Amino acid Biosynthesis in Chloroplasts

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The synthesis of amino acids depends upon the supply of reduced nitrogen and the provision of carbon skeletons, usually in the form of α-keto acids. The nitrogen supplied to leaves is either as nitrate or as amino acids. The nitrogen from the latter can be incorporated into other amino acids via transamination; this reduction and the subsequent incorporation of ammonia into α-amino nitrogen is strongly light-dependent [1]. Although isolated intact chloroplasts cannot fix $^{14}CO_2$ into amino acids in any significant amount [2], they can reduce and incorporate nitrite [3]. The enzymes required for this process and also acetolactate synthase, which is required for the synthesis of the carbon skeletons of leucine, *iso*leucine and valine, have been shown by density gradient separation techniques to be present in chloroplasts [4]. Although chloroplasts contain low levels of glutamate dehydrogenase [5], the level of glutamine synthase is much higher. The K_ms of the extracted enzymes also favour ammonia assimilation into glutamine rather than glutamate. It has recently been shown that the amido group of glutamine can be transferred to the amino group of glutamate by a ferredoxin-dependent glutamate synthase [6]. It is considered that this pathway, which does not involve glutamate dehydrogenase, is the major route of nitrogen assimilation in leaves. This hypothesis is consistent with the results of ^{15}N incorporation patterns [7].

1. Canvin, D. T. and Atkins, C. A. (1974) *Planta* **116,** 207.
2. Givan, C. V. and Leech, R. M. (1971) *Biol. Rev.* **46,** 409.
3. Miflin, B. J. (1974) *Planta* **116,** 187.
4. Miflin, B. J. (1974) *Plant Physiol.* **54,** 550.
5. Lea, P. J. and Thurman, D. A. (1972) *J. Exp. Biol.* **23,** 440.
6. Lea, P. J. and Miflin, B. J. (1974) *Nature* **251,** 614.
7. Lewis, O. A. M. and Pate, J. S. (1973) *J. Exp. Botany* **24,** 596.

Asparagine Metabolism in Higher Plants

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Asparagine has frequently been shown to be a major constituent of the soluble fraction of plant extracts [1]. A review of the literature has shown that asparagine is formed when there is excess ammonia in the plant, either as a result of protein degradation followed by oxidation of amino acids, or by external application [1]. Ammonia is converted to asparagine as a detoxification process; the ratio of 2N atoms to 4C atoms makes the molecule very economic for the storage of superfluous nitrogen.

The conversion of aspartate to asparagine has long been the subject of discussion, since many authors have found only very low incorporations of ^{14}C-Asp into asparagine *in vivo*. Fumarate, malate and, in particular, succinate have been shown to be the main precursors of asparagine when applied externally [2, 3]. Although numerous attempts have been made to detect the presence of an enzyme capable of converting aspartate to asparagine, only Streeter [3] and Rognes [4] were able to demonstrate a very unstable enzyme in crude preparations utilizing the amide group of glutamine. A glutamine-dependent asparagine synthetase has recently been purified and characterized from 6-day-old lupin seedlings, the enzyme has a very low K_m for glutamine compared to ammonia [5]. As glutamine is now thought to be the main entry point of ammonia into amino acids [6], the direct transfer of the amide group to aspartate would liberate glutamate for the acceptance of a further ammonia molecule.

Evidence from radioactive tracer studies have shown that asparagine is not metabolized in seedlings [7], although early work has shown that asparagine is metabolized in leaves in the light, and disappears in legumes during fruit formation [1]. Enzymes capable of transaminating asparagine to form α-keto-succinamic acid have been known for some years [8] but their role in asparagine breakdown is not understood; these enzymes forming glutamate and alanine are presently being investigated in leaves. Although asparagine is metabolized by isolated chloroplasts in the light, it is not a substrate for the transfer of the amide group to α-oxoglutarate in the presence of reduced ferredoxin [6]. An oxidation of NAD(P)H in the presence of asparagine and α-oxoglutarate, has been demonstrated by Dougall [9] in tissue culture extracts, although the products of the reaction have not been characterized. However, attempts in this laboratory to demonstrate a reduced coenzyme dependent transfer of the amide group to acceptor α-oxo acids in various plant extracts have so far been unsuccessful.

1. McKee, H. S. (1962) In *Nitrogen Metabolism in Plants.* Clarendon Press, Oxford.
2. Mitchell, D. J. and Bidwell, R. G. S. (1970) *Can. J. Botany* **48,** 2001.
3. Streeter, J. G. (1973) *Arch. Biochem. Biophys.* **157,** 613.
4. Rognes, S. E. (1970) *Fed. Eur. Biochem. Soc. Letters* **10,** 62.
5. Lea, P. J. and Fowden, L. (1975) in preparation.
6. Lea, P. J. and Miflin, B. J. (1974) *Nature* **251,** 614.
7. Lees, E. M., Farnden, K. J. F. and Elliott, W. H. (1968) *Arch. Biochem. Biophys.* **126,** 539.
8. Kretovitch, V. L. (1958) in *Advances in Enzymology* (Nord, F. F., ed.), Vol. 20, p. 319. Interscience, New York.
9. Dougall, D. K. (1974) *Biochem. Biophys. Res. Commun.* **58,** 639.

The Enzymatic Cleavage of the C–S Bond of Substituted Cysteines in Higher Plants

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The enzyme alliin lyase (E.C. 4.4.1.4) has been partially purified from garlic cloves, onion bulbs, and *Brassica* sp. [1–3]. The enzyme from each source is specific for the sulphoxide structure, having no activity on the thioether analogue. Pyridoxal phosphate appears to be a necessary cofactor. The enzymes differ as to their pH optima. The garlic and onion enzymes have similar Michaelis constants for the same substrates, but differ from the *Brassica* enzyme. *Brassica* species also can cleave L-cystine to cysteine persulfide, pyruvate, and ammonia [4]. At least two isoenzymes have been found which have cystine lyase and alliin lyase activity [5]. An enzyme in the hypocotyls of