Phytochemistry, Vol 26, No 3, pp 597–610, 1987
Printed in Great Britain

0031-9422/87 $3.00 + 0.00

REVIEW ARTICLE NUMBER 23

ROLE AND REGULATION OF L-GLUTAMATE DEHYDROGENASE ACTIVITY IN HIGHER PLANTS

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(*Received* 7 *July* 1986)

Key Word Index—Amino acid synthesis, ammonia assimilation, enzyme activity, enzyme regulation, higher plants, L-glutamate dehydrogenase

Abstract—L-Glutamate dehydrogenase catalyses the reversible conversion of 2-oxoglutarate and L-glutamate for the entry of ammonium into the organic cycle and for its release as well. Various isozymes of GDH are present ubiquitously in higher plant tissues. The enzyme, with a molecular weight of 208 000 to 270 000, is composed of four to six subunits, contains a free -SH group at the active centre, and is associated with metal ions. Some isozymes of GDH are inducible and vary according to the nutritional and environmental status of the tissues. The level and activity of enzyme in either direction is regulated by age, light/dark regime, inorganic and organic nitrogen, carbon and energy status, growth regulators and some other factors. The enzyme seems to be important in assimilation of ammonia under stress conditions such as dark starvation, high temperature, salinity, water stress, environmental pollution, senescence and other abnormalities.

INTRODUCTION

The inorganic nitrogen acquired by plants is ultimately converted to ammonium before being incorporated into organic molecules. Although several biochemical reactions involving ammonium as a reactant are known [1], the reductive amination of 2-oxoglutarate to glutamic acid has long been considered as a major route of ammonia assimilation. This reversible reaction is catalysed by the enzyme L-glutamate dehydrogenase [L-glutamate $NAD^+/NAD(P)^+$ or $NADP^+$ oxidoreductase (deaminating) GDH, EC 1.4.1.2–4]. In the seventies, however, the discovery of a new enzyme, glutamate synthase, from bacteria [2] and leaves of higher plants [3] changed this concept and it is now recognized that ammonium is first incorporated into glutamine by the action of glutamine synthetase (L-glutamate ammonia ligase, GS, EC 6.3.1.2) and subsequently into glutamic acid by glutamate synthase [L-glutamate, $NAD(P)^+$/ferredoxin oxidoreductase, GOGAT, EC 1.4.1.13–14 and 1.4.7.1] [4].

The path of ammonia assimilation, including studies on GDH, has been reviewed [1, 4–10]. However, the physiological role of large amounts of GDH present in the tissues of higher plants is still obscure. Although the GS-GOGAT pathway is considered to be the major route for ammonia assimilation in plants under normal growth conditions, the role of GDH under some environmental and nutritional conditions cannot be excluded and, therefore, the possible factors under which GDH may play a significant aminating or deaminating role in cell metabolism are yet to be discussed. Moreover, numerous new observations have appeared following the review on plant GDH by Stewart *et al.* [9]. Our aim in this article is to review the literature on higher plant GDH and to evaluate the possible physiological role of the enzyme in the nitrogen metabolism of higher plants, under different environmental and nutritional conditions.

DISTRIBUTION AND LOCALIZATION

L-Glutamate dehydrogenase has been found to be present universally in almost all types of organisms from microbes to higher plants and animals. In higher plants, the enzyme activity has been detected in almost all species tested.

Organ specific spectra

Although the enzyme is frequently distributed in various plant parts, its level and behaviour appear to be organ specific. The aminating (NADH-dependent) enzyme activity is higher in roots than in leaves of *Zea* [11, 12], *Secale* [13] and *Hordeum* [14]. In *Pisum* and *Pastinaca*, high GDH is found in the area of rapid growth and high phytochrome content [15] and in *Arachis* seedlings, the enzyme activity is higher in the cotyledons than in root or shoot portions [16]. Further, the developing pods and seeds of *Phaseolus* and *Cajanus* possess higher GDH activity than the leaves [17]. It may be noted that the concentration of ammonium in the seeds of *Cajanus* is also greater than that in the leaves. The enzyme activity also varies according to the age and tissue composition of the organ. For example, in *Zea* roots the enzyme is much more active in mature regions [18, 19] and in meristems than in apical regions [18]. In developing *Hordeum* grains, GDH activity is largely confined to

the endosperm in contrast to GS which is mostly in the testa pericarp [20].

The enzyme present in different tissues may be different in its isozymic pattern and consequently in the regulatory nature. In *Triticum*, the predominant form of the enzyme in senescent leaves is a different isozyme than the one present in young leaves [21]. In *Ricinus*, the leaf chloroplast and root enzymes segregate from the endosperm enzyme on polyacrylamide gel electrophoresis [22]. Organ specific isozymes are also reported in *Medicago*, although they show similar general and kinetic properties [23]. In *Glycine* roots and nodules, the enzyme is regulated differentially by urea [24]. Further, the patterns of GDH isozymes are different in nodules and roots of *Lupinus* [25]. Loyola-Vargas and de Jimenez [26] have reported the presence of different isozymes/conformers of GDH in root, callus and leaves of *Zea* which may vary depending on the nutritional requirement and state of differentiation. It has been suggested that the characteristic spectra of GDH isozymes in the endosperm, embryo and pericarp of *Zea* may be due to differential activity of genes controlling the enzyme syntheses in each tissue [27].

Cellular distribution

In *Zea*, *Gomphrena* and *Sorghum*, NADH-GDH is found to be localized in bundle sheath cells [28]. Further, in various C_4-species 69–87% of NADH-GDH is equally distributed between mesophyll and bundle sheath cells [29]. This finding is further confirmed by Harel *et al.* [30] in greening *Zea* leaves and by Moore and Black [31] in *Digitaria* leaves. In nodulated plants the relative distribution of GDH shows three possible types of patterns (i) the enzyme is distributed equally in cytosol and bacteriod fractions in *Lupinus* [32], (ii) it is primarily located in the cytosol fraction in *Glycine* [33] and in *Medicago* [34] and (iii) it is higher in bacteriods than in cytosol in *Phaseolus* [35]. A histological study shows that a higher level of GDH is present in the cells surrounding the nodules in *Lupinus* roots [36].

Intracellular localization

Various studies, using differential centrifugation and density gradient techniques, have demonstrated a mitochondrial location of the enzyme [22, 36–47]. Since the enzyme is easily dissolved, it is thought to be localized in the dissolved matrix of the mitochondria [48–50]. However, the method of disrupting mitochondria involved a freeze/thaw treatment followed by sonication in most cases, which could easily dissolve the membranes. Yamaya *et al.* [46] using a gentle disruption method (osmotic shock) demonstrated that the enzyme is loosely bound with the mitochondrial membrane. On the basis of Arrhenius plots of GDH activity from 5-day-old *Glycine* axes and its analogy to that of *Vigna* succinate oxidase (both consist of three phases), Duke *et al.* [51] deduced that GDH is associated with membrane lipids. A separate GDH species differing in certain properties from the mitochondrial enzyme has been characterized from the chloroplasts of many plants [22, 29, 39, 42, 52–54]. This enzyme is shown to be tightly bound to the chloroplast lamellae, and cannot be released by osmotic shock. It can, however, be dissolved by a detergent treatment [52]. There is some evidence for association of GDH activity with the plastids of roots as well [55, 56]. Many workers have reported the occurrence of GDH activity in the supernatant fraction in addition to mitochondrial fraction [18, 54, 57, 58]. It has been found that enzymes from both fractions possess similar physical and kinetic properties, and hence it is suggested that the cytoplasmic enzyme is due to leakage from mitochondria [57]. However, evidence for a separate cytoplasmic enzyme differing in certain kinetic properties and isozyme pattern has been demonstrated by some workers [18, 41, 58].

