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# REVIEW

# METABOLIC DETOXIFICATION OF AMMONIA IN TISSUES OF HIGHER PLANTS

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**Key Word Index**—Amides; ammonia; anaplerotic carboxylation; catabolism; keto-acids; nitrogen assimilation; regulation; respiration.

Why should plant scientists study metabolic processes associated with ammonia detoxification? The term 'detoxification' and the detoxification approach to ammonia assimilation studies emphasize two main points. (1) Although growing plants need to assimilate nitrogen, ultimately in the fully reduced form, excessive concentrations of ammonia or ammonium ions are toxic to plants. The toxicity of ammonia to higher plant tissues has been demonstrated repeatedly, even though the exact biochemical causes of toxicity and the specific concentrations causing toxicity are not always clear [1–4]. (2) Plants possess mechanisms which not only mediate normal rates of assimilation in the presence of non-toxic amounts of ammonia, but also permit the disposal of elevated levels of ammonia. It will be shown in this article that there are several ways in which plants may be able to assimilate and dispose of high concentrations of ammonia. In many cases plants may detoxify excessive ammonia by simply accelerating the pace at which they carry out nitrogen assimilation by the usual pathway. In other cases the normal assimilation pathway may possibly be supplemented by additional ammonia-utilizing reactions, initiated only at times when the plant is subjected to excessive levels of ammonia. Exceptionally large amounts of ammonia have to be disposed of when there is either a large exogenous source of ammonia, or, alternatively, an internal source within a plant tissue. Large amounts of ammonia may be endogenously generated during rapid nitrogen fixation or when proteins are being degraded. In the latter case there is usually a deamination of amino acids, the carbon skeletons then being used as respiratory substrates.

It is desirable to learn the details of mechanisms by which ammonia is efficiently metabolized so that it can be used for growth, or at least converted to an innocuous form, rather than accumulating to concentrations that become toxic to plant cells. Where possible, particular consideration should be given to those cases where plant tissues are exposed to much greater than normal amounts of ammonia. Why should metabolic responses to high ammonia levels be a matter of such great concern? Strains of the nitrogen-fixing bacterium *Rhizobium* will probably soon be developed which possess greatly elevated rates of ammonia production within the host tissues of legume root nodules [5]. Recent evidence clearly shows that in root nodules of legume plants, the host plant tissues—not the bacteroids—carry out the task of removing and assimilating the bulk of the ammonia generated by the bacteroidal fixation of nitrogen (e.g. [6]). In order to metabolize and prevent excessive accumulation of ammonia generated by bacteroidal nitrogen fixation, the host plant must have a very efficient assimilation mechanism to make productive use of all the ammonia generated. A further point to consider is that with the increasing use of nitrification inhibitors in agricultural fertilizing practice (cf. [7]), account needs to be taken of the effects of elevated ammonia levels on plant metabolism and of the mechanisms by which plants can effectively remove and metabolically utilize relatively high levels of soil ammonia or ammonium ions.

It is the purpose of this short review to examine the metabolic pathways that mediate assimilation and detoxification of ammonia, plus those factors which may actually determine the rate at which higher plants can utilize ammonia by assimilating it to organic compounds. Particular attention will be paid to the importance of the interplay between carbohydrate metabolism and the process of ammonia assimilation/detoxification. Some of the facts and concepts to be reviewed here are not new, but they have often received inadequate attention during recent years. Consideration of the interrelations between nitrogen assimilation and carbohydrate metabolism seems worth while in the context of current research. The summary which follows may prove useful particularly to new investigators entering the field. The list of literature citations is far from exhaustive but should nevertheless enable the interested reader to delve further. In discussing the metabolic reactions mediating ammonia detoxification I have deliberately omitted any treatment of the biochemical causes of ammonia toxicity, i.e. what the specific *deleterious* effects of ammonia are on plant metabolism. This question is complex and views differ. Readers interested specifically in deleterious effects of ammonia may consult references [1–4], [8] and [9]. In considering interrelationships between ammonia detoxification and carbohydrate metabolism, I have chosen three main aspects for discussion: (1) the enzymic reactions that operate to utilize normal and excessive amounts of ammonia, i.e. the assimilation reactions themselves; (2) metabolic factors regulating the rates of ammonia assimilation in higher plants; and (3) the ways in which carbohydrate metabolism is controlled and how it can respond to the requirements imposed by ammonia-utilization processes.

## AMMONIA ASSIMILATION REACTIONS

*(a) The glutamine synthetase/glutamate synthase cycle—the pathway operating at normal ammonium levels*

The glutamine synthetase/glutamate synthase cycle is now believed to be the usual pathway for the assimilation of ammonia at low (i.e. normal) intracellular concentrations. In the presence of glutamic acid the enzyme glutamine synthetase (GS) binds ammonia as glutamine. GS works in conjunction with glutamate synthase (or 'GOGAT', acronym for glutamine-oxoglutarate aminotransferase); the latter enzyme removes the newly-assimilated nitrogen atom from the amide group of glutamine and catalyses the formation of glutamic acid in the presence of the carbon substrate α-ketoglutarate. Equations I and II illustrate the reactions catalysed by GS and GOGAT. The functioning of this pathway in plants has been established only recently, thanks to the pioneering work of Dougall [10], Fowler and collaborators [11], Stewart and Rhodes [12], Lea and Miflin [13] and others. A detailed review article on the pathway of nitrogen assimilation in higher and lower plants has been published by Miflin and Lea [14].

