

# Characteristics of amino acid uptake in barley

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**Abstract** Plants have the ability to take up organic nitrogen (N) but this has not been thoroughly studied in agricultural plants. A critical question is whether agricultural plants can acquire amino acids in a soil ecosystem. The aim of this study was to characterize amino acid uptake capacity in barley (*Hordeum vulgare* L.) from a mixture of amino acids at concentrations relevant to field conditions. Amino acids in soil solution under barley were collected in microlysimeters. The recorded amino acid composition, 0–8.2  $\mu\text{M}$  of L-Serine, L-Glutamic acid, Glycine, L-Arginine and L-Alanine, was then used as a template for uptake studies in hydroponically grown barley plants. Amino acid uptake during 2 h was studied at initial concentrations of 2–25  $\mu\text{M}$  amino acids and recorded as amino acid disappearance from the incubation solution, analysed with HPLC. The uptake was verified in control experiments using

several other techniques. Uptake of all five amino acids occurred at 2  $\mu\text{M}$  and below. The concentration dependency of the uptake rate could be described by Michaelis–Menten kinetics. The affinity constant ( $K_m$ ) was in the range 19.6–33.2  $\mu\text{M}$ . These  $K_m$  values are comparable to reported values for soil micro-organisms.

**Keywords** Active uptake · Affinity constant · Amino acids in soil solution · Efflux · Nitrogen acquisition · Organic nitrogen

## Abbreviations

DM Dry matter

HPLC High-performance liquid chromatography

## Introduction

The ability of plant roots to absorb organic nitrogen (N) was observed already some time ago (Hutchinson and Miller 1911; Virtanen and Linkola 1946; Melin and Nilsson 1953; Wright 1962; Miller and Schmidt 1965). Still, the role of root absorbed amino acids in the plant N budget is poorly known. The importance of organic N as an N source has generally been emphasized for plants in N limited ecosystems having low temperature and slow mineralization rates (Schimel and Bennett 2004). Uptake of amino acids has, however, been recorded in various plant species from

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as diverse ecosystems as arctic tundra (Kielland 1994; Schimel and Chapin 1996), boreal forests (Näsholm et al. 1998; Öhlund 2004) and alpine ecosystems (Raab et al. 1996, 1999) as well as from subantarctic and tropical ecosystems (Schmidt and Stewart 1999). Agricultural plants have not been thoroughly studied but mycorrhizal grassland species (Näsholm et al. 2000) and non-mycorrhizal winter wheat (Näsholm et al. 2001) were demonstrated to take up double-labelled Glycine in field, and several plant species including  $N_2$ -fixing legumes were shown to absorb organic N in laboratory studies (Virtanen and Linkola 1946; Soldal and Nissen 1978; Schobert and Komor 1987; Jones and Darrah 1994). Recently, studies of the model plant *Arabidopsis thaliana* L. have identified two amino acid transporters, the Lysine Histidine Transporter 1 (Himer et al. 2006; Svennerstam et al. 2007) and the amino acid permease 1 (Lee et al. 2007), as components of the root amino acid uptake system. These findings provide a molecular background to the phenomenon of amino acids as nutrition for plants. No doubt plants have the ability to absorb amino acids. However, an unsolved question is whether plants can compete with microbes for N sources such as amino acids in natural environments. In this context, root uptake kinetics of amino acids may provide important information in the assessment of potential plant amino acid acquisition.

Concentrations of amino acids in the soil solution vary among ecosystems. Dissolved free amino acids were in the range 13–158  $\mu\text{M}$  in an alpine area (Raab et al. 1999) and 57–73  $\mu\text{M}$  in boreal forests (Öhlund 2004) while total soil amino acid concentrations were in the range 20–60  $\mu\text{M}$  and individual amino acids in the range 0.3–10  $\mu\text{M}$  in grasslands (Jones et al. 2005). These studies identified three to five amino acids as the most abundant free amino acids in soil solution; L-Glutamic acid, L-Serine, Glycine, L-Alanine and L-Aspartic acid. The amino acid pool in soil solution has been described as small but dynamic with large variation in measured half-lives, 0.75–20.8 h but most commonly less than 3 h in soils devoid of plants (Jones 1999; Lipson et al. 2001; Owen and Jones 2001; Jones and Kielland 2002; Jones et al. 2005).

In spite of the findings that soil amino acids generally occur in low  $\mu\text{M}$  concentrations, most uptake experiments have been carried out at considerably higher concentrations, both when characterising root uptake kinetics and when investigating

plant amino acid uptake in field. Hence, most laboratory studies have used amino acid concentrations in the range 100–8,000  $\mu\text{M}$  (Wright 1962; Raab et al. 1999; Schmidt and Stewart 1999; Owen and Jones 2001) and only a few have used field relevant amino acid concentrations, i.e. 0.1–10  $\mu\text{M}$  (Soldal and Nissen 1978; Schobert and Komor 1987; Kielland 1994). Some of these investigations were, however, performed on excised roots (Soldal and Nissen 1978; Kielland 1994), which have been found to display a different uptake compared to intact plants (Falkengren-Grerup et al. 2000). Moreover, most studies comprised only one amino acid at a time, predominantly Glycine, while only a few investigated simultaneous uptake of several amino acids (Persson and Näsholm 2001; Falkengren-Grerup et al. 2000).