STRUCTURE AND KINETIC PROPERTIES

Structure and isozyme pattern

The structure of GDH from animal and fungal systems has been extensively studied and the literature has been adequately reviewed [5, 10]. A general account of the plant enzyme was also presented by Stewart *et al.* in 1980 [9]. The present discussion, therefore, will be limited to more recent developments in the area. The plant enzyme is thought to be a metalloprotein having an M_r in the range 208 000–270 000 [4]. The enzyme is composed of identical subunits having M_r in the range of 46 000–58 500. There is some discrepancy in the literature regarding the number of subunits. As six electrophoretic bands appear after cross-linking of the enzyme with diimidates from *Pisum* seeds, the enzyme is suggested to be a hexamer [59]. However, Scheid *et al.* [60] using sodium dodecyl sulphate gel and by EM observations have demonstrated that GDH from *Lemna* and *Pisum* seeds is a tetramer (M_r 230 000) with four identical subunits (M_r 58 500). Although the hexameric and tetrameric configurations of the enzyme have been reported from various organisms [9], further investigations using modern techniques are required to elucidate whether enzymes of two different configurations exist in various plant tissues. Scheid *et al.* [60] have reported that the amino acid composition of the tetrameric enzyme from *Lemna* and *Pisum* is similar to those of various hexameric GDHs obtained from other organisms and the *N*-terminal amino acid of the *Pisum* enzyme is alanine. Further, the enzyme is considered to contain free -SH groups at active centres [61] which are apparently more abundant in the enzyme from light grown *Phaseolus* leaves than in those from dark grown leaves [62]. The presence of Zn^{2+} as an associated metal ion is reported for *Zea* root [63] and *Lycopersicum* chloroplast [64] enzymes. Other divalent cations such as Ca^{2+}, Mn^{2+}, Co^{2+}, Fe^{2+} also activate the enzyme activity [9].

Isozymic number of the enzyme varies with plant species as well as other nutritional and environmental conditions. Seven isozymic forms have been reported in *Pisum* [60, 65, 66], *Phaseolus* [38], *Vicia* [67] and *Ricinus* [68]. The number of isozymes, however, is increased by the addition of ammonium [25, 58, 69–71] and amino acids [25, 72], and decreased by the addition of sucrose [25]. Fourteen isozymes have been reported in *Pisum* [73], *Medicago* [74] and *Lupinus* [25] and the number increases to 17 in young *Pisum* shoots preincubated with tap water [49]. The number and banding pattern of isozymes are also influenced by growth stages [25, 65, 74], plant organs [23, 53], nitrogen nutrition [25, 58, 70–72], light–dark regime [70, 75–77], carbohydrate status [25, 66, 78] and effective symbiosis with *Rhizobium* [25, 79].

In legumes, it has been suggested that the GDH isozyme pattern is the result of an adaptation of the cell to nitrogen metabolism [25, 72]. Individual plant organs may develop a typical GDH isozyme pattern for each organ during the growth stages which may be correlated with the known metabolic activities of those organs as well as with external nutritional and environmental conditions available to the plant. This reveals a physiological role of GDH isozymes in the regulation of nitrogen metabolism. A hypothesis has been proposed for the anabolic and catabolic role of GDH isozymes in plants [25, 68, 73, 74] and Masurowa *et al* (25) have demonstrated in *Lupinus* that anabolic and catabolic forms of GDH isozymes may be present in two types of mitochondria, heavy mitochondria sedimenting at 3000 *g* with bacteriod fraction of the nodules contain anabolic isozymes, and light mitochondria sedimenting at 20000 *g* contain both anabolic and catabolic isozymes. Differences in enzymatic content between heavy and light mitochondria [80] and the presence of respiratory and synthesizing mitochondria [81] from higher plant tissues have also been shown. Further, evidence for glutamate synthesis in isolated mitochondria has also been presented [46, 49, 82]. However, in *Medicago* similar kinetic properties of the two forms of GDH isozymes, previously proposed as forms reflecting different physiological functions [23, 74] suggest that this hypothesis may not hold universally, or even in legumes. Hence the proposal of Masurowa *et al* [25] should be tested carefully in other plants also. *Pisum* GDH isozymes are reported to be the same molecule with different types of subunits [83]. On the basis of gene analysis, it has also been suggested that two non-allelic genes are responsible for the synthesis of two types of subunits which could arbitrarily associate in a hexameric complex giving rise to several isozymes [84]. However, the existence of definite isozymes in *Ricinus* which do not change after reprecipitation and reelectrophoresis of the enzyme [68] and the presence of identical subunits in the *Lemna* and *Pisum* enzymes [60] do not support this idea.

Coenzyme specificity, reaction mechanism and kinetic behaviour

Multiple forms of steady state and inducible GDH enzyme from various organs of higher plants can use NADH and NADPH in aminating and NAD^+ as well as $NADP^+$ in deaminating, as coenzymes. Most of the studies reveal that the enzyme utilizes NADH and NAD^+ preferentially over NADPH and $NADP^+$ to varying degrees [8, 22, 68, 85–89]. In developing seeds of *Glycine* three forms of GDH having different nucleotide specificity have been identified [53]. GDH-1, a seed enzyme, is active with both NADH and NADPH in aminating reaction and readily uses NAD^+ and $NADP^+$ in deamination, GDH-2, a chloroplastic enzyme prefers NADPH in amination but NAD^+ in deamination while GDH-3, a mitochondrial enzyme, is specific for NAD^+.

The reaction mechanism of plant GDH is proposed as the compulsory ordered binding of NADH, 2-oxoglutarate and ammonia followed by the ordered release of glutamate and NAD^+ and vice versa for either direction [90–92]. However, a partially random [23, 93] and even a fully random [94] mechanism for the GDH catalysed reaction have also been suggested. The kinetics of GDH catalysed reactions have been examined in several plants [9]. It has been indicated that the aminating enzyme has an unusually high K_m for ammonium (ranges from 5.2–70.0 mM), the fact often used to question the aminating role of the enzyme in plant tissues [1, 6]. However, more recent studies reveal that the K_m values for NH_4^+ and even for other substrates may be influenced by various external and internal factors and the values estimated may not be a true reflection of what might be happening *in situ*. It has been shown that the catalytic efficiency of GDH increases at low ammonium and low NADH concentrations, i.e. $K_{NH_4^+}$ decreases by many fold [22, 46, 49]. The apparent K_{NADH} values are also reduced at low NADH [23, 46], and considerably at low ammonium concentrations [22]. It may be noted therefore that various levels of substrates for aminating activity reveal a complex kinetic behaviour of the enzyme which is highly adaptive for the substrate level. The kinetics of the amination reaction of *Lupinus* nodule enzyme are also influenced by accompanying anions of NH_4^+ salts, as normal Michaelis Menton kinetics were obtained with $(NH_4)_2SO_4$ while NH_4Cl and ammonium acetate showed substrate inhibition [95, 96]. It has been suggested that anions bind to an allosteric site on the enzyme and cause a change in some of the rate constants of the reaction, i.e. decrease the rates of association of NADH and 2-oxoglutarate with the enzyme and increase the rate of dissociation of NAD^+.