Equation I

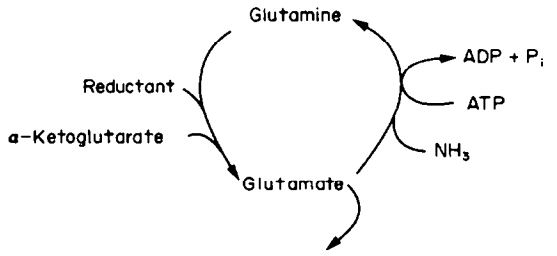
$$\text{L-Glutamate} + NH_3 + ATP \xrightarrow[\text{Synthetase (GS)}]{\text{Glutamine}} \text{Glutamine} + ADP + P_i$$

Equation II

$$\text{Glutamine} + \alpha\text{-ketoglutarate} + \text{reduced ferredoxin [or NAD(P)H]} \xrightarrow[\text{(GOGAT)}]{\text{Glutamate Synthase}} 2\ \text{L-Glutamate} + \text{oxid. ferredoxin [or NAD(P)}^+\text{]}$$

Recently, much attention has been given to certain factors, especially light and nutritional factors, which may control the rate at which this cycle turns over in higher plant tissues [15–20].

GS/GOGAT assimilation cycle




The substrate inputs to the GS/GOGAT cycle, in addition to ammonia, are α-ketoglutarate, ATP and reductant. The outputs consist of the amide glutamine, or alternatively glutamic acid (or other amino acids which can effectively draw off the assimilated amino-nitrogen by means of aminotransferase reactions). It is important to note that ADP and $P_i$ are also products of this assimilation cycle.

*(b) Alternative assimilation reactions possibly operating at high ammonium levels*

In addition to the GS/GOGAT system there are other potential routes of ammonia assimilation which might come into operation at high levels of ammonia. Assimilation reactions in this category all involve enzymes with a relatively low affinity for ammonium, i.e. they have a high $K_m$ ($NH_4^+$) value.

i. *The glutamate dehydrogenase reaction.* Glutamate dehydrogenase (GDH) mediates the combination of α-ketoglutarate with ammonia to yield glutamic acid (Equation III) and also readily catalyses the reverse reaction leading to oxidative deamination of glutamate. A reduced pyridine nucleotide is required to support the production of glutamic acid by this route. For many years GDH was considered the principal assimilatory enzyme in higher plants, but its role in assimilation is now thought to be far more limited.

Equation III

$$NH_3 + \alpha\text{-ketoglutarate} + NAD(P)H \underset{\text{Dehydrogenase}}{\overset{\text{Glutamate}}{\rightleftharpoons}} \text{L-glutamate} + NAD(P)^+$$

Having a $K_m$ ($NH_4^+$) upwards of 5 mM (e.g. [21]), this enzyme can probably assimilate ammonia only when the intracellular ammonia concentration is unusually high. A relatively prominent role of GDH at high ammonia levels is further suggested by the finding that considerable increases in the overall level of this enzyme often take place when plants are exposed to excessive concentrations of ammonia (e.g. [22, 23]). However, it is at the same time important to recognise that data obtained using chemical inhibitors of the GS/GOGAT cycle indicate that the GS/GOGAT mechanism may in fact still be operating as the major assimilatory pathway even at high ammonium levels [24].

ii. *The asparagine synthetase reaction.* Asparagine synthetase, characterized by Rognes [25], Lea and Fowden [26] and others, normally transfers the amide nitrogen from glutamine to aspartate, thereby producing asparagine (Equation IV). Asparagine synthetase can also react with ammonium directly (Equation V), but the $K_m$ ($NH_4^+$) is at least an order of magnitude higher than the $K_m$ (glutamine). Asparagine synthetase may therefore become a primary assimilating enzyme at excessively high ammonia levels. Whether this enzyme actually does act as a primary assimilator at high ammonium levels is, however, uncertain.

Equation IV

$$\text{Aspartate} + \text{Glutamine} + ATP \xrightarrow[\text{Synthetase}]{\text{Asparagine}} \text{Asparagine} + AMP + PP_i + \text{L-glutamate}$$

Equation V

$$\text{Aspartate} + NH_3 + ATP \xrightarrow[\text{Synthetase}]{\text{Asparagine}} \text{Asparagine} + AMP + PP_i$$

In addition to ammonia, the substrates in the putatively assimilatory asparagine synthetase reaction are aspartic acid and ATP. The carbon atoms destined for aspartate formation are probably supplied in the form of oxaloacetic acid (OAA), which can be directly transaminated (probably from glutamate) to give aspartate. OAA could be obtained from the mitochondrial tricarboxylic-acid cycle and additionally by carboxylation of pyruvate or phosphopyruvate [27]. OAA seems the most plausible source of asparagine carbon in most cases; however, labelling data have sometimes been inconclusive concerning the origin of asparagine carbon. Lea and Fowden

[26] have published a good summary of this topic, including discussion of the alternative route for asparagine formation via the beta-cyanoalanine pathway.

In view of the properties of the assimilation reactions which operate in plant tissues, it can be concluded that glutamine, and perhaps to a lesser extent asparagine and/or glutamic acid, are the most important primary products of ammonia utilization. As regards asparagine, its predominant role is more likely the very important one of serving as a secondary acceptor of assimilated nitrogen. Amide nitrogen derived from glutamine is readily transferred to asparagine by the asparagine synthetase reaction. Asparagine is frequently the dominant form of organic nitrogen exported from the nodules of legume roots (cf. [26, 28]. In legume nodules the amide N of asparagine is almost certainly obtained from glutamine rather than from free ammonia or ammonium ions.

