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EFFECT OF AMINO COMPOUNDS ON NITRATE UTILIZATION BY ROOTS OF DWARF BEAN

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Key Word Index—*Phaseolus vulgaris*; Papilionaceae; dwarf bean; nitrate uptake; nitrate reductase activity; amino compounds.

Abstract—Amino compounds (1 mM, pH 5) were given prior to, together with, or after the addition of nitrate to study their effect on nitrate uptake and *in vivo* nitrate reductase activity (NRA) in roots of *Phaseolus vulgaris*. The effect of amino compounds varied with the amino species, the nitrate status of the plant (induced vs uninduced) and the aspect of nitrate utilization. Cysteine inhibited the nitrate uptake rate and root NRA under all conditions tested. NRA in uninduced roots was stimulated by tryptophan, and arginine inhibited NRA under all conditions tested. Uptake was inhibited by aspartate and glutamate and stimulated by leucine when these amino compounds were given prior to or after completion of the apparent induction of nitrate uptake. In the presence of β-alanine and tryptophan, induction of uptake was accelerated.

INTRODUCTION

Regulation of NO_3^- utilization in the higher plant operates at the level of individual links in the chain of transport and conversion processes as well as at the level of their coordination [1]. Our previous work with dwarf bean (*Phaseolus vulgaris* L.) identified NO_3^- uptake and reduction of NO_3^- to NO_2^- in the roots as two key steps in the NO_3^- utilization sequence [2]. Our subsequent work focused on these two processes and their association. We reported on the effect of sugars [3], plant growth regulators [4], exogenous and endogenous NO_3^- concentration [5], NO_2^- [6] and NH_4^+ [7]. In the latter paper we concluded that the effect of NH_4^+ on NO_3^- uptake and reduction depends on NH_4^+ assimilation rather than on NH_4^+ *per se*. Possible mechanisms for the inhibitory action of NH_4^+ were suggested to be the high energy requirement of NH_4^+ incorporation via the glutamine synthetase–glutamate syntase cycle and the generation of regulatory compounds from assimilated NH_4^+, such as amides and amino acids. The aim of the present investigation was to examine the latter possibility by studying the effect of exogenous amino compounds on NO_3^- uptake rate (NUR) and *in vivo* NO_3^- reductase activity (NRA) before, during, and after the apparent induction of uptake and reduction in roots of nitrogen-depleted dwarf bean.

RESULTS

Pretreatments with amino compounds

A wide variation in the response of NO_3^- uptake to pretreatments with amino compounds (18 hr, 1 mM) was observed (Table 1). Steady-state NO_3^- uptake was inhibited by β-alanine (β-Ala) and cysteine by more than 50%. A 25–50% inhibition occurred after pretreatments with γ-aminobutyrate (γ-Abu), aspartate, glutamate, glutamine, glycine, serine, threonine and tyrosine. Less inhibitory (10–25%) pretreatments were those with lysine and proline. Alanine, arginine, asparagine, isoleucine, methionine, phenylalanine, tryptophan and valine were without effect. Leucine was the only amino compound tested that stimulated (25%) the NUR.

The effect of a number of amino compounds was tested on the time pattern of NO_3^- uptake (Table 2). The uptake rate rose continuously during incipient NO_3^- nutrition and became constant after *ca* 6 hr in control plants (cf. Fig. 1). The apparent induction was 2 hr slower with β-alanine. None of the amino pretreatments tested accelerated induction.

Roots of plants grown on basal medium were devoid of NO_3^- [2] and showed a very low NRA [8]. Pretreatments with β-alanine, arginine, aspartate, cysteine, glutamate, leucine, phenylalanine and tryptophan did not alter the NRA in these roots (data not given). After 6 hr of NO_3^- supply, the bulk of the plant's *in vivo* NRA, and presumably also of the plant's NO_3^- reduction, is root-borne [2, 8]. Root NRA after 6 hr was lowered by more than 70% by pretreatments with β-alanine, arginine, cysteine, glycine, serine and threonine, and was stimulated by aspartate and tryptophan (Table 1).

In general, the correlation between the pretreatment effect of an amino compound on the NRA and NUR was poor. No effect on both aspects of NO_3^- utilization was exerted by alanine, isoleucine or phenylalanine. Uptake and NRA were inhibited to the same extent by β-alanine, lysine and glutamine. The negative effect of cysteine, glycine, proline, serine and threonine on NRA exceeded that on the NUR. Arginine, asparagine, methionine and valine inhibited NRA but not the NUR, while γ-aminobutyrate, glutamate and tyrosine inhibited only the NUR. The data from Table 1 were used to select amino compounds for further experimentation.