The relatively few studies of amino acid uptake by intact crop plants performed with several amino acids simultaneously and at concentrations relevant for field conditions stimulated us to perform the present investigation. The aim of our work was to characterize amino acid uptake capacity in barley (*Hordeum vulgare* L.) from a mixture of amino acids at concentrations relevant to field conditions. To achieve this aim, we first measured amino acids in soil solution under barley. The recorded amino acid composition was then used as a template for hydroponic uptake studies in intact barley plants.

## Materials and methods

### Amino acids in soil solution

#### Site

Field studies were performed during 2003 on an estate belonging to the Swedish University of Agricultural Sciences (SLU), Umeå, Sweden (63° 45'N, 20° 17'E, 12 m a. s. l.). The plots (15×6 m) were part of a 6-year crop rotation comprising barley with undersowing of ley, first year ley, second year ley, green-fodder rape, potato and ryegrass. The same crop rotation and fertilization regimes had been used since 1965. Plots with six-row barley (*Hordeum vulgare* L. cv. Agneta) were sown on 29 May with undersowing of *Phleum pratense* L., *Festuca pratensis* L. and *Trifolium pratense* L. Herbicide treatment (Express 50T, 5.6 g ha<sup>-1</sup>) of barley was done on 24 June 2003.

Barley was harvested on 2 September 2003. One plot received N-P-K in the amounts 40-15-25 kg ha<sup>-1</sup> from chemical fertilisers while the second plot received N-P-K in the amounts 30-5-0 kg ha<sup>-1</sup> from chemical fertiliser and about 10-10-45 kg ha<sup>-1</sup> from stable manure. Chemical fertiliser was added in spring (22 May 2003) and stable manure was added in the preceding autumn (18 September 2002).

#### *Collection of soil solution with lysimeters*

Small lysimeters, Rhizon Soil Moisture Samplers (P2.30-1, Eijkkelkamp, Giesbeek, The Netherlands), with a pore size of 0.1 µm were used. A control experiment in the laboratory verified that the lysimeters were collecting 18 standard amino acids and NH<sub>4</sub><sup>+</sup> quantitatively and representatively from a mixture of these compounds. Lysimeters were installed at 45° angle to soil surface and spanned the soil depth 2–9 cm. Six lysimeters were installed in each plot on 7 June 2003. To facilitate close contact between soil and lysimeter water corresponding to 20 mm precipitation was added within a circular area 0.11 m<sup>2</sup> above each installed lysimeter. For collection of soil solution a sterile needle and a pre-evacuated sterile 10 ml glass tube fitted with a rubber stopper were attached to the lysimeter. Collection was done overnight (19.00 h until 9.00 h) on 9 June and 3 August, and in daytime (9.00 h until 18.00 h) on 6 October. Tubes were covered with aluminium foil (June, August) or shaded by vegetation (October) during collection. Tubes with collected liquid were kept in a cooler during transport (ca 1 h) and then immediately frozen, for later analysis by HPLC.

#### *Plant uptake studies*

##### *Plant material and growth conditions*

Seeds of six-row barley (*Hordeum vulgare* L. cv. Olsok) were soaked in tap water for 12–24 h and were then left to germinate on moist tissue paper in a glass petri dish covered with a plastic bag for 5–6 days. The petri dish was kept in daylight at approximately 20°C and the plastic bag was opened once daily to aerate the seedlings.

Seedlings with 2–4 cm high shoots were transferred to hydroponic culture in a greenhouse located in Umeå. Climate conditions in the greenhouse were

set to 20–25°C, 70% relative air humidity and 16–18 h of supplemental light (Osram HQI-BT, 400 W). Experiments were performed in April–June. Neither the growth conditions nor the experimental conditions were axenic. There was no mycorrhiza inoculation of the plants. Four seedlings were placed in a black plastic container (9.5×9.5×14 cm) filled with tap water. The water was aerated from an aquarium pump with 1–2 outlets per container. The seedlings were kept at the water surface by support from a 9×9×1 cm piece of floating black foam plastic with one seedling placed in a slot on each side of the squared piece.

After 5 days the water was replaced with a nutrient solution which was renewed 6 days later. The commercial nutrient solution Rika-S, Weibulls (Hammenhög, Sweden) was diluted to 1 mM total N (NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>) and supplemented with CaCl<sub>2</sub> to obtain the same mass of Ca and Mg in the solution. The nutrient solution thus contained (in mM) N: 1 (NO<sub>3</sub><sup>-</sup>-N: 0.62; NH<sub>4</sub><sup>+</sup>-N: 0.38); P: 0.07; K: 0.26; Ca: 0.03; Mg: 0.05; S: 0.04; B: 3.0×10<sup>-3</sup>; Fe: 3.0×10<sup>-3</sup>; Mn: 1.4×10<sup>-3</sup>; Zn: 0.4×10<sup>-3</sup>; Cu: 0.06×10<sup>-3</sup> and Mo: 0.06×10<sup>-3</sup> (values according to the manufacturer, plus additional CaCl<sub>2</sub>).

Barley plants were used in the uptake experiments after 13 days in hydroponics, 2 days after the latest renewal of nutrient solution. The plants had a dry matter content of 15%, a root fresh weight in the order of 0.1 g, and the longest root was on average 18 cm. The shoots had three to four leaves and were on average 20 cm high. The average dry weight was 69 mg for shoots and 21 mg for roots; average shoot/root ratio was 4.

