though rather less than that of Chl. The 4000*g* pellet has less than 6% of the total catalase, Cyt *C* oxidase, or fumarase (data not shown), another mitochondrial marker. Overall recovery for all the enzymes was between 80 and 112% of the activity in the original protoplast lysate, and mixing experiments did not indicate the presence of any inhibitors or inactivators of aspartate kinase.

Table II. *Distribution of Aspartate Kinase in Protoplast Lysate Fractions*

Data in Part B represents the means of two (pea) and six experiments (barley).

	Recovered Activity				
	Aspartate kinase	Chl	Nitrite reductase	Catalase	Cyt *c* oxidase
			%		
A. Barley (single experiment)[a]					
4000*g* Pellet	93	98.2	87	6	5
10,000*g* Pellet	0	1.5	2	24	91.5
Supernatant	7	0.3	11	69	3.5
Recovery	99	112	112	106	82
B. Barley					
4000*g* Pellet	81	96	87		
Supernatant	19	4	13		
Pea					
4000*g* Pellet	72	89	70		
Supernatant	28	11	30		

[a] Protoplast lysate activity was 2×10^5 cpm mg^{-1} Chl h^{-1}.

Table III. *Distribution of Homoserine Kinase and Threonine Synthase in Protoplast Lysate Fractions*

Data represent the mean of two (pea) and five experiments (barley). Mitochondrial and miocrobody contamination of the 4000*g* pellets was similar to Table IIA.

	Recovered Activity		
	Homoserine kinase	Chl	Nitrite reductase
		%	
A. Barley[a]			
4000*g* Pellet	83	90	78
Supernatant	17	10	22
Recovery	90–110		
B. Barley[b]	Threonine Synthase	Chl	Nitrite Reductase
4000*g* Pellet	88	96	94
Supernatant	12	4	6
Recovery	90–110		
Pea[b]			
4000*g* Pellet	91	97	93
Supernatant	9	3	7
Recovery	90–110		

[a] Protoplast lysate activity was 0.12 nkatal mg^{-4} Chl.

[b] Protoplast lysate activity in both barley and pea was 1×10^6 cpm mg^{-1} Chl h^{-1}.

From these experiments, it is concluded that *in vivo* aspartate kinase is confined to the chloroplast.

Table III shows the results of similar experiments with homoserine kinase and threonine synthase. Overall recovery for both enzymes was 90 to 110% as shown, and in these and all subsequent experiments, mitochondrial and microbody contamination of the 4000*g* pellet was essentially the same as that shown in Table IIA. Recovery of both enzymes in the 4000*g* pellet is essentially the same as that for nitrite reductase, again suggesting an *in vivo* location in the chloroplast alone. Threonine synthase was assayed in the presence of SAM, which gave a 12 to 20-fold stimulation of activity in both pea and barley, as shown previously (15, 22).

The results for cystathionine γ-synthase follow essentially the same pattern (Table IV), again indicating a chloroplast location for the enzyme. The pattern for cystathionine β-lyase is rather different, however. Up to 60% of the total activity could be found in the 4000*g* pellet, but there was significant activity in both the 10,000*g* pellet and the supernatant, considerably more than the proportion of nitrite reductase in these fractions. We did not undertake detailed substrate specificity studies to distinguish between cystathionine lyase and other C-S lyases. However, preliminary experiments showed that the enzyme in all fractions was active with djenkolic acid at similar rates to those found for cystathionine.

Methionine synthase was mostly recovered in the supernatant, although some activity was associated with the 10,000*g* pellet.

Table IV. *Distribution of Methionine and SAM Biosynthetic Enzymes in Barley Protoplast Lysates*

Data represent the mean of three to four experiments. Mitochondrial and microbody contamination of the 4000*g* pellet was similar to Table IIA. Protoplast lysate activity was as follows: Part A, 2.2×10^5 cpm mg^{-1} Chl h^{-1}; Part B, 0.3 nkatal mg^{-1} Chl; Part C, 1.2×10^6 cpm mg^{-1} Chl h^{-1}; and Part D, 1×10^6 cpm mg^{-1} Chl h^{-1}.