Table 1. Effect of pretreatments with amino compounds (18 hr, 1 mM, pH 5) on the NO_3^- uptake rate (NUR) and NO_3^- reductase activity (NRA) of roots of dwarf bean after 7 hr of NO_3^- supply (0.4 mM)*

	NUR	NRA
Pretreatment	(% of control)	
Control	100	100
Cys	40±3	18±2
Thr	53±6	18±4
Arg	101±11	21±3
β-Ala	24±3	23±3
Gly	69±8	25±3
Ser	61±5	28±4
Asn	99±8	38±3
Gln	56±7	44±5
Pro	79±11	58±5
Met	103±6	60±8
Lys	80±9	67±9
Val	98±10	75±4
Leu	125±11	97±13
Tyr	73±7	99±6
Glu	60±8	100±12
Phe	103±9	100±10
γ-Abu	66±10	103±15
Ile	98±7	104±8
Ala	98±6	104±11
Asp	74±8	144±20
Try	96±12	152±15

*Data are given as % of the control (no amino pretreatment) ±s.d.; $n = 3$ (NUR) or 5 (NRA). Absolute control values for NUR and NRA were in the range 27–36 and 6–9 μmol NO_3^- or NO_2^-/hr per g, respectively. Not all amino compounds could be compared in the same experiment. The coefficient of variation of the control was 7–10% for NUR and 11–15% for NRA in the various experiments.

Table 2. Time required to attain a constant rate of NO_3^- uptake by dwarf bean after pretreatment with various amino compounds (18 hr, 1 mM, pH 5)*

Pretreatment	Lag period (hr)
Control	5.9±0.5 ($n = 5$)
β-Ala	7.8±0.7 ($n = 4$)
Arg	6.8±0.4 ($n = 3$)
Asp	6.1±1.0 ($n = 3$)
Cys	6.0±0.8 ($n = 3$)
Glu	5.9±0.2 ($n = 3$)
Leu	5.4±0.3 ($n = 4$)
Phe	6.5±0.1 ($n = 3$)
Try	6.0±0.4 ($n = 3$)

*Data are given ±s.d. with the number of independent experiments for each compound in parentheses.

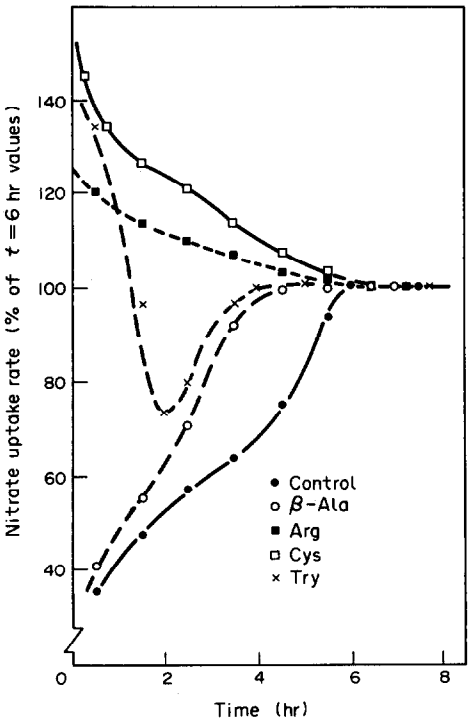

Fig. 1. Rate of NO_3^- uptake by dwarf bean. Plants were given NO_3^- (0.4 mM) with or without amino compounds (1 mM, pH 5) as indicated. Absolute rate of the control after 6 hr: 32.8 μmol/hr per g. For other absolute rates, see Table 3.

Concomitant supply of NO_3^- and amino compounds

When given at the onset of NO_3^- nutrition, β-alanine accelerated the apparent induction of NO_3^- uptake by 1–2 hr (Fig. 1). Arginine and cysteine caused a gradual decline of the NUR, resulting in a constant rate after 6–7 hr. The combination of NO_3^- and tryptophan initially caused a decreasing uptake rate, but the NUR increased after 2 hr to yield a pattern similar to that of β-alanine with a constant rate after 4 hr. No effect on the time pattern of NO_3^- uptake was observed with aspartate, glutamate, leucine or phenylalanine. The constant rates attained between 4 and 7 hr in Fig. 1 were maintained for at least 4 hr.

Uptake was curtailed in the presence of arginine and cysteine, but stimulated with glutamine (Table 3). No effect on the NUR was found with β-alanine, aspartate, glutamate, leucine, phenylalanine or tryptophan. Root NRA was 80% lower with cysteine, 40% lower with arginine, glutamate and glutamine, and 25% lower with aspartate (Table 3). In the presence of tryptophan, the root NRA was nearly doubled after 6 hr of NO_3^- supply.