#### *Experimental procedure*

The roots were gently blotted with tissue paper and then immediately dipped into three aliquots of 0.5 mM CaCl<sub>2</sub> to remove adsorbed compounds. Surplus solution was gently blotted with tissue paper after the third dipping and the plant roots were then immediately placed into the incubation solution. Each root system was in 15 ml incubation solution in an 18-ml vial. The vial with incubation solution and plant was rapidly weighed at start and end of the experiment to enable a correction for evapotranspiration losses during the experiment. During the uptake study the test solution was aerated from an aquarium pump via a sterile filter (0.22 µm) attached to a

0.8 mm diameter needle. Each uptake experiment lasted for 2 h. Uptake of amino acids was recorded as disappearance of amino acids from the incubation solution. An initial sample (160  $\mu$ l) was taken from the incubation solution immediately after placing the plant root into the solution and the sampling was repeated every 30 min. Samples were kept on ice during the experiment and were then stored in a freezer until analysed. The roots were again washed in 0.5 mM  $\text{CaCl}_2$  after the uptake experiment. The length of the longest root and the shoot height were measured and the number of leaves was counted. The shoot and root were then separated, kept on ice for 1 h and then stored in a freezer. Roots and shoots were later on dried at 60°C for 24 h and weighed.

### Amino acid uptake

Prevalent amino acids found in the soil solution served as guidance for choice of types and concentration ranges of amino acids in the amino acid uptake studies. The intention was also to include amino acids known to have a relatively high turnover rate in soil and having different properties with respect to the charge of their side chain at pH 7. The concentration of individual amino acids in the soil solution was in the range of 0–8.2  $\mu$ M (Table 1) (for analysis, see below). The incubation solutions used in the study of amino acid uptake were a mixture consisting of equal concentrations of the five amino acids L-Serine, L-Glutamic acid, Glycine, L-Arginine and L-Alanine in ultrapure water. All incubation solutions had a  $\text{CaCl}_2$  concentration of 0.5 mM, to ensure membrane integrity of the root cells (Epstein 1961). pH in the solution at start of the experiments was between 5.5 and 5.9.

The ability to take up amino acids was tested in pure amino acid incubation solutions at the four concentrations 2, 5, 10 and 25  $\mu$ M of each amino acid. The exact concentrations, measured at start of each experiment, are shown in Figs. 1 and 4 and are used for calculations in Fig. 2.

Four types of control experiments were carried out. Glycine-2- $^{13}\text{C}$ - $^{15}\text{N}$  was used in one experiment and the amount of labelled Glycine in the plants (root + shoot) was used to validate that disappearance of amino acids from the incubation solutions was due to root uptake. The composition of the incubation solution was the same as above except that 2  $\mu$ M Glycine-2- $^{13}\text{C}$ - $^{15}\text{N}$  (98% enrichment) was added to a total Glycine concentration of 4  $\mu$ M (2+2  $\mu$ M). Disappearance of amino acids from the incubation solution was studied as above.

Passive uptake of amino acids, amino acids leaked into the root cells and amount of amino acids bound to the apoplast was quantified by using the ATPase inhibitor CCCP (carbonyl cyanide m-chlorophenylhydrazone). By pre-treating the roots with this compound the disappearance of amino acids from the incubation solution is viewed as either a leakage into the root or a binding to the apoplast since active uptake, dependent on ATPase activity, is inhibited. The roots were pre-treated with 10 or 50  $\mu$ M CCCP in 0.5  $\mu$ M  $\text{CaCl}_2$  solution for 30 min prior to uptake experiments at the amino acid concentrations 0, 2 and 25  $\mu$ M together with 0, 2 and 25  $\mu$ M  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . Roots were washed between CCCP solution and amino acid solution. Uptake experiments were carried out as above.

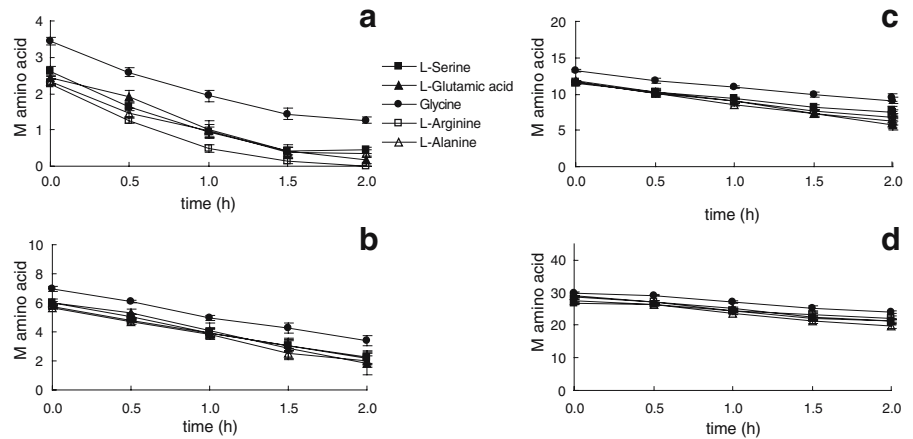
In order to exclude uptake of amino acids by microbes an interrupted uptake experiment was