It has also been suggested that SO_4^{2-} added as $(NH_4)_2SO_4$ acts as an activator of the enzyme [97]. Further, $K_{NH_4^+}$ is decreased about seven-fold when the pH is increased from 7.3 to 9.0 in green *Nicotiana* callus [98]. In *Phaseolus* leaves GDH has lower $K_{NH_4^+}$ value for the enzyme from dark grown leaves than that from light grown and it has been suggested that a more active GDH species is generated in the dark [99]. Nesselhut and Harnischfeger [100] and Furuhashi and Takahashi [98] have indicated that the actual substrate for GDH may be NH_3 rather than NH_4^+ and replotting of the data for NH_3 concentrations reduces the K_{NH_3} value. However, it is difficult to conclude that a real substrate for plant GDH is NH_3. The speed with which ammonia reacts with water makes it difficult to examine this hypothesis. However, the association of GDH with mitochondria may provide a lipid-rich 'micro-climate' in which NH_3 is more readily available to the dehydrogenase [82].

Saturation curves for three substrates to NADH-GDH showed normal Michaelis Menton kinetics in the presence of 1 mM Ca^{2+}, and in the absence of Ca^{2+} a marked substrate inhibition was observed [46, 98]. Since, plant mitochondria contain sufficient Ca^{2+} for this activation [46] it is evident that plant GDH is already activated with regard to Ca^{2+}. Moore and Akerman [101] have suggested that Ca^{2+} is a modulator of the mitochondrial NADH dehydrogenases *in vivo*, as Ca^{2+} increases NADH-oxidation in *Helianthus*. These observations, however, reveal a complex kinetic behaviour of the enzyme in plant mitochondria and its integrated function needs further clarification.

REGULATION

Plant age and rhythmicity

Levels of plant GDH vary according to plant age. A general increase in enzyme activity has been observed by several workers in roots [25, 102, 103], hypocotyl

[25], cotyledons [67], shoots [103] and leaves [99, 102, 104–106], during their initial growth phase. However, the period taken for enzyme induction appears to vary with plant species and is possibly affected by other factors as well. On the other hand, in plant cellcultures behaviour of the enzyme seems to be different. For example, it remains unchanged during the initial growth period in *Nicotiana* [107] and *Chenopodium* [108] cell cultures and increases during later phases, i.e. after 33 days. Further, in *Zea* [26] and in *Saccharum* [109] callus, GDH activity decreases considerably during the initial period of growth and differentiation on MS medium, but for non-shoot-forming callus of *Saccharum*, GDH activity increases considerably between 4–9 days of incubation and replaces the GS-GOGAT route for ammonia assimilation [109]. In *Lupinus* seedlings, individual organs develop a GDH isozyme pattern typical for the metabolic requirement of each organ during growth [25].

The enzyme is least studied in mature plants; in *Citrus* trees, GDH levels increase at the time of flowering and fruiting when the plants are supplied with nitrate as an N-source [110]. Further, in *Vigna* genotypes associated with different rhizobial strains the enzyme activity increases considerably at early flowering and early pod filling stages. This increase is most pronounced in genotype K_{851} associated with strain S_{24} [111]. However, in *Arabidopsis* roots and leaves both NADH- and NAD^+-GDH activities remained relatively constant until the development of flower buds and then increased, accompanied by a gradual decrease in GS activity [106].

GDH activity also changes during seed development. In *Triticum*, enzyme activity starts increasing 15 days after anthesis and reaches maximum at 40 days with three conspicuous peaks at 25, 40 and 55 days [20]. During development of *Pisum* embryos, the enzyme activity reaches its highest point in the seed coat at 27 days and in the cotyledons at 40 days [112]. A higher enzyme concentration in the seed coat is possibly associated with a higher ammonium content, as has been reported in *Phaseolus* [113], *Lupinus* [114] and *Pisum* [115, 116]. In different mutants of *Zea*, GDH activity increased during kernel development between 15 and 35 days after pollination, the period of intensive synthesis of storage protein [117]. The increase is, however, more pronounced in an opaque-2 mutant than in normal maize. It has been suggested that the opaque-2 gene increases GDH activity along with GOT and GPT and decreases lysine catabolism in the developing endosperm [117].

An increase in GDH activity during leaf senescence appears to be a general feature [77, 106, 118–122]. The increase is reported to be due to *de novo* synthesis of the enzyme in *Hordeum* during the first hour of senescence [121]. It has been suggested that ammonia produced by proteolysis during senescence could be responsible for *de novo* synthesis of the enzyme [71, 76, 118, 123]. It has been demonstrated recently that an increase in GDH activity follows a similar control mechanism which governs the course of senescence, and kinetin, which retards senescence, also retards the increase in GDH activity [122]. Since GS-GOGAT enzymes decrease rapidly during senescence [122], the activity of GDH may possibly serve as an adaptation of leaves to detoxify the ammonium generated during senescence. The considerable increase in NAD^+-GDH (deaminating) during leaf senescence [106] demonstrates that the enzyme also operates in the direction of energy generation. Since the proline content also increases greatly in detached *Oryza* leaves during senescence [124], a part of the increase in GDH activity in either direction, i.e. aminating or deaminating, may be caused by proline accumulation, as it has been shown in excised *Zea* leaves that total NADH- and NAD^+-GDH activity increases considerably when proline is added to the medium [125]. Seasonal variations have also been recorded for GDH activity. In *Pyrus*, the highest enzyme activity is found in leaves and wood in October, and in stem bark in May [126]. In *Deschampsia* and *Zerna* species growing on acidic or calcareous soils, maximum levels of GDH activity are observed in February/March followed by a decline to a lower level which remains fairly constant throughout the year [127].

In light grown *Pisum* cell cultures a 48 hr rhythm is observed for GDH activity which is, however, irregular in the dark grown cells [128]. A circadian rhythm, in *Pisum* roots [129] and in *Triticum* leaves (104) and ultradian rhythms in *Chenopodium* seedlings [130] have also been reported. In *Glycine* and *Zea* seedlings Duke *et al* [51] demonstrated some correlation of peaks in GDH activity with soluble protein content of the seedling, reflecting that the periodicity in enzyme activity is of physiological significance. They also believe that dark stimulated activity of GDH may be part of a circadian periodicity [51]. Further, it has been suggested that phytochrome level oscillations modulate circadian rhythm by coupling with the high frequency oscillation of various biochemical and physiological functions [131, 132] and the rhythm is presumably due to alternations in the anabolic and catabolic balance of organic compounds [133].