A corresponding effect on the rates of uptake and reduction of NO_3^- was exerted only by β-alanine, leucine and phenylalanine (no effect) and arginine (*ca* 50% inhibition).

Effect of amino compounds on induced plants

After 3 hr of amino supply, the NUR of NO_3^--induced plants was inhibited by arginine, aspartate, cysteine or

Table 3. Nitrate uptake rate (NUR) and NO_3^- reductase activity (NRA) of roots of dwarf bean after 6 hr of concomitant supply of NO_3^- (0.4 mM) and an amino compound (1 mM) at pH 5*

	NUR	NRA
Treatment	(% of control)	
Control	100±10	100±14
β-Ala	105±10	95±11
Arg	48±6	57±7
Asp	109±9	74±8
Cys	50±7	18±4
Gln	204±17	56±8
Glu	99±11	60±7
Leu	107±6	91±11
Phe	103±8	92±12
Try	98±10	184±15

*Data are given as % of the control ± s.d., $n = 3$ (NUR) or 5 (NRA). Absolute control values for NUR and NRA were 25.2 ± 2.5 and 5.9 ± 0.8 μmol NO_3^- or NO_2^-/hr per g, respectively.

Table 4. Nitrate uptake rate (NUR) of roots of NO_3^--induced (18 hr, 2 mM) dwarf bean after 3 hr of amino compound supply (1 mM, pH 5) and after 3 subsequent hr without amino compounds*

	NUR	
	3 hr	6 hr
Treatment	(% of control)	
Control	105±6	101±8
β-Ala	100±7	95±10
Arg	78±6	75±9
Asp	76±4	89±7
Cys	48±5	52±7
Glu	75±7	60±5
Leu	132±12	117±9
Phe	143±9	164±10
Try	94±8	104±7

*Amino compounds were given at $t = 0$ to media containing NO_3^- at 0.4 mM. After 3 hr the roots were washed (2 × 1 min) in, and supplied with, fresh basal medium + NO_3^- (0.4 mM). Data are given as % of the control rate measured prior to amino addition ± s.d. ($n = 3$). Control value: 38.2 ± 2.8 μmol NO_3^-/hr per g.

glutamate (Table 4). Inhibition by arginine occurred gradually, while an immediate and constant inhibition was caused by aspartate, cysteine and glutamate. Leucine and phenylalanine increased the NUR, and this stimulation progressed with time.

Three hr after a short wash to remove exogenous amino compounds, the NUR was not altered in plants previously supplied with β-alanine, arginine, cysteine, leucine or tryptophan (Table 4). Removal of the amino compound stimulated the NUR of plants treated with aspartate and phenylalanine, but further reduced the NUR of glutamate-affected plants. The NUR of plants kept on NO_3^- without amino compounds was essentially constant for at least 6 hr, and washing *per se* did not alter the NUR of control plants.

Root NRA of induced plants was not changed 6 hr after supply of β-alanine, aspartate, glutamate, leucine, phenylalanine or tryptophan (Table 5). However, cysteine and—to a lesser extent—arginine inhibited root NRA significantly.

Uptake and NRA were similarly affected after 3 hr supply of β-alanine, arginine, cysteine or tryptophan. In the absence of an effect on root NRA, NUR was stimulated by leucine and phenylalanine but inhibited by aspartate and glutamate.

DISCUSSION

Nature of the effects of the amino compounds

The effect of amino compounds on various aspects of NO_3^- utilization varies with the NO_3^- history, amino history and amino species. The observed effects are probably mainly metabolic since no consistent relationship emerged between the physicochemical properties of the amino compounds, e.g. charge at pH 5, dissociation constant and isoelectric point, on the one hand, and their effects on NUR and NRA, on the other.

Amino compounds are co-transported with protons, and the kind and degree of coupling seem to vary with the acidic or basic nature of the transported compound [9]. Sahulka and Lisá [10] found that in roots of *Pisum sativum* NRA was inhibited at low concentrations of glutamic acid, but that higher concentrations were able to reverse negative effects of other amino compounds on NRA. It is not known whether proton-linked transport phenomena played a role in their observation. In our experiments, identical effects were caused by neutral, acidic and basic amino compounds, and the ambient pH fluctuated within narrow limits, so that the effect of H^+

Table 5. Nitrate reductase activity (NRA) of NO_3^--induced (18 hr, 2 mM) dwarf bean roots, 3 and 6 hr after the supply of amino compounds (1 mM, pH 5)*

	NRA	
	3 hr	6 hr
Treatment	(% of control)	
Control	108±11	106±10
β-Ala	106±12	110±9
Arg	84±8	76±7
Asp	92±12	104±8
Cys	47±6	21±5
Glu	105±10	108±9
Leu	103±8	95±7
Phe	95±9	100±12
Try	104±11	92±8

*NRA is expressed as % of control measured prior to amino addition ($t = 0$) ± s.d. ($n = 5$). Control value: 17.1 ± 2.0 μmol NO_2^-/hr per g.

co-transport does not seem to interfere significantly with our results.

