Miflin B J & Lea P J. The pathway of nitrogen assimilation in plants. *Phytochemistry* 15:873-85, 1976.
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The review article describes the isolation of both ferredoxin and NADPH dependent glutamate synthase from a range of plant tissues. The role of glutamate dehydrogenase (GDH) in ammonia assimilation is questioned and additional ^{15}N-labelling and inhibitor studies are used as evidence for the operation of the glutamate synthase cycle. [The *SCI*® indicates that this paper has been cited in over 220 publications since 1976, making it the 2nd most-cited paper published in this journal.]

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October 31, 1983

"In the early 1960s, Sims and Folkes[1] clearly showed the role of the enzyme glutamate dehydrogenase (GDH) in the assimilation of [$^{15}NH_3$] in the food yeast *Candida utilis*. Such was the elegance of their work that other people broadened their conclusions to include the whole of the plant kingdom. Although in 1969, Brown,[2] working at Newcastle University in an adjacent department to B.J. Miflin, did suggest a novel pathway of ammonia assimilation in bacteria, it was thought at the time to only operate under conditions of severe nitrogen limitations. The primary features of this pathway, which has become known as the glutamate synthase cycle, are the assimilation of ammonia into the amide position of glutamine, catalyzed by glutamine synthetase [E.C. 6.3.1.2] followed by the reductive transfer of this group to the C-2 of 2-oxoglutarate to give the amino acid glutamate. This second step is catalyzed by the enzyme glutamate synthase (or L-glutamate:$NADP^+$ [or ferredoxin] oxidoreductase, E.C. 1.4.1.13 [or E.C. 1.4.7.1]). Thus, one molecule of glutamate continuously recycles, while ammonia is incorporated into the 2-amino position of an amino acid.

"In 1973, Miflin became the head of the biochemistry department at Rothamsted after spending a sabbatical with Harry Beevers in Santa Cruz, California. P.J. Lea joined the department after spending three years working with Sir Leslie Fowden at University College, London, on the aminoacylation of t-RNA, but had done his PhD research on plant GDH. Both of us were disillusioned with the low levels of chloroplast GDH, as we knew that intact chloroplasts could readily convert nitrite to amino acids in a light dependent reaction and contain high levels of glutamine synthetase.[3] After many long hours of discussion we concluded that chloroplasts must be able to carry out the glutamate synthase reaction. A major stumbling block was separating glutamine from glutamate, and we eventually fell back on the tried and tested (but extremely unpleasant) method of phenol/NH_3 paper chromatography used so successfully by Fowden for the separation of nonprotein amino acids. Using isolated intact pea chloroplast and extracts derived from them we were able to show that they could convert glutamine and 2-oxoglutarate to two molecules of glutamate. The reaction was catalyzed by a glutamate synthase enzyme active with reduced ferredoxin (similar to nitrite reductase) rather than reduced pyridine nucleotides.

"The review article considered as a *Citation Classic*™ marks our first attempt to draw together all the available evidence to confirm the role of the glutamate synthase cycle in plants. Once the existence of the pathway was realised, a lot of previously published work slipped neatly into place allowing us to present a reasonably complete and coherent picture of ammonia assimilation which we argue took place through the glutamate synthase cycle rather than via GDH. This view has been confirmed many times by subsequent work; the role of the glutamate synthase cycle in ammonia assimilation in nitrogen fixing root nodules[4] and in photorespiration[5] in C_3 leaves is still an important area of study."

1. **Sims A P & Folkes B F.** A kinetic study of the assimilation of [^{15}N]-ammonia and the synthesis of amino acids in an exponentially growing culture of *Candida utilis*. *Proc. Roy. Soc. London Ser. B* 159:479-502, 1964. (Cited 75 times.)
2. **Tempest D W, Meers J L & Brown C M.** Synthesis of glutamate in *Aerobacter aerogenes* by a hitherto unknown route. *Biochemical J.* 117:405-7, 1970. (Cited 150 times.)
3. **O'Neal D & Joy K W.** Localisation of glutamine synthetase in chloroplasts. *Nature* 246:61-2, 1973.
4. **Cullimore J V, Lara M, Lea P J & Miflin B J.** Purification and properties of two forms of glutamine synthetase from the plant fraction of *Phaseolus* root nodules. *Planta* 157:245-53, 1983.
5. **Wallsgrove R M, Keys A J, Lea P J & Miflin B J.** Photosynthesis, photorespiration and nitrogen metabolism. *Plant Cell Environ.* 6:301-9, 1983.