**Table 1** Amino acid, ammonium and nitrate concentrations measured in soil solution under barley, irrespective of fertiliser

Amino acid	9 June			3 August			6 October		
	Mean	Median	Max	Mean	Median	Max	Mean	Median	Max
L-Serine	1.7	0.4	8.2	0.1	0.0	0.2	0.4	0.3	1.1
L-Glutamic acid	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.1
Glycine	0.7	0.3	3.3	0.2	0.2	0.3	0.3	0.3	0.5
L-Arginine	0.1	0.0	0.6	0.0	0.0	0.1	0.0	0.0	0.1
L-Alanine	0.5	0.2	2.1	0.4	0.3	0.5	0.3	0.3	0.6
Sum aa	5.5	2.0	22.2	1.4	1.4	2.0	1.8	1.8	3.6
$\text{NH}_4^+$	8.4	10.2	15.8	7.1	5.5	16.1	7.9	7.2	12.9
$\text{NO}_3^-$	1732	2082	2833	10	5	40	1	1	2

All values are in  $\mu$ M. Sum aa=sum of 16 amino acids analysed. Values are from 6 lysimeters sampled on three occasions.

**Fig. 1** Amino acid uptake measured as amino acid disappearance from incubation solution. Mean  $\pm$ SE (unless smaller than the symbol) for  $n=5$ . **A**, initial amino acid concentration 2  $\mu$ M; **B**, 5  $\mu$ M; **C**, 10  $\mu$ M and **D**, 25  $\mu$ M. Note different scales on y-axes



carried out where the plants were removed from the amino acid incubation solution after half the experimental time but measurements of concentration change in the test solution continued.

Efflux and/or leakage of amino acids from barley roots was recorded as appearance of amino acids in a solution of 0.5 mM  $\text{CaCl}_2$  in water during 2 h.

#### Analyses

##### *Amino acids and ammonium*

The frozen samples were thawed and immediately prepared for analysis by reversed-phase liquid chromatography using a Waters HPLC system with a Waters 470 fluorescence detector (Näsholm et al. 1987). The individual components were separated on a LiChroCART 250-4 Chrompher 100 RP-18 column using the following gradient: 0–10 min 40% MeOH, 10–20 min 50% MeOH, 20–30 min 68% MeOH, 30–33 min 100% MeOH, the balance being provided by 1 ml triethylamine and 1 ml HAc per 1000 ml of

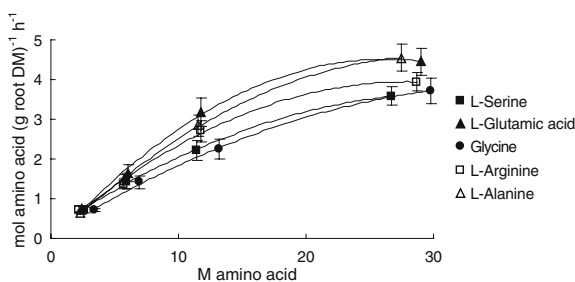
ultrapure water, pH 4.2. The samples were prepared with homocysteic acid (5–10  $\mu$ M) as internal standard with 40  $\mu$ l added to 160  $\mu$ l test solution (100  $\mu$ l + 100  $\mu$ l for soil solution samples) to a total of 200  $\mu$ l and then 50  $\mu$ l 1 M borate solution, pH 6.4, was added as a buffer. For fluorescence detection 250  $\mu$ l 9-fluorenylmethylchloroformate (FMOC-Cl, 15 mM in acetone) was added. After 10 min excess reagent was removed with N-heptan (1 ml). All samples were centrifuged before chromatography.

##### *Nitrate*

Concentration of  $\text{NO}_3^-$  in soil solution samples was analysed with a Nitrate/Nitrite Colorimetric Assay (Catalog No. 780001, Cayman Chemical Company, USA). Nitrate concentration was measured indirectly as nitrite. Total NO ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ) was analysed as nitrite through the use of nitrate reductase. Original nitrite concentrations were analysed in absence of nitrate reductase and then subtracted from total NO. Nitrite was analysed photometrically at 540 nm.

##### $^{15}\text{N}$ and $^{13}\text{C}$

Plants supplied with Glycine-2- $^{13}\text{C}$ - $^{15}\text{N}$  were dried as above and milled in a ball mill (Mixer Mill MM 301, Retsch®) to a fine powder. The  $^{13}\text{C}$  and  $^{15}\text{N}$  in the plant material was then analysed with a CN analyser (ANCA-NT system, liquid preparation module, PDZ Europa, Cheshire, U.K) coupled to an Europa 20–20 isotope ratio mass spectrometer as described by Ohlsson and Wallmark (1999). The values of excess  $^{15}\text{N}$  were converted to mole  $^{15}\text{N}$  excess  $\text{g DM}^{-1}$ . Excess  $^{15}\text{N}$  in the plant could then be compared with



**Fig. 2** Relationship between uptake rate, expressed per root dry mass, and measured initial amino acid concentration. Mean  $\pm$  SE (unless smaller than the symbol) for  $n=5$ . The curves show the polynomial regression fitted to the data

the loss of  $^{15}\text{N}$  from the solution.  $^{15}\text{N}$  disappearance from the incubation solution was analysed as Glycine concentration with HPLC.