One conclusion seems clear from an examination of the substrate-input requirements for the reactions utilizing ammonia and catalysing the synthesis of glutamine and asparagine: in addition to the relevant assimilating enzymes, supplies of ATP, plus oxaloacetate and/or α-ketoglutarate must be available if any of the assimilatory reactions is to proceed at a high enough rate to permit the utilization of large amounts of ammonia.

## AMIDE ACCUMULATION AND ITS REGULATION DURING AMMONIA ASSIMILATION/DETOXIFICATION

A great deal of evidence indicates that amides are key detoxification products when plants are exposed to ammonia from external sources. Either glutamine or asparagine may be the major form of assimilated nitrogen accumulating under these conditions. Numerous investigators, e.g. Vickery and coworkers [29], working on beets, Yemm and Willis [30] (barley roots), Weissman [31] (soya bean and sunflower), and Barash and collaborators [32] (excised oat leaves) have demonstrated amide accumulation during assimilation of ammonium from an exogenous source. The accumulated amides thus act as storage reservoirs or sinks for assimilated ammonia as well as being early products of assimilation. Amide accumulation is known to be especially pronounced when a plant has to detoxify large amounts of ammonia [29, 32], and this generalization frequently applies also to the cases where ammonia is generated internally within plant tissues. Amide accumulation, for example, appears to provide the primary means of combatting ammonia toxicity during leaf senescence, when proteolysis has released soluble amino acids which are then deaminated and respired, with consequent release of ammonia (cf. [33]).

In walnut seeds, a quite different detoxification mechanism has been postulated. Grosse [34] has demonstrated the accumulation of serotonin (5-OH tryptamine) in cotyledons of walnut seeds. Serotonin is accumulated mainly during the period after abscission of the fruit from the tree. Proteolysis is thought to take place during this period. Ammonia derived from deamination of amino acids is presumably initially converted to glutamine by GS present in the cotyledons. Glutamine is then utilized in the anthranilic-acid synthetase reaction, with the formation of tryptophan, and ultimately serotonin, taking place subsequently. Grosse's results indicated that serotonin, unlike tryptophan, did not cause serious feedback inhibition of the anthranilate-synthetase reaction, the step that controls flux through the biosynthetic pathway leading to tryptophan. Hence, accumulation of serotonin did not inhibit its further synthesis. Serotonin could, therefore, accumulate as an ammonia-detoxification product.

Since the most widespread detoxification mechanisms involve amide accumulation, we must consider the factors which control the rate of amide synthesis to remove free ammonia. It appears that one decisive factor is the availability of the carbon substrates, or possibly ATP, needed for amide synthesis. There is, of course, an indispensable requirement for adequate levels of the assimilating enzymes, and it is known that the levels of such enzymes may change very significantly in response to variations in the type, or concentration, of nitrogen compounds supplied to plants (e.g. [19, 22]). However, adequate levels of these enzymes are not alone sufficient to permit assimilation, and other factors may be critical in controlling the rate of ammonia assimilation.

A number of basic observations underlie the view that ammonia utilization may often be critically limited by the availability of the various co-substrates required as inputs for the synthesis of amides. The points summarized below suggest the importance of the availability of these substrates, which are derived primarily from reserve carbohydrate, supplemented by anaplerotic $CO_2$ assimilation.

(a) When plants are exposed to excessive levels of soil ammonia (or ammonium ions), the synthesis of amides takes place principally in the roots. Likewise, the host tissue of the legume-root nodule is responsible for the bulk of the amide synthesis taking place during the fixation of nitrogen by rhizobial symbionts. Data obtained over many decades (e.g. [6, 29, 31]) all seem to have verified the point that the amount of ammonia directly transported from root to shoot is relatively low. Although ammonia may begin to accumulate in shoot tissues after severe and prolonged exposure of a plant to toxic levels of soil ammonia, most of the ammonia accumulating in shoots under these conditions actually results from proteolysis of leaf protein, followed by deamination of amino compounds. The old, but admirably comprehensive work of Vickery and coworkers [29] may be consulted for a detailed elaboration of this conclusion. An important conclusion from Vickery's analyses was that the superior detoxifying and amide-synthesizing ability of roots could be ascribed as much to the high carbohydrate-reserve levels of roots as to any special enzymic properties of root tissue.

(b) Careful study of the performance of hydroponically grown barley plants has also pointed towards a very important relation between carbohydrate availability and the ability of plants to utilize ammonium for growth and to avoid toxic effects. Arnon [35] found that both high illumination levels and good aeration of the roots were necessary to permit ammonium-grown plants to perform as well as those cultured on nitrate, and to avoid ammonium toxicity. These results seem to agree with the view that good availability of carbohydrate reserves is necessary to prevent the toxic effects of ammonia. Arnon's results further indicate the requirement for active oxidative metabolism (which may reflect an ATP requirement).