Light, radiation and photoperiod

Light has shown variable effects on GDH activity in different plant species. In *Pisum* roots [134], *Cucumis* cotyledons [135] and the leaves of *Triticum* [77, 104], *Cucurbita* [76], *Syzygium* [136], *Arabidopsis* [106] and *Phaseolus* [62, 137], enzyme activity is increased in darkness. The inhibitory effect of light on GDH is often explained in terms of increased carbohydrate level in the tissue, as exogenously supplied sucrose and some other sugars inhibit enzyme activity [49, 106, 134, 138–142]. Postius and Jacobi [76] observed a parallel increase in GDH activity and endogenous ammonium concentration during dark stress in *Cucurbita* leaves and concluded that ammonium is the inducing factor during darkness. It has also been shown that dark alters the isozyme pattern of GDH [75, 143] and induces *de novo* synthesis of one isozyme, responsible for this alteration [77], which is identified as the same isozyme which is synthesized when exogenously supplied ammonium [71]. However, in *Phaseolus* leaves the increase in enzyme activity in darkness is observed in both the absence and presence of NH_4^+-salt, and the dark induced enzyme is apparently different in its kinetic constants for various substrates, in elution profile through Sephadex G-200 [99] and in its reaction to thiol modulating compounds such as DTT and DTNB [62]. It has been proposed that darkness induces the synthesis of a more active GDH species which has lower $K_{NH_4^+}$ than the enzyme synthesized in light [62, 99].

On the other hand, in *Avena* leaves, NH_4^+-dependent induction of a new isozyme of GDH is reported to be proportional to the light intensity and the induction is inhibited by DCMU [85]. Kar and Feierabend [122] have also shown that the senescence-induced increase in leaf

GDH activity in *Triticum* is higher in light than in the dark. A light dependent NAD^+-GDH is also demonstrated in *Arabidopsis* [106]. Further, in *Pisum* and *Pastinaca* roots, GDH activity increases after illumination with far-red [15]. The duration of the photoperiod also seems to affect the enzyme activity. In *Urtica* leaves [133] and in *Linum* roots [102], longer photoperiods increase the enzyme activity but in *Linum* leaves the activity is not affected by the change in photoperiod [102]. Although the mechanism of this increase has not been followed it appears that the regulation of GDH by light/dark is a complex process and different isozymes of GDH may be synthesized in either condition depending on the metabolic status of the cell. If ammonia accumulates in higher amounts it induces detoxification by the GDH isozyme in both dark and in light.

Inorganic nitrogenous salts

The supply of ammonium, nitrate and urea as nitrogen sources influences GDH activity in plants. Ammonium generally increases enzyme activity, although in a few cases it has either no effect or even inhibits the enzyme activity (Table 1). Apparently the variable effects of ammonium are determined by several other factors such as duration of ammonium supply, tissue studied, age of seedlings as well as presence of other salts and metabolites.

Table 1. Effect of nitrogen salts on glutamate dehydrogenase activity

Nitrogen source (mM)		Duration of incubation (d/hr)	Plant tissue	Function of the enzyme activity	Effect	References
Ammonium,						
up to	14.3	2 d	*Oryza* roots	Aminating	Increase	[58]
	15.0	3 d	*Lemna* plantlets			[148]
	15.0	3 d	*Avena* leaves			[149]
up to	21.5	3 d	*Rosa* callus			[145]
	10.0	5 d	*Lemna* plantlets			[150]
	7.0	3 d	*Lupinus* embryonic axes			[141]
	10.0	4 d	*Zea* roots and shoots			[12]
	9.0	14 d	*Citrus* roots and leaves			[110]
	15.0	3 d	*Triticum* leaves			[71]
	10.0	9 d	*Zea* leaves	Aminating and deaminating		[12]
	6.0	15 d	*Zea* roots and shoots			[103]
	20.0	3 d	*Zea* roots			[26]
	15.0	3 d	*Arabidopsis* leaves			[106]
	15.0	3 d	*Arabidopsis* roots	Aminating		[106]
	10.0	4 d	*Zea* roots and shoots	Deaminating	Decrease	[12]
	10.0	9 d	*Zea* leaves			[12]
	15.0	3 d	*Arabidopsis* roots			[106]
	20.0	3 d	*Zea* leaves	Aminating and deaminating		[26]
	20.0	3 d	*Zea* callus			[26]
	10.0	4 d	*Glycine* cell cultures	Aminating	No effect	[146]
	20.0	18 hr	*Pisum* shoots			[49]
	10.0	5 hr	*Zea* roots			[19]
	10.0	3 hr	*Zea* roots and shoots			[12]
	8.0	20 d	*Hordeum* roots			[14]
	20.0	3 d	*Zea* roots			[26]
	9.0	14–42 d	*Citrus* roots and leaves		Increase	[110]
Nitrate,	3.0	16 d	*Plantago* roots and leaves			[151]
	6.0	15 d	*Zea* roots and leaves			[110]
	10.0	9 d	*Zea* leaves			[12]
	10.0	3 hr	*Zea* roots and leaves	Deaminating		[12]
	10.0	3 d	*Zea* leaves			[26]
	10.0	5 hr	*Zea* roots	Aminating	Decrease	[19]
	40.0	3 d	*Zea* roots and leaves			[26]
	20.0	5 d	*Phaseolus* leaves			[152]
	10.0	3 hr	*Zea* roots and leaves	Deaminating		[12]
	40.0	3 d	*Zea* callus	Aminating and deaminating		[26]
	10.0	4 d	*Zea* roots	Aminating	No effect	[120]
	10.0	3 d	*Arabidopsis* roots and leaves			[106]
	10.0	3 hr	*Zea* shoots and leaves			[12]
up to	14.3	4 d	*Oryza* roots			[58]
up to	8.0	20 d	*Hordeum* roots			[14]
Urea	10.0	20 d	*Glycine* roots	Deaminating	Increase	[24]
	8.0	3 d	*Zea* roots and leaves			[26]
	8.0	3 d	*Zea* roots and leaves	Aminating	No effect	[26]
	8.0	3 d	*Zea* callus	Aminating and deaminating	Decrease	[26]
	10.0	20 d	*Glycine* nodules			[24]

etc. In *Lupinus* embryonic axes, NH_4^+ caused no effect during a 24 hr incubation but increased enzyme activity during 72 hr treatment [141]. Further, in *Zea* roots, shoots and leaves a continuous prolonged supply of ammonium caused increase supply than a 3 hr treatment [12]. However, in the roots of mature *Citrus* plants, ammonium increased the enzyme level only during the first 2 weeks treatment, and thereafter, up to 6 weeks, there was no significant effect [110]. Differential effects of ammonium on different tissues such as callus, nodules, roots, shoots and leaves have also been demonstrated by various workers [12, 24, 26, 106]. For example, in *Glycine*, ammonium, nitrate or urea increased root enzyme while the same sources decreased nodule GDH activity [24]. Further various forms of GDH activity, i.e. NADH-, NADPH- and NAD^+-dependent activities respond differently even in primary and secondary leaves of the same age [12].

The effects of ammonium vary in magnitude with different concentrations of ammonium in most cases [58, 89, 145]. However, in *Glycine* cell culture [146] and in some grassland species [147] the concentration response is not so marked and it appears, therefore, that some plants, tissues are not adapted to changes in available ammonia. It is also evident from the above-mentioned studies that not only the nature but also the magnitude of the effect of ammonium ions on GDH is affected by many other factors as well. The effect of ammonium upon the specific functions of GDH activity also varies, as it increases NADH-GDH activity in *Zea* roots, while decreasing NAD^+-GDH activity [12]. Loyola-Vargas and de Jimenez [26] have also shown different responses of two activities, in various *Zea* tissues, to a supply of NH_4^+ and suggested that the NAD^+:NADH ratio tends to decrease with the supply of ammonium and urea. This enzyme behaviour reflects a complex mechanism for ammonium regulation.