	Recovered Activity		
	Cystathionine synthase	Chl	Nitrite reductase
		%	
A.			
4000*g* Pellet	88.3	92.8	92.9
10,000*g* Pellet	6.8	5.1	1.8
Supernatant	4.8	2.1	5.3
Recovery	94–102		
	Cystathionine β-Lyase		
B.			
4000*g* Pellet	54.6	91.6	87.0
10,000*g* Pellet	15.8	6.3	0.6
Supernatant	29.7	2.1	12.4
Recovery	87–115		
	Methionine Synthase		
C.			
4000*g* Pellet	4.1	93.0	91.7
10,000*g* Pellet	12.2	4.5	2.1
Supernatant	83.7	2.5	6.2
Recovery	90–100		
	Methionine Adenosyltransferase		
D.			
4000*g* Pellet	2.2	90.6	79.8
10,000*g* Pellet	7.6	6.6	6.4
Supernatant	89.3	2.8	13.8
Recovery	100–115		

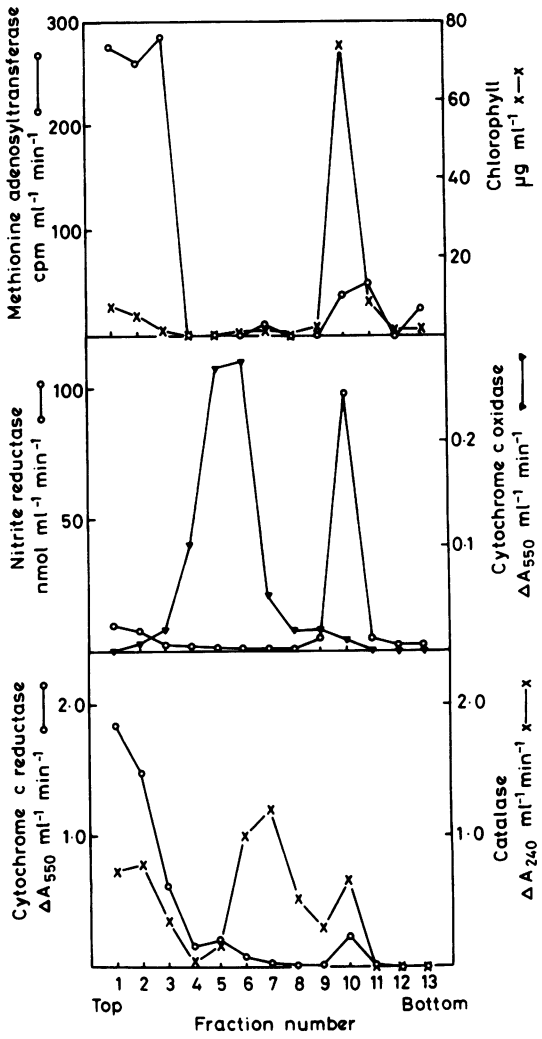

FIG. 1. Distribution of methionine adenosyltransferase and marker enzymes on sucrose density gradient centrifugation of barley leaf protoplast lysate.

Table V. *Stability of [^{3}H]SAM in the Methionine Adenosyltransferase Assay*

Fraction	Recovered [^{3}H]
	% control
Protoplast lysate	87.4
4000*g* Pellet	92.7
10,000*g* Pellet	116.1
Supernatant	75.8

Similarly, almost 90% of the methionine adenosyltransferase was recovered in the supernatant. For this latter enzyme, sucrose density gradient fractionation was used to give a clearer separation of organelles, in order to determine whether activity was truly associated with mitochondria or chloroplasts. Some problems were experienced in obtaining reproducible results, and it became clear that both the assay and the subsequent electrophoresis had to be performed as rapidly as possible to keep background radioactivity in the SAM down to acceptable levels. This problem had not been experienced with the much smaller number of fractions from differential centrifugation experiments. With these precautions, the results shown in Figure 1 were typical. In all experiments, up to 10% of the recovered activity was within the gradient, but its exact position varied with one or two minor peaks anywhere