Even earlier than Arnon's work was a noted study

carried out by Prianischnikow [36] on the ability of etiolated plants to deal with ammonium nitrogen. Granted that this work has been subjected to criticisms [37], Prianischnikow nevertheless demonstrated that etiolated tissues, poor in carbohydrate, were also relatively poor at synthesizing amides and utilizing ammonium. When glucose was supplied along with a source of ammonium, amide synthesis was substantially increased and the accumulation of ammonia reduced. Much more recent work, by Takahashi's laboratory [2], has shown, similarly, that supplying α-ketoglutarate to higher plant tissues can greatly reduce their internal $NH_3$ concentration and can thus alleviate toxicity due to exogenously supplied ammonium ions.

(c) Carbon substrates used during assimilation of ammonia supplied from the soil or from nodular fixation appear to be derived from reserve carbohydrate. These substrates do not originate directly from photosynthetic intermediates formed prior to sugars. This fact is of course obvious with respect to roots, which normally have no photosynthetic metabolism. However, this conclusion may also be in part applicable to shoot tissues carrying out detoxification of artificially supplied ammonia (e.g. [32, 38]), though it must be remembered that apparently very little ammonia supplied to roots reaches the shoots directly as ammonia [31]. As regards leaves, it is very important to distinguish the case of high levels of (supplied) exogenous ammonia from the cases where the chloroplasts are themselves actively generating ammonia derived from nitrate transported from the root, or where leaf mitochondria are generating $NH_3$ in carrying out photorespiratory conversion of glycine to serine. In these latter two cases the ammonia produced is probably efficiently assimilated by the leaf GS enzyme. Leaf GS may be both chloroplastic and cytosolic in subcellular location [39, 40]. GS, by virtue of its high activity and low $K_m$ ($NH_4^+$) [41, 42] may well ensure the absence of a significant build-up of free ammonia in the leaf. Equally here, in the case where leaf cells are assimilating endogenously-generated ammonia at presumably a very low ambient ammonia concentration, there is probably a requirement for carbon substrate derived from extra-chloroplastically catalysed intermediary reactions, if not necessarily from extraplastid reserves. Current evidence indicates that plastids can generate neither OAA nor α-ketoglutarate from Calvin-cycle compounds (cf. [43, 44]). Moreover, if high ammonia levels requiring detoxification ever did occur in leaves (and it should again be stressed that this may well be an exceptional circumstances though possibly important if the rate of photorespiration is high [73], these high ammonia levels would be likely to uncouple photophosphorylation and therefore eliminate chloroplasts as an ATP source for amide synthesis [16, 17].

## RESPIRATION AND CARBOHYDRATE CATABOLISM DURING ASSIMILATION/DETOXIFICATION OF AMMONIA

### (a) *Respiratory rates during ammonia assimilation*

In a very careful and comprehensive study on barley roots, Willis and Yemm [45] demonstrated enhancement of both respiratory $O_2$ uptake and utilization of reserve carbohydrate during the assimilation of ammonium (or of other nitrogen sources reducible to ammonia). Net $CO_2$ output was markedly stimulated even though a great deal of carbohydrate-derived carbon was simultaneously channelled into amide synthesis. The respiratory stimulation observed to accompany nitrogen assimilation was much greater than that which took place upon exposure of the tissue to other ions. A particularly significant comparison was the one made between the effect of ammonium and potassium, both monovalent cations and both often playing similar roles at the enzyme level (see below). The stimulation of respiration brought about by ammonium was much greater than that elicited by potassium or other ions. This finding was interpreted to mean that the ammonium stimulation was probably not a direct ion effect comparable to so-called 'salt respiration'. The authors concluded, though without much direct evidence, that the assimilation of ammonium might well have led to release of ADP during amide synthesis, and that the increased ADP availability would stimulate carbohydrate breakdown [30]. Somewhat similar observations were made by Syrett [46] on unicellular algal systems.

### (b) *Biochemical characterization of the accelerated respiration during ammonia assimilation*

i. Respiratory-quotient data [45] have confirmed that carbohydrates were the principal respiratory substrates when ammonium ions were assimilated by growing barley root tissues. This conclusion is probably generally applicable to many young or mature tissues. In the case of senescent material the situation is no doubt different, for here there will be extensive respiration of amino acids derived from hydrolysed protein.

ii. There has been considerable evidence that the intracellular levels of tricarboxylic acids decline during the assimilation of ammonia, and that this loss of organic acids is correlated with an accumulation of glutamine, and sometimes other amino acids. Recent work of Bergmann and collaborators [47] highlighted this phenomenon in tissues of *Nicotiana* grown in liquid suspension culture. Addition of ammonium ions to cultures previously grown on nitrate nitrogen led to an immediate and sharp rise in cellular glutamine level, plus a rather less pronounced accumulation of alanine. A precipitous decline in the level of malate took place immediately following addition of ammonium to the cultures, during the period of marked glutamine accumulation. Major declines in levels of tissue organic acids have also been found in cases of intact plants grown in the presence of high levels of ammonium nitrogen (H. J. Evans, personal commun. [74]). This type of observation suggests that during periods of ammonia assimilation a very high level of demand is placed on carbohydrate degrading reactions to provide the necessary substrates—so high a demand that the carbohydrate-dissimilating reactions are evidently sometimes unable to maintain the organic acids at adequate levels. While enhanced carboxylation of phosphopyruvate to OAA probably operates and helps to meet this demand (e.g. [75]), depletion of organic acids may nevertheless occur.

iii. Intracellular concentrations of adenine nucleotides have been found to vary according to whether plants are grown on ammonium or nitrate. Weissman [31], working on soya bean roots, found that ADP and AMP levels in plants grown on ammonium exceeded the

levels found in nitrate grown plants. The amount of ATP in the nitrate- and ammonium-grown plants was approximately the same. The 'energy charge' [48] value was, therefore, somewhat reduced in ammonium-grown plants as compared to plants grown on nitrate. In similar experiments carried out on sunflower, however, Weissman found essentially no difference in energy charge between ammonium- and nitrate-grown tissues. The concentrations of ammonium supplied to Weissman's plants were not toxic. Since Weissman's studies did not include respiratory measurements, it is unclear whether the measured differences in adenine-nucleotide levels (giving differences in calculated energy-charge values) had any specific repercussions as regards the respiratory metabolism of the tissues. It is, however, quite possible that such alterations in cellular adenine nucleotide levels could be relevant to adjustments in respiratory metabolism taking place to support rapid amide synthesis.