### *Amino acids in plants*

Amino acid content was analysed in extracts of plant roots after efflux measurement in 0.5 mM  $\text{CaCl}_2$ . Frozen roots were kept frozen in liquid  $\text{N}_2$  and milled to a fine powder in the ball mill. Milled samples (30–32 mg) were extracted in 1 ml of 0.01 M HCl for 1 h under agitation at 4°C. The supernatant (40  $\mu\text{l}$ ) was collected after centrifugation at 14000 rpm for 10 min and prepared for HPLC analysis as above but with 160  $\mu\text{l}$  of 10  $\mu\text{M}$  homocysteic acid.

### *Data handling and statistics*

The uptake rate of each amino acid was calculated from linear regression between amino acid concentration in solution and experimental time (0–2 h). Each regression was based on five sampling times. Uptake rates were approximated as linear but should be asymptotic if the concentration dependency is following Michaelis–Menten kinetics. The relation between uptake rate and initial concentrations was described by polynomial regression. The maximum uptake rate ( $V_{\text{max}}$ ) and the half-saturation constant or the uptake affinity ( $K_m$ ) were determined by Hanes–Wolf plot. In control experiments linear regression was used to distinguish CCCP inhibited uptake.

## Results

### Amino acid uptake

Barley took up amino acids at all initial concentrations (2–25  $\mu\text{M}$ ) when measured as disappearance from the amino acid solution (Fig. 1). Uptake capacity was, however, shown at even lower concentrations than 2  $\mu\text{M}$  since the uptake continued during the experimental time, in some plants until the solution was totally depleted. Uptake of amino acids was almost constant over time and variation among plants was small. Mean amounts of amino acids taken up during the 2 h incubation time was 1.3  $\mu\text{mol}$  L-Alanine ( $\text{g root DM}^{-1}$ ), 1.4  $\mu\text{mol}$  ( $\text{g root DM}^{-1}$ ) of Glycine, L-Serine, and L-Glutamic acid and

1.5  $\mu\text{mol}$  ( $\text{g root DM}^{-1}$ ) of L-Arginine at the 2  $\mu\text{M}$  initial concentration. Larger amounts of amino acids were taken up in experiments with higher initial concentrations, up to 8.6  $\mu\text{mol}$  ( $\text{g root DM}^{-1}$ ) of L-Glutamic acid and L-Alanine at the initial concentration of 25  $\mu\text{M}$  (Fig. 1).

Uptake rates were calculated from disappearance of amino acids from the incubation solutions, including all sampling times (Fig. 1). For each amino acid the uptake rate increased with increased concentration of the amino acid solution (Fig. 2). The dependency of uptake rate on amino acid concentration could be described by Michaelis–Menten kinetics which includes that uptake could be described by polynomial regression in conformity with enzyme kinetics. Uptake over time should therefore be entailed to be asymptotic. We have, however, approximated a linear uptake for uptake rate estimations in this study. Linear approximations of uptake rates are convenient when the uptake of amino acids during the incubation time is small in relation to the initial concentration. Thus, uptake rate of each amino acid at each initial concentration was estimated from linear regression of the values in the time series for each replicate plant (Fig. 1). All slopes for the regression were significant and  $R^2$  values were in the range 0.8–1.0. For the 2  $\mu\text{M}$  concentration a deviation from the linearity caused by decreased uptake measured in the final samples was visible while at higher initial concentrations (5–25  $\mu\text{M}$ ) the deviation was very small (Fig. 2). This deviation leads to an underestimation of the uptake rate for the 2  $\mu\text{M}$  concentration. Uptake rates ranged between 0.6 and 4.6  $\mu\text{mol amino acid (g root DM}^{-1} \text{ h}^{-1}$ ) for the different concentrations and was in the range 0.6–0.8  $\mu\text{mol amino acid (g root DM}^{-1} \text{ h}^{-1}$ ) (L-Alanine, L-Glutamic acid) for the 2  $\mu\text{M}$  initial concentration (Fig. 2).

Uptake of amino acids from the incubation solutions was further characterised by calculating uptake affinity. The affinity constant ( $K_m$ ) was in the range 19.6–33.2  $\mu\text{M}$  and maximum velocity ( $V_{\text{max}}$ ) was in the range 6.3–10.2  $\mu\text{mol (g root DM}^{-1} \text{ h}^{-1}$ ) (Table 2).

### Control experiments

To verify that the disappeared amounts of amino acids from the incubation solutions were absorbed by plant roots, Glycine-2- $^{13}\text{C}$ - $^{15}\text{N}$  uptake was measured as  $^{15}\text{N}$

**Table 2** Calculated maximum uptake rates and affinity constants for amino acid uptake

Amino acid	Charge	$K_m \pm \text{SEE}$ ( $\mu\text{M}$ )	$V_{\text{max}} \pm \text{SEE}$ ( $\mu\text{mol (g root DM)}^{-1} \text{ h}^{-1}$ )
L-Serine	uc	20.1 $\pm$ 1.0	6.3 $\pm$ 0.2
L-Glutamic acid	nc	21.4 $\pm$ 5.3	7.9 $\pm$ 1.2
Glycine	uc	33.1 $\pm$ 3.0	7.9 $\pm$ 0.5
L-Arginine	pc	19.6 $\pm$ 4.5	6.7 $\pm$ 0.9
L-Alanine	uc	33.2 $\pm$ 6.0	10.2 $\pm$ 1.3