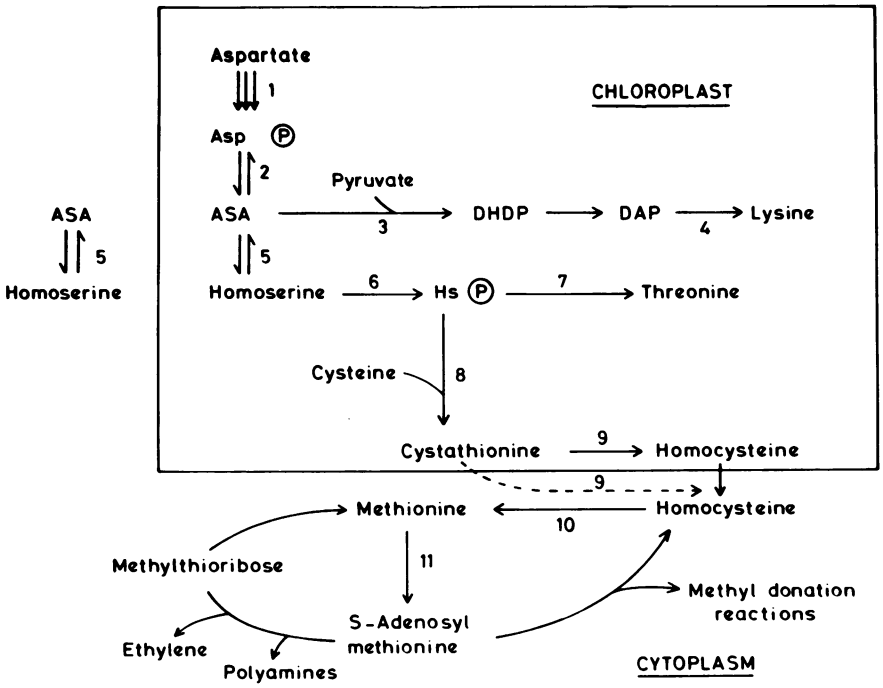

FIG. 2. The localization of the aspartate pathway in leaf cells. Enzymes: 1, aspartate kinase; 2, ASA dehydrogenase; 3, DHDP synthase (EC 4.2.1.52); 4, diaminopimelate decarboxylase (EC 4.1.1.20); 5, homoserine dehydrogenase; 6, homoserine kinase; 7, threonine synthase; 8, cystathionine γ-synthase; 9, cystathionine β-lyase; 10, methionine synthase; 11, methionine adenosyltransferase. Asp P, aspartyl phosphate; ASA, aspartic β-semialdehyde; DAP, diaminopimelate; Hs P, *O*-phosphohomoserine.

between fractions 7 and 13. In no case was a clearly defined peak of activity found to coincide with any of the marker enzymes, suggesting that enzyme activity was not associated with intact chloroplasts, mitochondria, or microbodies.

The methionine adenosyltransferase assay used required long incubation times with what are essentially crude leaf extracts. It is thus possible that activity could be seriously underestimated in some fractions due to further metabolism or degradation of SAM. The overall recoveries obtained, and the results of mixing experiments, gave no indication of a serious problem, but the stability of SAM in the reaction conditions was studied as an additional control. [^{3}H]SAM, (10 μCi) was incubated in the standard reaction mix (without [^{35}S]Met) with 40 μl of each barley protoplast fraction for 45 min at 30°C. The reaction was stopped and the products were separated and counted as for the standard assay. Results are shown in Table V. In the absence of extracts, the [^{3}H]SAM was stable, and neither 4000*g* nor 10,000*g* pellet showed evidence of appreciable SAM degradation. Lowest recovery was found with the supernatant fractions, but was still in excess of 75%. Thus, it is very unlikely that activity in any fraction is being significantly underestimated. These results taken together suggest that methionine adenosyltransferase is not associated with any organelle and is a soluble cytoplasmic enzyme.

DISCUSSION

The results presented here and earlier work (26, 28) indicate that in green leaves the enzymes responsible for the synthesis of lysine and threonine from aspartate are confined to the chloroplast in green leaves. The second enzyme of the pathway, aspartate-semialdehyde dehydrogenase (EC 1.2.1.11), has not yet been fully localized, but has been demonstrated in chloroplast extracts (Verbruggen and Wallsgrove, unpublished). Although the nonregulatory isoenzyme of homoserine dehydrogenase is cytoplasmic (18), it is not thought to be involved in homoserine synthesis. It would thus seem that the light-stimulated synthesis of lysine and threonine from aspartate in chloroplasts (16) is the sole source of these amino acids in the leaf cell.

For methionine and SAM synthesis, the situation is more complex. Cystathionine synthase is chloroplast located, as is the bulk of the cell's cystathionine lyase. However, the enzymes responsible for further metabolism of homocysteine to methionine and SAM cannot be shown in the chloroplast, in contrast to an earlier report (19). The inability of plastids to synthesize SAM has also been found in studies of Chl biosynthesis. In isolated cucumber etioplasts, methylation of Mg-protoporphyrin IX is dependent on exogenous SAM, and this requirement cannot be met by methionine + ATP (12). Earlier suggestions that the mitochondria contained the enzymes necessary for the final steps of methionine and SAM synthesis (9) cannot be confirmed. From our results, it is possible that mitochondria have some capability to methylate homocysteine, but it is clear that they contain no methionine adenosyltransferase activity. This enzyme and methionine synthase would appear to be cytoplasmic enzymes, though it should be noted that in our system we cannot differentiate between cytoplasmic and vacuolar constituents.