Matsumoto and Wakiuchi [49] reported up to a two-fold *rise* in ATP content when cucumber tissues were subjected to toxic concentrations of ammonium; however no data were provided on the effect of ammonium on the levels of other adenine nucleotides. These authors suggested that an acceleration of respiration was initiated in response to ammonium in order to provide the increased supply of organic acids required for ammonia detoxification.

iv. As regards long-term changes in enzyme levels, treatment of plant tissues with ammonium has been found to result in gradual rises in the amount of extractable activity of several carbohydrate-degrading enzymes [50]. There is also evidence that the synthesis of new *assimilatory* enzyme may play a role in detoxification of ammonia [22, 23]. Increased synthesis of carbohydrate-degrading enzymes, while probably facilitating the production of essential carbon substrates in the long term, is certainly not the sole mechanism by which carbohydrate metabolism responds to ammonia treatment. The studies of Barash and coworkers [32] demonstrated that cycloheximide, which prevented enzyme synthesis, did not greatly inhibit the initial rate of conversion of ammonia nitrogen to glutamine in excised leaf tissues. Over longer periods, however, less ammonia accumulated in control tissues than in tissues treated with cycloheximide. There is, therefore, evidence that enzyme synthesis may play a role in ammonia detoxification, but it is difficult to assess the relative importance of synthesis of new nitrogen-assimilating enzyme as against carbohydrate dissimilating enzymes. Moreover, it seems probable that enzyme synthesis is only involved in longer term ammonia detoxification, and that the primary or initial metabolic adjustments to elevated ammonia levels may take place in the absence of *de novo* enzyme synthesis.

## CONTROL OF CARBOHYDRATE CATABOLISM IN HIGHER PLANTS

A complete discussion of the regulation of carbohydrate catabolism in higher plants is outside the scope of the present article and this subject has been very well reviewed elsewhere (e.g. [51–53]). Here I shall attempt only to emphasize those aspects which may be important in relation to the gearing between carbohydrate metabolism and the assimilation/detoxification of ammonia.

A combination of *in vivo* and *in vitro* analyses has been employed in attempts to learn how rates of carbohydrate dissimilation are controlled in higher plants. An important conclusion emerging both from *in vivo* and *in vitro* studies is that very often the glycolytic enzyme phosphofructokinase (PFK) is a rate-limiting step which plays a key regulatory role. Levels of extractable activity of this enzyme are frequently significantly lower than those of other glycolytic enzymes (e.g. [54]). Moreover, *in vivo* analyses of intact tissue systems have demonstrated that PFK, an essentially irreversible enzymic step, is generally greatly displaced from thermodynamic equilibrium. This conclusion has been drawn as the result of measurements done on tissue extracts. It has been found that the mass-action ratio for the PFK reaction differs greatly from the theoretical equilibrium (e.g. [55–57]). Evidence has also been obtained indicating that positive regulation (i.e. a stimulation) of PFK takes place *in vivo* under certain conditions where glycolysis is accelerated, owing apparently to alteration in intracellular concentrations of adenine nucleotides or $P_i$. This conclusion is based on the observation that the pathway substrate for the PFK reaction, fructose-6-phosphate, has been found to decline when the flux through the PFK step appeared to increase (e.g. [55, 56, 58]). The pyruvate-kinase (PK) reaction also has been concluded to play a regulatory role, based on similar criteria [55].

*In vitro* studies on the control of plant glycolysis have been of two types, both of which have generally reinforced the chief conclusions drawn from *in vivo* studies. Some *in vitro* studies have involved examination of the properties of individual enzymes; other investigations have dealt with complete multi-enzyme systems able to convert hexose phosphate to ethanol [59–61]. The *in vitro* studies have indicated various additional factors which *may* be relevant to *in vivo* control mechanisms. Among the major *in vitro* findings are that (a) orthophosphate can accelerate glycolysis at either the triose-phosphate dehydrogenase step or at an earlier step (likely PFK) depending on the concentration of magnesium ions [59]; (b) citrate can inhibit the PFK and PK enzymes, as can ATP above a certain concentration [59, 62–64]; (c) lack of ADP retards *in vitro* glycolysis principally at the PK step [65]; and (d) operation of the hexose-monophosphate shunt pathway may be accelerated by an increase in the $NADP^+$:NADPH ratio, with glucose-6-phosphate dehydrogenase being the chief regulatory step (cf. [51]).