un, uncharged; nc, negatively charged; pc, positively charged amino acid at pH values used in the uptake experiment; SEE, standard error of estimate

labelling of plants.  $^{15}\text{N}$ , but not  $^{13}\text{C}$ , was clearly detected in plants and therefore only  $^{15}\text{N}$  could be used to assess plant uptake and compare it with rates of depletion from the incubation solutions. A shift in  $^{13}\text{C}$  pertaining to uptake of tracer was not possible since dilution of  $^{13}\text{C}$  in plants is prominent especially when using low substrate concentrations. Mean plant N pertaining to uptake of Glycine during 2 h incubation equalled 0.31 $\pm$ 0.02  $\mu\text{mol N (g DM)}^{-1}$  (mean $\pm$ SE). Disappearance of Glycine from the solution during the 2 h incubation was 0.43  $\mu\text{mol N}\pm$ 0.04 (g DM) $^{-1}$  (mean $\pm$ SE). This shows that disappearance of Glycine from the incubation solution was indeed due to plant uptake.

In the interrupted uptake trial, the concentration of amino acids in the incubation solution remained constant following removal of the plant (Fig. 3). The uptake during the first hour, when the plant was present, was similar to the uptake in experiments when the plant was present throughout the experimental time (Fig. 1).

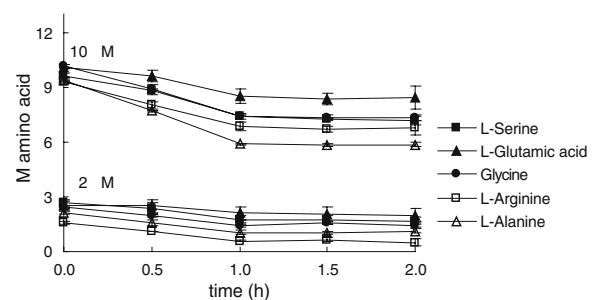
Results from pre-treatment with 50  $\mu\text{M}$  CCCP suggested that passive uptake was insignificant for nearly all amino acids (Table 3). We could measure a significant amino acid uptake also in the CCCP treated plants for L-Arginine at 2  $\mu\text{M}$  amino acid concentration and L-Serine, L-Glutamic acid and L-Alanine at 25  $\mu\text{M}$  amino acid concentration. The uptake was however low, the variation was large and the main part of the amino acid uptake was inhibited, except for L-Glutamic acid. The disappearance of L-Glutamic acid during treatment without CCCP was maintained in the treatment with CCCP (Table 3).

When barley roots were placed in the incubation solution containing only 0.5 mM  $\text{CaCl}_2$  we recorded an appearance, although at low concentration, of all

five amino acids in the solution (Fig. 4). The concentration of L-Glutamic acid increased slightly during the first 30 min but then it decreased. The concentration of L-Arginine was nearly constant and close to zero during the incubation. In comparison with amino acid uptake (Fig. 1), total efflux was small, no more than 0.15  $\mu\text{mol (g root DM)}^{-1}$  (Fig. 4, L-Alanine). There was no obvious relation between the concentration increase of amino acids in the incubation solution during the efflux experiment and the endogenous amino acid concentrations in the roots of the same plants (data not shown).

## Discussion

The soil solution of agricultural ecosystems may contain a range of organic N compounds, including amino acids. We used microlysimeters in an attempt to study the content of free L-amino acids in the soil solution with minimal disturbance of the soil. Amino acids were present at concentrations up to c. 10  $\mu\text{M}$  under barley (Table 1) which is in agreement with findings by Jones et al. (2005) from grassland (*Lolium perenne* L. and *Trifolium repens* L.). We addressed the question if plants can acquire amino acids when these are present at concentrations relevant for the root environment in field. Our results clearly show that barley has the capacity to take up all tested amino acids at concentrations as low as 2  $\mu\text{M}$  (Fig. 1). Our data also indicate that root amino acid uptake occur at concentrations lower than 2  $\mu\text{M}$ , since uptake of all amino acids by barley continued below 2  $\mu\text{M}$ , and reached concentrations below 0.5  $\mu\text{M}$  during the 2 h incubation for all amino acids except Glycine. Earlier studies of root amino acid uptake have commonly



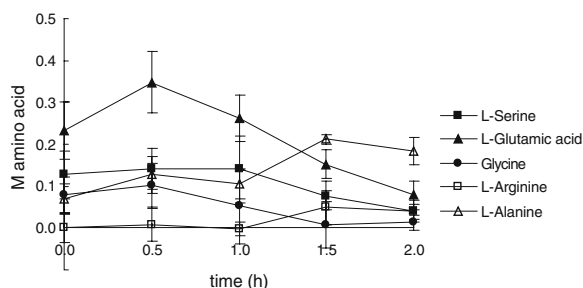
**Fig. 3** Disappearance of amino acids in incubation solution during interrupted uptake trials at the initial concentrations 2  $\mu\text{M}$  and 10  $\mu\text{M}$ . Mean  $\pm$  SE (unless smaller than the symbol) for  $n=3$ . Plants were removed from the solutions after 1 h

**Table 3** Results from regression of plant root uptake of amino acids over time after treatment without CCCP and with CCCP