Ammonium may increase enzyme activity by increasing the amount of enzymic protein or/and by modulating the activity of existing enzyme molecules. *De novo* synthesis of GDH in response to ammonium has been demonstrated in *Lemna* [148] and in *Arabidopsis* plantlets [106], *Oryza* roots [58], *Avena* [70] and *Triticum* leaves [71]. Evidence for *de novo* synthesis of the enzyme protein has been derived from time course studies, the supply of protein synthesis inhibitors, density and radioactive labelling techniques, gel electrophoresis [58, 70, 106, 148] and more recently using immunochemical approaches [71]. The synthesis of new isozyme is believed to take place at 80S ribosomes and an increase in enzyme is observed only in the soluble fraction and not in the mitochondrial fraction [58]. Further, the inhibitors of 70S ribosomes, such as D-threo-chloramphenicol, lincomycin and erythromycin, do not prevent the rise in GDH of *Lemna* [148] whereas puromycin [148] and cycloheximide [12], inhibitors of protein synthesis at 80S ribosomes, inhibit ammonium induced increases in *Lemna* and *Zea*, respectively.

It is believed that a new isozyme appears to detoxify the excess ammonium absorbed by the tissue because its accumulation is harmful to cell metabolism [123]. Moreover, the positive effect of ammonium on enzyme activity may not involve only *de novo* synthesis of the enzyme, as the increase in activity is also observed during *in vitro* incubation of the enzyme with ammonium [12]. Further, the inhibitors of protein synthesis are able to block increases in enzyme activity only to a limited extent, after a prolonged treatment of 2 days [148]. The aminating and deaminating activities of the enzyme also respond in different manners in *Zea* [12, 26]. However, in *Arabidopsis*, cycloheximide completely suppresses the ammonium-induced increase in the enzyme [106]. Although the exact mechanism of enzyme activation by ammonium is not known, the possibility of an allosteric regulation cannot be ruled out [12]. At least *Neurospora* $NADP^+$-GDH shows a complex interaction with NH_4^+ and it is suggested that ammonium is acting both as a substrate and as an activator of the enzyme [153]. A similar action of ammonium on higher plant enzyme may also be proposed because it has been shown that $K_{NH_4^+}$ for the enzyme is modified by the concentration of NH_4^+ itself (see kinetic behaviour). However, further experiments using modern techniques are required to elucidate the proposed mechanism.

The effect of nitrate on GDH activity also varies with species, tissue, time of incubation and some other factors (Table 1). Generally nitrate is either inhibitory [12, 19, 154] or has no effect [14, 43, 58] on enzyme activity during short term supply to excised tissues. However, prolonged treatment of intact seedlings with nitrate increases enzyme activity in various plants [12, 103, 151, 155]. It is likely that the stimulatory effect of nitrate during prolonged treatment is indirect, possibly through ammonium produced from the reduction of nitrate. It is also evident from various studies that the effect of nitrate is not as pronounced as that of ammonium [12, 103, 156, 157]. However, in one study, the presence of flowers and fruits caused the GDH level in mature *Citrus* plants receiving NO_3^- to rise to a level similar to those receiving ammonium [110]. In *Plantago lanceolata*, a species from a relatively nutrient-poor habitat, NADH-GDH increased during 4 days after a switch from 2% to 100% NO_3^--N nutrition [151], whereas in *Plantago major*, a grassland species from relatively nutrient rich habitat, it remained relatively constant [158]. Urea, another nitrogenous nutrient, is less studied, probably because it is easily hydrolysed to produce ammonium and thus its effects are expected to be the same as those of ammonium. This expectation, has, in fact, been realized in *Glycine* and *Zea* [24, 26].

Amino acids, amides and amino acid analogues

Amino acids have been reported to variably affect both aminating and deaminating activities of GDH in various tissues of plants. Glutamate increases the aminating enzyme activity in *Lemna* [150] and the deaminating enzyme activity in *Zea* roots [125] and leaves [125, 159]. However, it hardly affects aminating activity of the enzyme in *Lemna* [148] and *Pisum* shoot [49] or even inhibits the same in *Lemna* plantlets [160, 161], *Pisum* roots [162], *Camellina* rootlets [163], *Zea* roots [26], *Zea* leaves [125], *Lemna* plantlets [150], *Lupinus* embryonic axes [141] and deaminating enzyme activity in *Zea* roots [26, 125] and leaves [125]. It has been suggested that glutamine would exert a positive control on GDH but a negative one on GS, and therefore it may determine the entry of ammonia via either pathway [141, 150, 156]. On the other hand, glutamine inhibits NADH-GDH in *Zea* roots [125], and both activities (NADH and NAD^+) in *Zea* leaves and callus tissue [26] possibly because of some

other interactions. Among other amino acids, threonine and alanine increase aminating enzyme activity in *Camellina* rootlets [163]. Aspartate also increases aminating enzyme activity in *Lemna* plantlets [150] and deaminating enzyme activity in *Zea* roots and leaves [125]. However, it has no effect on aminating enzyme activity of *Zea* leaf tissue and inhibited it in roots [125]. Asparagine increases aminating GDH activity in *Lupinus* embryonic axes [141] and in *Lemna* plantlets [150] and deaminating activity in *Zea* leaves [125]. However, asparagine inhibits the NADH-GDH of root and leaf and NAD^+-GDH of root in *Zea* tissues [125]. Alanine, glycine and serine do not effect GDH activity in *Lemna* [161]. In excised *Zea* roots, NADH-GDH is inhibited by exogenously supplied arginine, lysine, proline, cysteine, leucine, phenylalanine and tyrosine but as far as NAD^+-dependent activity is concerned only proline, leucine and aromatic amino acids, i.e. phenylalanine and tyrosine, inhibit the enzyme activity significantly, whereas cysteine has no effect and arginine and lysine increase enzyme activity [125]. In *Zea* leaves, on the other hand, these amino acids increase deaminating enzyme activity but for cysteine and tyrosine. While lysine increases aminating activity of the leaf, cysteine inhibits it and the other amino acids mentioned above have no significant effect. It has also been shown that the presence of ammonium in the medium generally increases the enzyme level and prevents the inhibitory effect of amino acids to some extent [125].

These discrepancies in the literature reveal that the response of amino acids in different tissues is possibly due to differences in the regulatory nature of the enzyme. It also appears from various studies that the responses of the amino acids are also dependent on the concentration, period and procedure of application. For example, the effect of glutamate is concentration-dependent in *Zea* roots and leaves [125] and glutamate or glutamine supplied to excised tissues for 24 hr showed different responses from those when they were supplied consistently for longer periods [26, 125, 141, 150]. Although the literature regarding the mechanism of the action of amino acids on GDH is scanty and in spite of the fact that most workers have studied the effect of non physiological concentrations of the acid, it has been suggested that glutamate acts as a competitive inhibitor with respect to 2-oxoglutarate and also as an allosteric modulator of the enzyme molecule either directly or via some metabolic product(s). Besides the general effect of various amino acids, cysteine or some metabolic product(s) of cysteine possibly acts as a specific regulator of the GDH molecule [125].