## THE MECHANISM BY WHICH CARBOHYDRATE CATABOLISM IS ACCELERATED DURING AMMONIA DETOXIFICATION/ASSIMILATION

Given the existence of a certain background of information about how carbohydrate degradation is regulated, it now remains to consider how an immediate activation of carbohydrate breakdown might take place in response to the initiation of ammonia assimilation/detoxification processes. Unfortunately, there are many more questions than answers here. The problem can usefully be divided into two subheadings: (a) what *specific steps* of carbohydrate breakdown become activated during a period of rapid ammonia assimilation

by plant tissues; (b) what cellular cofactors or metabolites actually modulate the functioning of glycolytic enzymes to stimulate their catalytic activity during ammonia assimilation?

*(a) Steps of carbohydrate catabolism activated during ammonia assimilation*

Until very recently there were virtually no data on higher plants bearing on this question. Bassham's group [38, 76] have now found that treatment of green leaf tissue with ammonium brings about changes in levels of $^{14}C$-labelled glycolytic metabolites indicating enhancement of flux through the PK reaction. These authors suggested that ammonium might be the causative agent of the observed PK stimulation. Such a conclusion would appear to be basically similar to an earlier one made with respect to the alga *Chlorella* [66]. Further data on other higher plant tissues will be needed in order to ascertain the general applicability of this result. It seems particularly urgent to extend such studies to root tissues, since roots play the central role in assimilating ammonia derived either from bacteroid nitrogen fixation or from fertilizer ammonia supplied via the soil. That such analyses would be feasible on root systems is indicated by recent work on the influence of nitrate supply on the levels of glycolytic metabolites in root tissues [67].

*(b) Identity of molecules effecting acceleration of glycolysis during ammonia assimilation*

At present we have no direct evidence as to the specific identity of the compound(s) which interact with a rate-controlling glycolytic enzyme (or enzymes) during ammonia assimilation. One possibility would be a direct stimulatory effect of ammonium ions on the rate of catalysis by one or more key enzymes of carbohydrate catabolism. Alternatively, the compound actually causing acceleration of catabolism might not be ammonium itself, but another compound whose concentration changes when ammonium assimilation is initiated.

A direct regulatory effect of ammonium on key enzymes of glycolysis is theoretically conceivable, and has sometimes been proposed (e.g. [68]). Both PFK and PK are important rate-controlling enzymes in plant glycolysis. Both are activated by the ammonium ion (cf. [52, 69]). Thus, an elevated intracellular level of ammonium might directly accelerate either or both of these enzymic steps. Against this hypothesis it can logically be argued that direct ammonium activation of glycolytic enzymes would be unlikely if a plant were already adequately supplied with potassium ions. Potassium and ammonium ions tend to have similar effects at the enzyme level (cf. [52]). While either of these ions alone can stimulate the activity of plant PK, there is evidence that ammonium may in fact be somewhat inhibitory in the presence of adequate amounts of potassium [70]. Hence, any interpretation based on direct activation of a glycolytic enzyme by ammonium has to account for the observation that the stimulation of respiration elicited by exposing tissues to ammonium is much greater than the stimulation elicited by potassium or other monovalent cations [45].

As an alternative to a direct ammonium-ion effect, the positive regulation of glycolytic flux during ammonia assimilation could well be based on ammonium induced fluctuations in intracellular concentrations of adenine nucleotides, orthophosphate, or possibly organic acids. As pointed out earlier in this article, both the assimilation of ammonia nitrogen into glutamine and the subsequent transfer of the amide nitrogen to asparagine are ATP utilizing reactions. It is, therefore, quite possible that depletion of ATP may take place in higher-plant tissues during periods of active assimilation/detoxification of ammonia, with a concomitant rise in intracellular concentrations of orthophosphate, pyrophosphate, ADP and AMP. Recently published evidence has documented a drop in cellular ATP levels upon the initiation of nitrogen assimilation in blue-green prokaryotes [71] and yeasts [72]. It is known that adenine-nucleotide and orthophosphate concentrations may play an important role in modulating the activity of the key PFK and PK enzymes. Thus, changes in $P_i$ and adenine-nucleotide levels caused by the initiation of rapid ammonia assimilation might enhance the rate of catalysis by PFK and PK. The activities of PFK and PK are further integrated; phosphopyruvate-induced inhibition of PFK is probably relieved when PK activity increases and the phosphopyruvate level drops in consequence [63].

The mechanism causing an increase in the rate of glycolytic pyruvate formation during ammonia assimilation can only be regarded as conjectural for the time being. Nevertheless, it seems clear than an additional supply of pyruvate, once generated, could be metabolized to α-ketoglutarate via the tricarboxylic acid cycle. Moreover, it is apparent that pyruvate, or its immediate precursor phosphopyruvate, must and indeed will undergo enhanced anaplerotic carboxylation to give malate or oxaloacetate, which can support the synthesis of asparagine via aspartic acid [76] and generally help maintain the levels of cellular organic acids. In order to resolve the question of how carbohydrate catabolism is accelerated during rapid ammonia assimilation, it will be necessary to carry out comprehensive kinetic analyses on the metabolic response of root tissues to ammonia, with particular reference to the alterations in nucleotide, $P_i$, and metabolite levels. This might give some further information as to the control mechanism actually operating to hasten carbohydrate breakdown. It will be of further interest to elucidate the mechanisms by which enhanced anaplerotic $CO_2$ fixation is initiated during periods of high ammonia assimilation. Such fixation is needed to counteract the decline in organic acids, so that the tricarboxylic-acid cycle may continue to function at rates sufficient to support synthesis of ATP needed for ammonia assimilation and for other endergonic cellular processes.