Initial amino acid concentration ( $\mu\text{M}$ )	Amino acid	Uptake rate ( $\mu\text{mol (g root DM)}^{-1} \text{h}^{-1}$ )		$R^2$		$p$		Inhibition by CCCP (%)
		Without CCCP	With CCCP	Without CCCP	With CCCP	Without CCCP	With CCCP	
2	L-Serine	1.2	0.2	0.88	0.10	0.00	0.26	100
2	L-Glutamic acid	1.1	0.6	0.90	0.13	0.00	0.18	100
2	Glycine	1.5	0.3	0.83	0.12	0.00	0.20	100
2	L-Arginine	1.2	0.4	0.82	0.30	0.00	0.03	67
2	L-Alanine	1.2	-0.6	0.72	0.15	0.00	0.15	100
25	L-Serine	4.2	1.3	0.61	0.30	0.00	0.03	68
25	L-Glutamic acid	3.3	3.6	0.84	0.55	0.00	0.00	-8
25	Glycine	6.1	2.0	0.63	0.08	0.00	0.29	100
25	L-Arginine	4.7	1.4	0.32	0.10	0.01	0.25	100
25	L-Alanine	5.2	1.9	0.86	0.36	0.00	0.02	63

Mean for  $n=3$ , except for 25  $\mu\text{M}$  amino acids and without CCCP where  $n=4$ .

used concentrations far higher than those recorded in studies of soil solutions (e.g. Wright 1962; Raab et al. 1999; Schmidt and Stewart 1999; Owen and Jones 2001). Our study thus indicates that root uptake of amino acids will occur also when soil solution concentrations of such compounds are in the lower end of the  $\mu\text{M}$  range. We used two different methods to verify that the depletion of amino acids in the test solutions could be used as a proxy for root amino acid uptake. Firstly, rates of  $^{15}\text{N}$  labelling of plants exposed to Glycine-2- $^{13}\text{C}$ - $^{15}\text{N}$  showed that a major fraction of the loss of Glycine from the incubation solution was recovered in plants. Secondly, when plants were removed from the incubation solution, the decrease in amino acid concentration was halted which suggests that microbes in the incubation solution did not make a measurable contribution to the amino acid depletion of the solution. Furthermore,



**Fig. 4** Amino acid concentrations following immersion of barley roots into 0.5  $\mu\text{M}$   $\text{CaCl}_2$ . Mean  $\pm$  SE (unless smaller than the symbol) for  $n=4$

uptake of all amino acids except L-Glutamic acid were inhibited when plants were pre-treated with the protonophore CCCP, suggesting that amino acid depletion in the solution was due to active uptake of roots. Thus, we argue that amino acid uptake of barley roots can be inferred from studies of depletion of root incubation solutions. This was also established in a study of root amino acid uptake in Scots pine seedlings (Persson and Näsholm 2001).

Because competition for N, and in particular for organic N such as amino acids, is strong in many terrestrial ecosystems, information on root uptake characteristics is critical to assess the possibility of plant organic N uptake. Concentration of amino acids does not necessarily reflect the fluxes between different pools of amino acids in the soil. Both the flux and the influence of binding of amino acids to soil particles are important for quantifying amino acid uptake in plants. However, the concentration of amino acids in the root environment is of importance when defining the root uptake affinity. Our data show that rates of root uptake increase with increasing concentrations of amino acids which suggests that concentration dependencies of root uptake rates of amino acids comply with Michaelis–Menten kinetics. Earlier studies on root uptake kinetics where done on excised roots (Kielland 1994; Soldal and Nissen 1978) and this has been questioned by Falkengren-Grerup et al. (2000) who found that presence–absence of shoot gave highly variable results. Our aim was therefore to

characterize root uptake in intact plants. Interestingly our data on barley are in agreement with Soldal and Nissen (1978) and corroborate that uptake is due to active transport, mediated by specific transporters. Published values on affinity constants of amino acid uptake (Table 4) vary from 9.6  $\mu\text{M}$  (L-Arginine; Soldal and Nissen 1978) up to 296  $\mu\text{M}$  (L-Glutamic acid; Kielland 1994). We found  $K_m$  to vary between 19.6  $\mu\text{M}$  (L-Arginine) and 33.2  $\mu\text{M}$  (L-Alanine) which is in the lower range of most published values (cf. Table 4). The variation in reported values of affinity constants may, however, primarily reflect differences in experimental conditions; in particular the range of concentrations used, and does not necessarily reflect differences between plant species. Recent studies have shown that root amino acid absorption is, at least partly, mediated by the Lysine Histidine Transporter 1 (LHT1) (Hirner et al. 2006; Svennerstam et al. 2007) and the amino acid permease 1 (AAP1) (Lee et al. 2007). LHT1 has been characterized as a high affinity transporter, mediating

transport of a wide range of amino acids but with relatively low affinity for basic amino acids. The affinity of LHT1 for e.g. L-Proline and L-Glutamic acid was found to be c. 10 and 14  $\mu\text{M}$ , respectively, as determined from studies of yeast expressing the Arabidopsis ortholog, (Hirner et al. 2006). Although  $K_m$  values might differ dependent on the system used (in this case plants versus yeast), the  $K_m$  of barley root L-Glutamic acid transport was estimated to be c. 21.4  $\mu\text{M}$ , i.e. slightly higher, but still in the same range, as the estimate of AtLHT1 mediated uptake of L-Glutamic acid in yeast. This could suggest that a LHT1 homolog is at least partly responsible for the uptake in barley.