The effect of non-protein amino compounds like γ-aminobutyrate and β-alanine was mimiced by some protein amino compounds, indicating that a potential protein status is not essential for an amino effect on NO_3^- utilization.

According to their biosynthetic pathways, amino compounds may be grouped into families [11]. Pretreatments with members of the serine family (Cys, Gly, Ser) repressed both the NUR and, even stronger, the NRA after 7 hr of NO_3^- supply (Table 1). Members of the pyruvate family (Ala, Ile, Leu, Val) did not affect, or stimulated (Leu) the NUR, and only in the case of valine was the NRA moderately (25%) inhibited. Amino compounds of the aromatic family (Phe, Try, Tyr) did not affect, or stimulated root NRA and only tyrosine moderately (25%) repressed uptake. These findings suggest the existence of common regulators, generated by amino metabolism, that equally or differentially affect various NO_3^- utilization processes. However, this explanation does not cover all observations and 'family effects' were extensively tested only after pretreatments (Table 1).

NO_3^- *uptake*

The apparent induction of NO_3^- uptake was retarded by a pretreatment with β-alanine (Table 2), but accelerated when this compound was given at the start of NO_3^- nutrition (Fig. 1). Under the latter conditions, tryptophan also accelerated the induction of NO_3^- uptake. Pretreatment with β-alanine also sharply reduced the NUR after 7 hr of NO_3^- supply (Table 1). To our knowledge, there are no published data on the effect of amino compounds on the induction of NO_3^- uptake in higher plants. In *Neurospora crassa* the induction is inhibited by casein amino acids [12].

Independent of the presence of NO_3^-, the NUR was severely inhibited by treatments with cysteine. Arginine was also a strong inhibitor but only in the presence of NO_3^- (Table 3). Full induction of uptake and reduction was obviously required for the stimulation of the NUR by phenylalanine (Table 4). Leucine stimulated both as a pretreatment and in induced plants. Glutamate and aspartate inhibited the NUR before and after, but not during, induction of NO_3^- uptake. It is remarkable that the NUR of amino-affected induced plants recovered after removal of aspartate and further increased when phenylalanine was withdrawn. Similar to the effect of NH_4^+ [7] and NO_2^- [6], the NUR was decreased when glutamate was removed. This increased inhibition may reflect differential regulation by endogenous and exogenous metabolities.

From the few literature data on the amino compound–NUR interaction no uniform picture arises. All data point to no or inhibitory effects and stimulations have not been reported. Glutamate and asparagine inhibit the NUR in the fungi *N. crassa* [12] and *Penicillium chrysogenum* [13]. Glutamine also inhibits uptake in the latter species, and in the bacterium *Pseudomonas fluorescens* [14], but not in *N. crassa* [12]. Conway [15] postulated that the total level of endogenous amino compounds controls the NUR of algae. In the diatom *Skeletonema costatum*, however, a number of amino compounds did not inhibit the NUR [16].

In cultured *Nicotiana tabacum* cells, casein hydrolysate inhibited the NUR, independent of the effect of tungstate, indicating that NO_3^- reduction did not mediate this effect [17]. Doddema and Otten [18] tested the effect of some amino compounds on the NUR of *Arabidopsis thaliana* and found the inhibition by glycine, serine and arginine to be slight, moderate and severe, respectively.

NO_3^- *reduction*

Amino pretreatments failed to induce root NRA in NO_3^- free roots. The pretreatments caused a strong stimulation (Asp, Try), no effect, or various degrees of inhibition of root NRA after 7 hr of NO_3^- supply (Table 1). When given together with NO_3^-, again tryptophan stimulated and arginine, cysteine and glutamine inhibited the development of root NRA after the initial 6 hr of NO_3^- supply (Table 3). The inhibition by arginine and cysteine was also apparent in fully-induced plants 3 and 6 hr after amino supply (Table 5). Tryptophan, in contrast, seems to affect the induction of NRA rather than the induced activity. Tryptophan and a number of other amino compounds stimulated the induction of NRA in *Ipomoea* cells [19]. These other compounds, however, had no or a negative effect on NRA induction in roots of dwarf bean, e.g. isoleucine, leucine, methionine, phenylalanine and proline (Table 1).