## SUMMARY AND CONCLUSIONS

Plants possess enzymic machinery allowing the assimilation of relatively large amounts of supplied or internally generated ammonia. The ability to assimilate and dispose of excessive levels of ammonia is no doubt useful to the plant as a means of preventing ammonia toxicity.

Low levels of ammonium, such as would normally occur in soils of high nitrifying capacity, or would be produced by nitrate reduction, are currently thought to undergo assimilation largely by the GS/GOGAT pathway. Several lines of evidence support this interpretation,

but a prime consideration is the high affinity of the GS enzyme for $NH_4^+$.

Two additional reactions—the glutamate dehydrogenase and asparagine synthetase reactions—can potentially convert ammonia or ammonium ions to organic nitrogen. These two enzymes have a relatively low affinity for ammonium, but there is a reasonable possibility that they could fulfil an important role in disposing of abnormally high ammonia levels.

Experimental observations made over a period of several decades have indicated that amides are usually the principal nitrogenous products accumulating during periods of ammonia detoxification. The evidence further suggests that the capacity of plants to synthesize amides, and thus to detoxify ammonia, can be critically governed by the availability of carbon substrates, (or possibly ATP). Such availability is determined by the supply of reserve carbohydrate and by the rate of its metabolic degradation. Anaplerotic $\beta$-carboxylation must also play a role in determining rates of amide synthesis. It is known that the rate of carbohydrate catabolism increases greatly during periods of active ammonium assimilation and there is some evidence also for enhanced anaplerotic assimilation of $CO_2$. One important goal of ammonia detoxification research should be to establish the details of the mechanism by which very rapid metabolic adjustments can take place to accommodate the assimilation and disposal of large amounts of ammonia.