Some amino acid analogues have been reported to be important in determining the path of ammonia assimilation in higher plants. L-Methionine sulphoximine (MSO), a potent inhibitor of GS-catalysed reactions, and the glutamine analogues, albizzine and azaserine, specific inhibitors of GOGAT activity, are reported to have no effect on GDH activity supplied either *in vitro* [1] or *in vivo* for 24 hr [89]. However, MSO inhibited the aminating enzyme activity to some extent during a 24 hr supply to excised *Zea* tissues [89]. In *Arabidopsis* roots and leaves NAD^+-GDH increased considerably and NADH-GDH to a limited extent when 2 mM MSO was supplied to the seedlings for 72 hr with 15 mM nitrate or ammonium [106]. It appears therefore that the effect of MSO depends on the feeding time. *p*-Fluoro-DL-phenylalanine (0.55 mM) and azetidine-2-carboxylic acid (0.1 mM) decreased the enzyme activity drastically if included with ammonium during incubation [148]. Delta aminolevulinic acid, a precursor in chlorophyll biosynthesis, did not affect enzyme activity in *Zea* leaves, although it increased nitrate reductase activity considerably [164]. Inhibition of GDH activity by derivatives of isophthalic acid, which are structurally related to glutamate, is reported for *Pisum* root enzyme [165]. 5-A-Substituted derivatives of aminoisophthalic acid completely inhibit the enzyme activity at concentrations equal to 2-oxoglutarate and glutamate. It is suggested that a compound with a minimum of three carbon atoms attached linearly to the amino group, is required for significant enzyme inhibition [165].

Carbohydrates, organic acids and energy level

The activity of GDH is inhibited by exogenously supplied sugars in most cases [42, 49, 106, 138, 142]. The effect of glucose, however, is variable. While it increases enzyme activity in chloroplast rich fractions of *Lemna* [134] and in excised *Phaseolus* leaves [99], it represses it in *Pisum* root [140] and shoots [49] and in cell cultures of *Asparagus* [142]. The repression of enzyme by glucose is not counteracted by the addition of glutamate, glutamine or ammonia [49]. However, it is overcome by the addition of cyclic adenosine 3',5'-monophosphate to the culture [142]. In addition to glucose, fructose [138, 140] and sucrose [49, 141] have also been reported to inhibit GDH activity in *Pisum* shoot and *Lupinus* embryonic axes, respectively.

It has been shown in *Lemna* that sucrose inhibits *de novo* synthesis of the enzyme [134]. Tassi *et al.* [142] have also shown that GDH is repressed by increasing glucose concentration in the culture medium. They have suggested that in cells of higher plants a regulatory mechanism exists with some similarities to the 'catabolite repression' mechanism operating in several prokaryotes and lower eukaryotes. A new isozyme has been shown to appear in *Lupinus* embryo when sucrose is withdrawn from the medium [72]. Sahulka and Lisa [139] have suggested that the effect of sugars on the enzyme is direct and not through their metabolites, as metabolites of the glycolysis, PPP and the TCA pathways do not effect the enzyme activity significantly. The studies reveal that the exact mechanism of action of sugars and its interaction with other metabolites on GDH is complex and it is difficult to draw conclusions on the basis of inadequate data. However, it seems logical to conclude that a supply of sugars increases the energy level and consequently the GS activity in the tissue, which in turn represses the GDH activity. This conclusion is supported by the studies of Oaks *et al.* [19], Ratajczak *et al.* [141] and Cammaerts and Jacobs [106].

Addition of iodoacetic acid, an organic acid, increases GDH activity in excised *Pisum* root, presumably because of increased H^+ in the medium, as the increase is negated by the addition of bases [42, 166]. As expected, the structural analogues of 2-oxoglutarate, glutarate and glyoxal, inhibited enzyme activity in excised *Zea* roots and leaves during a 24 hr *in vivo* supply [89].

The effect of nucleotides on enzyme activity has been extensively discussed by Stewart *et al.* [9] and it has been proposed that GDH may be controlled by the NAD^+/NADH ratio in the mitochondria as well [9, 46].

Adenosine triphosphate at concentrations of 1 mM and above is an inhibitor of GDH activity in both directions [90] However, when 0 2 mM AMP, ADP and ATP were supplied to *Asparagus* cell culture for 7 days, AMP reduced the enzyme activity while the other two had no effect [142] It has been suggested that when biosynthetic potential is low, the energy charge reduced and levels of NH_4^+ high in the tissue, GDH may play a more important role in the assimilation of ammonia than GS [19, 106, 161]

Environmental stresses

Temperature Lower temperature adversely affects GDH activity in the roots of *Triticum* [167], *Glycine* [168, 169, 170], *Pisum* [171] and *Zea* [172] A decrease in enzyme activity in *Glycine* roots grown at lower temperature is possibly caused by a relative decrease in enzyme protein content as well as by changes in the ratio between NADH:NADPH forms [168, 173] It also appears that the aminating activity of GDH may not be significant at lower temperature as the energy of activation [E_a] and $K_{NH_4^+}$ are higher at 12° than at 25° in *Triticum* roots [173] The enzyme activity is, however, hardly affected when plants are grown at high cultivation temperatures such as in *Glycine* [170] and *Triticum* [173] roots and in *Zea* leaves [174] The enzyme from *Agave* leaves has been reported to be heat stable at 50–70° [175] and *Triticum* root GDH has been found to be more thermostable than GS [173] Because of very limited studies dealing with characteristics of the enzyme at different temperatures and lack of information regarding deaminating GDH activity, it is difficult to draw any definite conclusions regarding the physiological significance of the thermostability of the enzyme However, a possible role of this enzyme at higher temperature may be proposed as an adaptation of plant tissue for ammonia detoxification when GS is inactivated at higher temperature

Water stress A consistent decrease in GDH activity is reported in the seedlings of *Brassica juncea* and *B campestris* [176], in roots and nodules of *Cajanus* [177] and in roots of *Poterium* [127] in response to increasing water stress Water stress has also been reported to inhibit the absorption of ammonium and nitrate [178] On the other hand, in root nodules of *Medicago* both aminating and deaminating GDH activities increase at higher levels of water stress (i e leaf water potential − 1 to − 2 MPa) invoked by withholding water [179] This discrepancy in the literature is possibly because the response to water stress depends upon the stage at which the stress is created and may vary with the plant species For example, no significant change in GDH activity is reported in the leaves of *B juncea* when wilting is created at the siliqua stage but wilting at the flowering stage decreases GDH activity [180] In contrast to this, stress at the flowering stage in *B campestris* increases GDH activity while that at the siliqua stage decreases activity [180] Further, in shoots of *Poterium* [127] and in *Medicago* root nodules [179] water stress created by PEG has only a marginal effect on enzyme activity while stress caused by withholding water increases it The increase in enzyme activity is believed to be a measure of detoxification of ammonia released as a breakdown product of proteins and amino acids during water stress [179] In addition to this, proline accumulated during water stress [181, 182] may also increase GDH activity as an increase in NAD^+ and NADH-GDH activities caused by proline has been reported for excised *Zea* leaves [125]

Salinity In the halophyte *Suaeda*, the enzyme activity is activated by 25 mM NaCl and inhibited by higher (over 100 mM) concentrations of the salt [183] An increase in enzyme activity with a low level of salinity has also been observed in *Oryza* embryo [184] and in *Lemna* [185] This increase in enzyme activity under saline conditions may be the consequence of increased ammonia and amide levels which accumulate under these conditions [186] possibly because of the inability of the GS-GOGAT route of ammonia assimilation to function under saline conditions [185, 187, 188] However, high salinity inhibits GDH activity in the roots of halophytes [187] and *Pisum* [189, 190] possibly by modifying the affinity of the enzyme for substrates and the catalytic potency of the enzyme [183] It appears, therefore, that GDH may detoxify NH_4^+ only under low salinity levels