Figure 2 shows the suggested localization of the aspartate pathway in green leaves. All the enzymes necessary for lysine, threonine, and homocysteine biosynthesis are contained in the chloroplast, which is also the site of the incorporation of inorganic sulfur into cysteine (14). The three arrows for aspartate kinase in Figure 2 represent the three isoenzymes present, at least in barley, two sensitive in lysine + SAM and one threonine sensitive (7). We have not carried out detailed inhibition studies on the protoplast fractions, but there is no indication that any of these isozymes is outside the chloroplast. Although cystathionine synthase is on a branch point of the pathway, there is no evidence of feedback regulation of this enzyme (3), in contrast to DHDP synthase, threonine synthase, and homoserine dehydrogenase. As homocysteine is effectively the end product for the chloroplast, it could possibly act as a feedback regulator for this enzyme, but we could find no inhibition of cystathionine synthase by up to 2 mM homocysteine (data not shown). Instead, there is now evidence

that the level of enzyme activity is altered, by induction/repression or activation/deactivation in response to the methionine pool in the cell, or that of some methionine metabolite (23).

Synthesis of methionine and SAM takes place in the cytoplasm. These two compounds play a central role in many processes in the cell quite separate from the incorporation of methionine into protein. Methionine is involved in initiation of protein synthesis, and SAM, as well as being perhaps the sole donor for methyl transfer reactions, is a precursor of ethylene and the polyamines spermine and spermidine. Shown in Figure 2 is the recycling of SAM, either via homocysteine after methyl donation or via methylthioribose in ethylene and polyamine synthesis (5, 30). Due to the action of these cycles, the demand for net methionine synthesis from aspartate is essentially restricted to that required for protein synthesis.

Transport of amino acids across the chloroplast membranes is very important: lysine and threonine must be moved out to sites of extrachloroplastic protein synthesis and methionine transported in for chloroplast protein synthesis (especially significant in the young, greening leaf cell). Studies with isolated chloroplasts have shown that exogenous methionine is readily incorporated into chloroplast protein (10) and that exogenous lysine and threonine enter the regulatory metabolic pools inside the chloroplast (16). In addition, SAM must rapidly equilibrate across the chloroplast membranes to gain access to its regulatory sites. The studies on etioplasts already quoted show that SAM does enter these organelles (12).

Chloroplasts, and probably plastids in general, have a central role in the synthesis of the nutritionally essential amino acids. In addition to the aspartate-derived amino acids, the aromatic amino acids (6, 11) and isoleucine (16) are synthesized in the chloroplast. Quantitatively, these may not represent major photosynthetic reactions, but qualitatively they are of enormous significance. For the aspartate pathway, it is interesting to note that the only enzymes found in animal cells, methionine synthase and methionine adenosyltransferase, are precisely those that are not located in the chloroplast in plants.