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## REFERENCES


1. Nightingale, G. T. (1948) *Botan. Rev.* **14**, 185.
2. Matsumoto, H., Wakiuchi, N. and Takahashi, E. (1971) *Physiol. Plant.* **25**, 353.
3. Vines, H. M. and Wedding, R. T. (1960) *Plant Physiol.* **35**, 820.
4. Puritch, G. S. and Barker, A. V. (1967) *Plant Physiol.* **42**, 1229.
5. Evans, H. J. and Barber, L. E. (1977) *Science* **197**, 332.
6. McParland, R. H., Guevara, J. G., Becker, R. R. and Evans, H. J. (1976) *Biochem. J.* **153**, 597.
7. Fowden, L. (1979) in *Nitrogen Assimilation of Plants* (Hewitt, E. J. and Cutting, C. V., eds.) 6th Long Ashton Symposium. Academic Press (in press).
8. Horsman, D. C. and Wellburn, A. R. (1976) in *Effect of Air Pollutants on Plants* (Mansfield, T. A., ed.) p. 185. Cambridge Univ. Press, Cambridge.
9. Raven, J. A. and Smith, F. A. (1976) *New Phytol.* **76**, 415.
10. Dougall, D. K. (1974) *Biochem. Biophys. Res. Commun.* **48**, 639.
11. Fowler, M. W., Jessup, W. and Sarkissian, G. S. (1974) *FEBS Letters* **46**, 340.
12. Stewart, G. R. and Rhodes, D. (1976) *FEBS Letters* **64**, 296.
13. Lea, P. J. and Miflin, B. J. (1974) *Nature* **251**, 614.
14. Miflin, B. J. and Lea, P. J. (1976) *Phytochemistry* **15**, 873.
15. Santarius, K. A. and Stocking, C. R. (1969) *Z. Naturforsch. Teil B* **24**, 1170.
16. Mitchell, C. A. and Stocking, C. R. (1975) *Plant Physiol.* **55**, 59.
17. Givan, C. V. (1975) *Planta* **122**, 281.
18. Givan, C. V. (1976) *Plant Physiol.* **57**, 623.
19. Rhodes, D., Rendon, G. A. and Stewart, G. R. (1976) *Planta* **129**, 203.
20. Anderson, J. W. and Done, J. (1977) *Plant Physiol.* **60**, 504.
21. Lea, P. J. and Thurman, D. A. (1972) *J. Exp. Botany* **23**, 440.
22. Shepard, D. V. and Thurman, D. A. (1973) *Phytochemistry* **12**, 327.
23. Barash, I., Sadon, T. and Mor, H. (1975) *Plant Physiol.* **56**, 856.
24. Rhodes, D. and Stewart, G. R. (1979) in *Nitrogen Assimilation of Plants* (Hewitt, E. J. and Cutting, C. V., eds.) 6th Long Ashton Symposium. Academic Press (in press).
25. Rognes, S. E. (1975) *Phytochemistry* **15**, 873.
26. Lea, P. J. and Fowden, L. (1975) *Biochem. Physiol. Pflanzen* **168**, 3.
27. Christeller, J. T., Laing, W. A. and Sutton, W. D. (1977) *Plant Physiol.* **60**, 47.
28. Atkins, C. A., Pate, J. S. and Sharkey, P. J. (1975) *Plant Physiol.* **56**, 807.
29. Vickery, H. B., Pucher, G. W. and Clark, H. E. (1936) *Plant Physiol.* **11**, 413.
30. Yemm, E. W. and Willis, A. J. (1956) *New Phytol.* **55**, 229.
31. Weissman, G. S. (1972) *Plant Physiol.* **49**, 138.
32. Barash, I., Sadon, I. and Mor, H. (1974) *Plant Cell Physiol.* **15**, 363.
33. Chibnall, A. C. (1939) *Protein Metabolism in the Plant*. Yale University Press, New Haven, Connecticut.
34. Grosse, W. (1977) *Z. Pflanzenphysiol.* **83**, 249.
35. Arnon, D. I. (1937) *Soil Sci.* **44**, 91.
36. Prianischnikow, D. (1922) *Ber. Dtsch. Bot. Ges.* **40**, 242.
37. Burkhart, L. (1938) *Plant Physiol.* **13**, 265.
38. Platt, S. G., Plaut, Z. and Bassham, J. A. (1977) *Plant Physiol.* **60**, 739.
39. O'Neal, D. and Joy, K. W. (1973) *Nature New Biol.* **246**, 61.
40. Miflin, B. J. (1974) *Plant Physiol.* **54**, 550.
41. O'Neal, D. and Joy, K. W. (1974) *Plant Physiol.* **54**, 773.
42. O'Neal, T. D. and Joy, K. W. (1975) *Plant Physiol.* **55**, 968.
43. Givan, C. V. and Harwood, J. L. (1976) *Biol. Rev.* **51**, 365.
44. Leech, R. M. and Murphy, D. J. (1976) in *The Intact Chloroplast* (Barber, J., ed.) p. 365. Elsevier, Amsterdam.
45. Willis, A. J. and Yemm, E. W. (1955) *New Phytol.* **54**, 163.
46. Syrett, P. J. (1956) *Physiol. Plant.* **9**, 19.
47. Bergmann, L., Grosse, W. and Koth, P. (1976) *Z. Pflanzenphysiol.* **80**, 60.
48. Atkinson, D. E. (1966) *Annu. Rev. Biochem.* **35**, 85.
49. Matsumoto, H. and Wakiuchi, N. (1974) *Z. Pflanzenphysiol.* **73**, 82.
50. Wakiuchi, N., Matsumoto, H. and Takahashi, E. (1971) *Physiol. Plant* **24**, 248.
51. Beevers, H. (1974) *Plant Physiol.* **54**, 437.
52. Turner, J. F. and Turner, D. H. (1975) *Annu. Rev. Plant Physiol.* **26**, 159.
53. ap Rees, T. (1974) *MTP Int. Rev. Sci. Biochem.* **11**, 89.
54. Fowler, M. W. (1971) *J. Exp. Botany* **22**, 715.
55. Kobr, M. J. and Beevers, H. (1971) *Plant Physiol.* **47**, 48.
56. Faiz-ur-Rahman, A., Trewavas, A. J. and Davies, D. D. (1974) *Planta* **118**, 195.
57. Shimizu, T., Clifton, A., Komamine, A. and Fowler, M. W. (1977) *Physiol. Plant* **40**, 125.
58. Givan, C. V. (1968) *Plant Physiol.* **43**, 948.
59. Givan, C. V. (1972) *Planta* **108**, 29.
60. Hatch, M. D. and Turner, J. F. (1958) *Biochem. J.* **59**, 495.
61. Greenway, H., Setter, T. and Hackling, K. (1977) *J. Exp. Botany* **28**, 534.
62. Dennis, D. T. and Coultate, T. P. (1966) *Biochem. Biophys. Res. Commun.* **25**, 187.
63. Kelly, G. J. and Turner, J. F. (1969) *Biochem. J.* **115**, 481.
64. Tomlinson, J. D. and Turner, J. F. (1973) *Biochim. Biophys. Acta* **329**, 128.
65. Givan, C. V. (1974) *Phytochemistry* **13**, 1741.
66. Kanazawa, T., Kanazawa, K., Kirk, M. R. and Bassham, J. A. (1972) *Biochim. Biophys. Acta* **256**, 656.
67. Stephan-Sarkissian, G. and Fowler, M. W. (1978) *Biochem. Physiol. Pflanz.* **172**, 1.
68. Steer, B. T. (1974) *Plant Physiol.* **54**, 758.
69. Miller, G. W. and Evans, H. J. (1957) *Plant Physiol.* **32**, 346.
70. Peterson, J. B. and Evans, H. J. (1978) *Plant Physiol.* **61**, 909.
71. Ohmori, M. and Hattori, A. (1978) *Arch. Microbiol.* **117**, 21.
72. Cocucci, M. C. (1977) *Plant Cell Physiol.* **18**, 1217.

73. Keys, A. J., Bird, I. F., Cornelius, M. J., Lea, P. J., Wallsgrove, R. M. and Miflin, B. J. (1978) *Nature* **275**, 741.
74. Kirkby, E. A. (1968) *Soil Sci.* **105**, 133.
75. Lawrie, A. C. and Wheeler, C. T. (1975) *New Phytol.* **74**, 437.
76. Paul, J. S., Kornwell, K. L. and Bassham, J. A. (1978) *Planta* **142**, 49.
77. Skokut, T. A., Wolk, C. P., Thomas, J., Meeks, J. C., Shaffer, P. W. and Chien, W.-S. (1978) *Plant Physiol.* **62**, 299.

## NOTE ADDED IN PROOF

Recent work [77] on $^{13}NH_4^+$ assimilation in cultured *Nicotiana tabacum* cells, ±inhibitors of GS and GOGAT, indicates operation of the GS/GOGAT pathway under all conditions, but probably with increased assimilation via GDH in $NH_4^+$-grown cells. No significant role is indicated for asparagine synthetase in primary assimilation.