A mixture of amino compounds or casein hydrolysate stimulates the induction of NR in *Ipomoea* cells [19], leaves of *Zea mays* [20] and in roots of *Phaseolus aureus* [21], but inhibits the induction in cultured cells of *Nicotiana* [22] and *Datura* [23], and in other cells and tissues ([24] and refs. cited therein). Heimer and Riklis [25] suggested that in *Nicotiana* cells NR is de-repressed by an amino compound in a post-transcriptional way. In *N. crassa*, glutamine inhibits the synthesis of m-RNA coding for NR [26].

No effect of a large variety of amino compounds was observed on the induction of NRA in roots of *Hordeum vulgare* [27]. Aspartate stimulated NR induction in roots of dwarf bean (Table 1) as well as those of *Gossypium hirsutum* [28]. Interactions between individual amino compounds, and between classes of amino compounds in their effect on NRA were indicated by Srivastava [24] and Zink [19].

Of the amino compounds tested on roots of induced dwarf bean (Table 5), only cysteine consistently had a negative effect on NRA in a number of other materials, e.g. *Lycopersicon esculentum* and *Nicotiana tabacum* [29] and cells of *Ipomoea* [19] and *Datura* [30]. A mixture of amino compounds or casein hydrolysate inhibited NRA in roots of *Zea mays* [31, 32] and *Pisum sativum* [10], and in *Nicotiana* cells [33], whereas no effect was noticed in mould fungi [34] or in the diatom *S. costatum* [35]. We have demonstrated that altered NO_3^- uptake may only incidentally explain the amino effects on root NRA. A similar conclusion was reached by Radin [28] and Premakumar *et al.* [26]. In general, the reverse explanation, i.e. an influence on uptake as a consequence of an amino effect on NO_3^- reductase, is also untenable.

Regulation of NO_3^- *utilization*

Once absorbed, the fate of exogenously supplied amino compounds may differ from that of endogenously synthesized amino compounds because they are stored in different cellular compartments [36]. Nevertheless, the

significant response of various aspects of NO_3^- utilization in roots of dwarf bean to exogenous amino compounds shows that this tissue has the capacity to respond differentially to naturally occurring amino compounds. We deduce therefore that NO_3^- uptake and NO_3^- reduction may be intimately linked to aspects of nitrogen metabolism subsequent to the entry and primary conversion of NO_3^-, e.g. the incorporation of NH_4^+ into amino groups or amino compound conversions. Besides this aspect of regulation, NO_3^- [8], NO_2^- [6] and NH_4^+ [7] are also potential regulators of NO_3^- utilization, indicating that NO_3^- uptake may be under feedback control of a number of remote links in the chain of utilization processes.

Cysteine inhibited the rate of uptake and reduction of NO_3^- by roots of dwarf bean under various conditions and therefore may function as an important metabolite in the regulation of NO_3^- utilization. It is not known, however, what the concentration dependence of the cysteine effect is and whether the endogenous cysteine level resulting from 1 mM exogenous cysteine bears any relation to physiological levels in roots. Other important regulatory compounds could be tryptophan and arginine, working especially on NRA, and aspartate, β-alanine, glutamate and leucine, mainly affecting uptake.

EXPERIMENTAL

Phaseolus vulgaris L. cv Witte Krombek was grown in an N-free liquid basal medium for 7 ± 1 days at 20° and a 16 hr photoperiod as detailed previously [6]. Experiments were started by adding amino compounds (1 mM) with or without $Ca(NO_3)_2$ at a concn specified in the Results. All initial pHs were 5, or brought to 5 with KOH or H_2SO_4. During experiments, pH changes were less than 0.4 unit. Induced plants were obtained by an 18 hr pretreatment with NO_3^- (2 mM). Transfer of plants from a soln with, to a soln without amino compounds was always accompanied by a short wash (2×1 min) with the latter soln.

Reduction of NO_3^- in the roots was estimated by an anaerobic *in vivo* NRA test with intact root systems. Results of this method and a similar method for leaf discs were in accord with the true reduction rate ($^{15}NO_3^-$ incorporation) in whole plants [2]. Uptake of NO_3^- was measured at 0.4 mM by ambient depletion, either continuously or discontinuously with various NO_3^- assays [7]. The assays for NO_2^- (NRA) and NO_3^- (NUR) were affected by some amino compounds, and the results were corrected accordingly. All experiments were repeated at least twice and data of representative experiments are given. All results are expressed per unit of root dry matter.

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