In general, differences in root uptake characteristics between amino acids were small (Fig. 1). Provided that root amino acid transporters have a broad substrate selectivity (Hirner et al. 2006; Svennerstam et al. 2007; Lee et al. 2007) it is possible that it is the total amino acid concentration rather than the concentration of single amino acids which is

**Table 4** Examples of kinetic parameters of amino acid uptake in plants and in soil microbes

Study object	Amino acid	Concentration ( $\mu\text{M}$ )	$K_m$ ( $\mu\text{M}$ )	$V_{\max}$	Reference
Intact plants					
<i>Ricinus communis</i>	L-Arginine	10–10,000	30–50		Schobert and Komor 1987
	L-Proline	10–10,000	30–50		
Excised roots					
<i>Hordeum vulgare</i>	L-Proline	0.9–28	61	2.81 $\mu\text{mol g}^{-1}$ FW $\text{h}^{-1}$	Soldal and Nissen 1978
	L-Lysine	0.1–4	3.9	0.031	
		4–55	40	0.17	
	L-Methionine	0.2–15	20	0.14	
	L-Arginine	10–25	9.6	0.45	
7 tundra species	Glycine	10–500	7–96	1.2–6.8 $\mu\text{mol g}^{-1}$ DM $\text{h}^{-1}$	Kielland 1994
	L-Aspartic acid	10–500	81–96	0.5–4.1	
	L-Glutamic acid	10–500	30–296	0.4–1.2	
Source of microbes					
Quaternary foothill	Glycine	100–5,000	1,950–2,550	0.039–0.119 $\mu\text{mol g}^{-1}$ soil $\text{h}^{-1}$	Vinolas et al. 2001
Sediment	L-Glutamic acid	100–5,000	1,210–2,580	0.030–0.176	
Sandy loam	L-Lysine	100–5,000	1,780–2780	0.049–0.086	Jones and Hodge 1999
	L-Lysine	10–10,000	518	0.068 $\text{nmol g}^{-1}$ soil $\text{h}^{-1}$	
	Glycine	10–10,000	590	0.045	
Loess parent material	L-Glutamic acid	10–10,000	900	0.028	Jones and Kielland 2002
	L-Glutamic acid	50–1,000	927–4,269	0.241–0.825 $\mu\text{mol g}^{-1}$ soil $\text{h}^{-1}$	
	Glycine	50–1,000	377–1178	0.050–0.321	
Alpine dry meadow	L-Lysine	50–1,000	756–6056	0.168–1.067	Lipson et al. 1999
	L-Glutamic acid	10–100	39	390 $\text{nmol g}^{-1}$ soil $\text{h}^{-1}$	
	Glycine	10–100	46	240	

decisive for rate of amino acid acquisition. However, L-Glutamic acid displayed a different pattern with respect to CCCP inhibition of uptake (Table 3) because rates of uptake of this amino acid were not affected by the inhibitor. The reason for this deviation is unclear.

Measured efflux (Fig. 4) was small or negligible in comparison to amino acid uptake (Fig. 1). Again, L-Glutamic acid displayed a different pattern compared to the other tested amino acids with an initial efflux during the first 30 min followed by a steady rate of uptake during the rest of the experiment. Several studies have addressed the question of root exudation of amino acids (Paynel et al. 2001; Paynel and Cliquet 2003; Phillips et al. 2004). Our results do not lend support to a prominent efflux of amino acids under the experimental conditions we used.

Relative affinities for uptake are one important factor in evaluating competitive capacities of various soil organisms. Similar to plants, estimates of affinity constants for amino acid uptake in micro-organisms vary by several orders of magnitude (e.g. Jones and Kielland 2002; Vinolas et al. 2001; Jones and Hodge 1999; Lipson et al. 1999, Table 4). The large variability in  $K_m$  estimates might be related to the presence of two distinct transport systems (high and low affinity) with an estimated transition between 50–150  $\mu\text{M}$  (Jones and Hodge 1999). When uptake has been characterized at lower concentrations,  $K_m$  values for uptake of various amino acids have been approximated in the range 20–50  $\mu\text{M}$ , i.e. within the same scale as plant roots (Table 4). Thus, if plants are inferior to micro-organisms with regard to competition for soil amino acids, this is probably not due to differences in affinities of uptake systems.

Quantification of amino acid uptake rates under field conditions is difficult and the most commonly used method is based on injections of isotope labelled amino acids into the soil. Studies that have used these methods have shown that plants can take up intact amino acids under field conditions (Schimel and Chapin 1996; Näsholm et al. 1998, 2000, 2001; Miller and Bowman 2003) but have been criticised for using high amino acid concentrations. Field studies have a critical function in assessing the ability of plants to absorb amino acids under field conditions but may not be suitable for assessing root uptake characteristics. However, our study shows that plants have uptake capacity at low amino acid concentrations.

In conclusion, we have shown that intact plants of barley can take up all the five studied amino acids occurring together at concentrations relevant for field conditions. The  $K_m$  values calculated for the amino acid uptake are comparable to measured values for soil micro-organisms. Further studies under realistic soil conditions are however needed in order to evaluate the competitive abilities in plants versus microbes and to assess the quantitative importance of amino acids in plant nutrition.

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