Pollution The aminating activity of GDH is quite sensitive to air pollutants and as such it has been advocated to be the best enzymic indicator of pollution stress [191, 192] The enzyme activity increases during exposure to SO_2 [193–196], NO_2 and NH_3 [197, 198] and H_2S [199] In *Phaseolus* leaves low levels of NO_2 do not affect NADH-GDH activity [152] Further, the pollutant (NO_2) is assimilated via the GS-GOGAT route, although assimilation at a higher level of the pollutant may involve the GDH pathway [152] Although the exact mechanism of increasing GDH by air pollutants is not known, some postulates have been advanced Since the effect of SO_2 can be simulated by acid treatment of isolated GDH, it is believed that SO_2 (and possibly NO_2 as well) acts by creating acidity in the enzymic environment [200] The pollutants may also increase enzyme activity through altered membrane permeability It has also been shown that SO_2 changes the isozyme pattern and electrophoretic mobility of GDH [83] The effect of other pollutants on the enzyme activity, however, warrants further investigation to ascertain the possible role of this enzyme during pollution stress

Pathogenic infection Pathogenic infections also affect GDH activity In *Linum* cotyledons infected with flax rust fungus, enzyme activity shows a biphasic increase, during the first 24 hr of infection and again after 7 days [201] The enzyme during each stage, however, differs in its properties and also possibly in its relative efficiency in deaminating and aminating reactions It is suggested, however, that in one-day-infected cotyledons, GDH appears to be an enzyme of glutamate degradation while in 7-day-infected cotyledons it plays a minor role in glutamate synthesis Berville *et al* [202] have demonstrated that a toxin produced by *Helminthosporium* inhibits NAD^+ transport in *Zea* mitochondria, which may consequently influence the dehydrogenases More detailed studies may give some insight on GDH regulation in plants during diseases However, in these studies care should be taken to assure that the enzymes of host and pathogen are measured independently

Growth regulators and others

A few studies have been performed concerning the effect of growth regulators on GDH activity In *Pisum* roots, IAA and other auxins increase GDH level to some

extent but kinetin has either no influence [145, 203, 204] or inhibits it [122, 205]. The enzyme is also stimulated slightly but consistently when 0·01 mM salicylic acid is included in the incubation medium although higher concentrations of the acid inhibit the enzyme activity [206]. Chloroethylphosphonic acid (CEPA), a well known growth regulator, greatly stimulates enzyme activity when supplied in a concentration range of 60–480 ppm to *Pennisetum* seedlings [207]. It is proposed, however, that CEPA decomposes in plant tissues to release ethylene which may retard growth and enhance enzyme activity. The exact mechanism is yet to be elucidated. Applications of 2,4-dichlorophenoxyacetic acid (2,4-D) also increase GDH activity in *Pisum* and *Zea* roots at low concentrations (2×10^{-6} to 2×10^{-5} M) but inhibit the same at higher concentrations [208]. The shoot enzyme from *Pisum* is also stimulated strongly at lower concentrations of the herbicide, although that from *Zea* is unaffected [208]. It has been suggested that under the influence of 2,4-D, ammonium accumulates in the root and shoots of both plants, stimulating enzyme activity [208]. Again foliar applications of 2-chloro-4,6-bis(ethylamino)-*s*-triazine, 2-methylmercapto-4-ethylamino-6-isobutylamino-*s*-triazine and 2-methoxy-4-isopropylamino-*s*-triazine and 2-methoxy-4-isopropylamino-6-butylamino-*s*-triazine (2 mg l^{-1}) increase NADH-GDH levels along with nitrate reductase and protein in *Pisum* and *Zea* leaves [209]. They postulate that sublethal concentrations of the *s*-triazines stimulate general nitrogen assimilation and protein synthesis. DCMU, an uncoupler of photophosphorylation, inhibits NADH-GDH in excised *Zea* leaves, possibly by interfering with NADH production [89].

The enzyme activity is stimulated by β-mercaptoethanol in the leaves of *Urtica* and *Spinacea* [210]. 5,5-Dithiobis(2-nitrobenzoate) (DTNB) completely inhibits enzyme activity at 1 mM in *Agave* leaves [211]. However, the activity is partially restored by cysteine, dithiothreitol, reduced glutathione and β-mercaptoethanol when supplied to either excised *Phaseolus* leaves or to the enzyme preparation [62, 99]. Inhibition by DTNB is more pronounced for the enzyme from light grown tissues than that from dark grown tissues [62]. It has been reported that the inhibition is due to interaction of DTNB with sylphydryl groups present at the active centre of the enzyme [61], which are apparently more abundant in the enzyme from light grown leaves than in that from dark grown [62]. Since the *in vitro* inhibition of enzyme by DTNB is dependent upon NADH concentration in the incubation mixture, it has been postulated that the enzyme first forms a complex with NADH and then this complex is acted upon by DTNB [62]. The deaminating enzyme activity in *Pisum* seedlings is inhibited by *p*-chloromercuriphenylsulphonic acid (PCMPSA) and phenylmercuric acetate (PMA), although the activity is restored by the addition of glutathione [212].

Metal binding agents, *O*-phenanthroline, LL-dipyridyl, EDTA, zincon, ferron, nitroso-R salts and 8-hydroxyquinoline are also reported to inhibit both the activities of GDH and these activities are restored by the addition of divalent metal ions [212]. Pyridoxal 5-phosphate inhibits the enzyme activity in *Lupinus* nodules [86] and *Pisum* mitochondria [213]. This behaviour reflects the association of metal ions with the active form of GDH.

PHYSIOLOGICAL SIGNIFICANCE IN VARIOUS TISSUES

Root nodules

Ammonia is the first stable product of dinitrogen fixation in nodule bacteriods. A large portion of it is excreted from the bacteriod to the nodule cytosol where it is assimilated into organic compounds [4, 123, 214]. Although the observed kinetic studies have revealed that ammonia produced from symbiotically fixed dinitrogen is assimilated primarily via the GS-GOGAT pathway, the significance of a large amount of NADH-GDH present in nodule cytosol [214, 215] is not understood.

Some evidence, based on pulse labelling, for the involvement of GDH in the assimilation of ammonium has been presented for nodule tissues of several legumes [216–218]. In *Lupinus* root nodules, 14 isozymes of GDH are considered to be physiologically significant and also change with the nitrogen and sugar content of the tissue [25]. The involvement of GDH in ammonia assimilation, especially at the early flowering and active pod filling stages, has also been shown in *Vigna* genotypes symbiotically associated with various rhizobial strains [111]. On the basis of pulse labelling and inhibitor studies with nodules of *Alnus*, a non-leguminous angiosperm, and in *Glycine*, Schubert and Cooker [219] suggested that GDH may play a major role in the assimilation of exogenously supplied ammonium. Further, NH_4^+ supply increased GDH activity considerably between 4 and 24 days of application with a maximum at 13 days in an ineffective *Medicago* clone, MnPL-480 [220].