LITERATURE CITED

1. AARNES H 1976 Homoserine kinase from barley seedlings. Plant Sci Lett 7: 187–194
2. AARNES H 1977 Partial purification and characterization of methionine adenosyltransferase from pea seedlings. Plant Sci Lett 10: 381–390
3. AARNES H 1980 Biosynthesis of the thioether cystathionine in barley seedlings. Plant Sci Lett 19: 81–89
4. AARNES H, SE ROGNES 1974 Threonine-sensitive aspartate kinase and homoserine dehydrogenase from *Pisum sativum*. Phytochemistry 13: 2717–2724
5. ADAMS OA, SF YANG 1977 Methionine metabolism in apple tissue. Plant Physiol 60: 892–896
6. BICKEL M, L PALME, G SCHULTZ 1978 Incorporation of shikimate and other precursors into aromatic amino acids and prenylquinones of isolated spinach chloroplasts. Phytochemistry 17: 119–124
7. BRIGHT SWJ, JSH KUEH, J FRANKLIN, SE ROGNES, BJ MIFLIN 1982 Two genes for threonine accumulation in barley seeds. Nature (Lond) 299: 278–279
8. BRYAN JK 1980 Synthesis of the aspartate family and branched chain amino acids. *In* BJ Miflin, ed, The Biochemistry of Plants, Vol 5. Academic Press, New York, pp 403–452
9. CLANDININ MT, EA COSSINS 1974 Methionine biosynthesis in isolated *Pisum sativum* mitochondria. Phytochemistry 13: 585–591
10. ELLIS RJ 1977 Protein synthesis by isolated chloroplasts. Biochim Biophys Acta 463: 185–215
11. FEIERABEND J, D BRASSEL 1977 Subcellular localization of shikimate dehydrogenase in higher plants. Z Pflanzenphysiol 82: 334–346
12. FUESLER TP, CM HANAMOTO, PA CASTELFRANCO 1982 Separation of Mg-protoporphyrin IX and Mg-protoporphyrin IX monomethyl ester synthesized *de novo* by developing cucumber etioplasts. Plant Physiol 69: 421–423
13. GIOVANELLI J, SH MUDD 1971 Transsulfuration in higher plants. Partial purification and properties of β-cystathionase of spinach. Biochim Biophys Acta 227: 654–670
14. GIOVANELLI J, SH MUDD, AH DATKO 1980 Sulphur amino acids in plants. *In* BJ Miflin, ed, The Biochemistry of Plants, Vol 5. Academic Press, New York, pp 453–505
15. MADISON JT, JF THOMPSON 1976 Threonine synthase from higher plants stimulation by *S*-adenosylmethionine and inhibition by cysteine. Biochem Biophys Res Commun 71: 686–691
16. MILLS WR, PJ LEA, BJ MIFLIN 1980 Photosynthetic formation of the aspartate family of amino acids in isolated chloroplasts. Plant Physiol 65: 1166–1171
17. ROGNES SE, PJ LEA, BJ MIFLIN 1980 *S*-Adenosylmethionine, a novel regulator of aspartate kinase. Nature (Lond) 287: 357–359
18. SAINIS JK, RG MAYNE, RM WALLSGROVE, PJ LEA, BJ MIFLIN 1981 Localization and characterization of homoserine dehydrogenase isolated from barley and pea leaves. Planta 152: 491–496
19. SHAH SP, EA COSSINS 1970 Pteroylglutamates and methionine biosynthesis in isolated chloroplasts. FEBS Lett 7: 267–270
20. SHEWRY PR, BJ MIFLIN, BG FORDE, SWJ BRIGHT 1981 Conventional and novel approaches to the improvement of the nutritional quality of cereal and legume seeds. Sci Prog Oxf 67: 575–600
21. Deleted in proof
22. THOEN A, SE ROGNES, H AARNES 1978 Biosynthesis of threonine from homoserine in pea seedlings. II. Threonine synthase. Plant Sci Lett 13: 113–119
23. THOMPSON GA, AH DATKO, SH MUDD, J GIOVANELLI 1982 Methionine biosynthesis in *Lemna*. Studies on the regulation of cystathionine γ-synthase, *O*-phosphohomoserine sulfhydrylase, and *O*-acetylserine sulfhydrylase. Plant Physiol 69: 1077–1083
24. TOLBERT NE 1974 Isolation of subcellular organelles of metabolism on isopycnic sucrose gradients. Methods Enzymol 31: 734–746
25. VEGA JM, J CARDENAS, M LOSADA 1980 Ferredoxin nitrite reductase. Methods Enzymol 69: 255–270
26. WALLSGROVE RM, M MAZELIS 1980 The enzymology of lysine biosynthesis in higher plants. FEBS Lett 116: 189–192
27. WALLSGROVE RM, PJ LEA, BJ MIFLIN 1979 The distribution of the enzymes of nitrogen assimilation within the pea leaf. Plant Physiol 63: 232–236
28. WALLSGROVE RM, PJ LEA, WR MILLS, BJ MIFLIN 1979 The regulation and subcellular distribution of enzymes of the aspartate pathway in *Pisum sativum* leaves. Plant Physiol 63: S-26
29. WALLSGROVE RM, AJ KEYS, IF BIRD, MJ CORNELIUS, PJ LEA, BJ MIFLIN 1980 The localisation of glutamine synthetase in leaf cells and its role in the reassimilation of ammonia released in photorespiration. J Exp Bot 31: 1005–1017
30. YUNG KH, SF YANG 1982 Methionine synthesis from 5-methylthioribose in apple tissue. Biochem Biophys Res Commun 104: 771–777