Defoliation and other types of stress induce nodule senescence in a wide range of legumes [221–223]. Root nodules of *Medicago* have an adaptive capacity to undergo temporary localized senescense in response to harvesting and applied N-fertilizer [223, 224]. It has been suggested that, although nodule NADH-GDH is not closely associated with N_2 fixation, it may be associated with ammonia assimilation during induced nodule senescence [224]. Further, when *Medicago* nodules are subjected to water stress, the GS/NADH-GOGAT cycle is operational in normal or even mildly stressed plants. When drought progresses NADH-GOGAT is inhibited, and NAD^+-and NADH-GDH increase (ψw = 1·7 M Pa), in the absence of a N-supply. The activities are maintained at higher level when plants are supplied with 20 mM nitrate [179]. It has been reported that NAD^+-GDH is relatively higher in root nodules in comparison with other tissues, and the ratio of $NADH/NAD^+$ is lower [179, 220, 224]. It has been suggested, therefore, that ammonium released as result of protein hydrolysis, amino acid oxidation or increased NR activity during nodule senescence may be assimilated by the co-action of NADH-GDH and GS and their relative contributions may possibly be dependent on the nitrogen, carbon and energy status of the tissue [179, 225]. Further, when the coupling of NADH-GOGAT with GS is interrupted during higher water stress, GDH is activated to provide glutamate for GS activity [179].

It appears, therefore, that although GS-GOGAT is the main route for the entry of symbiotically fixed NH_4^+ into the organic cycle, GDH, present in large amounts in the nodule cytosol, may also play some role under some nutritional and environmental conditions, depending upon plant species and type of symbiotic association. Moreover, there are a number of N_2-fixing symbionts

including non-legume angiosperms, where little is known about the biochemistry of ammonia assimilation and nitrogen transfer. In *Azolla*, for example, a large amount of GDH is present compared to GS. However, GS-GOGAT activities increase when association with *Anabaena* occurs [226].

Roots and tissue cultures

Ammonium in the root is generally derived from absorption or assimilation of inorganic salts available in the soil. It does not accumulate in the plant tissues because of its toxic nature [123]. Roots have been demonstrated to be the major site for ammonia assimilation in *Hordeum*, where 93% of externally supplied $^{15}NH_4^+$ was transported to the shoot in the form of organic nitrogen [227]. In *Pisum* roots both NR and GDH are higher in root tips [15, 138, 228], whereas in *Zea* roots GDH activity, as well as the levels of NO_3^- and NH_4^+, are higher in mature root portions [19]. Generally, the supply of NH_4^+ and to some extent NO_3^- increases aminating GDH activity considerably, which potentiates further with treatment time (see Table 1). A good correlation between aminating GDH activity and soluble and protein nitrogen fractions [12] and increased free amino acids [229] during inorganic nitrogen supply have also been shown.

One of the major objections regarding the operation of GDH in the aminating direction is a higher $K_{NH_4^+}$. However this needs reconsideration as various factors have been reported to influence the $K_{NH_4^+}$ for GDH (see kinetic behaviour). Pulse labelling and inhibitor studies support the operation of the GS-GOGAT route in normal growth conditions. However, in most studies glutamate is labelled along with glutamine and it is difficult to exclude the contribution of GDH in assimilation of labelled ammonium [19]. It may be realized that when methionine sulphoxide (MSO) is supplied with nitrate/ammonium, a very low level of ammonium accumulation occurs in the roots of several C_3 and C_4 plants [230]. Further, MSO increases GS and NADH/NAD^+-GDH when supplied with 15 mM NO_3^-, and NADH/NAD^+-GDH activities when supplied with 15 mM NH_4^+ [106]. These studies indicate that GDH is involved in ammonia assimilation at higher levels of nitrogen and at the same time it generates energy via the deamination reaction, for the activity of GS to assimilate physiological levels of nitrogen in the roots [106]. Further, it also appears that GDH is a more stable enzyme than GS in stress conditions (see above) and it may play a significant role in either direction to maintain plant metabolism during these conditions. For example, GDH has been shown to be more thermostable than GS [173, 175] and it increases during water stress [179] and salinity [183–185, 188]. Further, GS and GOGAT enzymes are more labile and they are inactivated or their coupling disturbed during stress conditions [173, 179, 183, 185, 187, 188]. In cell cultures of *Saccharum*, GDH has been shown to operate when normal growth and differentiation do not occur and GS is very low [109]. It would be interesting to study the primary amination reaction in xerophytes with this perspective.

Green shoots and leaves

The major source of ammonia in the shoots and leaves is the reduction of nitrate *in situ* [231]. In addition a large amount of ammonia can be generated *in situ* during the photorespiratory conversion of glycine to serine in mitochondria of green tissue [232, 233] and the breakdown of asparagine either via transaminase [234] or asparaginase [235, 236]. Many studies show that ammonia or/and nitrate supplied during seedling growth increase GDH activity considerably in shoot and leaf tissues (see Table 1). Although several studies have demonstrated the operation of the GS-GOGAT pathway for ammonia assimilation in shoot and leaf tissues [1, 4, 9], they do not exclude the role of GDH completely. It has been shown that the equilibrium of mitochondrial GDH is in the direction of glutamate formation, and isolated *Pisum* shoot mitochondria are able to incorporate ^{15}N from either 2 mM of $^{15}NH_4$ or ^{15}N glycine to glutamate [46, 237, 238]. Neeman *et al.* [239] have shown in their ^{15}N nuclear magnetic resonance studies that GS and GDH are both active for reassimilation of ammonia released during photorespiration in *Nicotiana* protoplasts. As mentioned earlier, GDH increases during various environmental stresses such as darkness, high temperature, water stress and air pollution (see environmental stresses) in leaves also. In *Phaseolus* leaves, the enzyme from dark grown samples is more active than that from light grown [62]. It may be proposed that during stress conditions, when the GS-GOGAT pathway is inefficient, GDH is activated, possibly to detoxify accumulated ammonia.

Seed development and germination

During the later stages of seed development, amino acids from senescing tissues are an important portion of the total nutrient supply to the pods and developing seeds. The *Glycine* seed GDH has been shown to be capable of operating in both aminating and deaminating directions and could possibly provide a suitable reversible link between carbon and amino acid metabolism [53, 240]. The enzyme has also been shown to be important during kernel development in *Zea* genotypes and is more abundant in a high lysine variety, i.e. opaque-2 [117, 241]. A general increase in the enzyme activity during seed germination (see plant age and rhythmicity section above) also suggests some role of the enzyme in ammonia metabolism during this process. A high concentration of ammonia in seed coats [113–116] and an increase in GDH activity in seed coat and cotyledons [112] and endosperm [20] may indicate a possible role of GDH in assimilating seed ammonia during germination.

ADDENDUM

Mutants of *Arabidopsis* deficient in leaf GOGAT activity [242] and *Hordeum* lacking chloroplast GS [243] have been shown to survive only under the conditions of non-photorespiration and high atmosphere, respectively, rather than the normal air/growth conditions. Further in senescent *Triticum* leaves ammonia released during photorespiration is assimilated through leaf GS rather than GDH [244]. These observations imply that GDH has no significant role in reassimilation of photorespiratory ammonia even in mutants lacking enzymes for the GS/GOGAT route and during leaf senescence.

Acknowledgements We are extremely thankful to Professor D P Ormrod for thorough editorial check up and review We also thank Ms Kristina Hladun for 'processing' the manuscript

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