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Lysine Transport in Two Barley Mutants with Altered Uptake of Basic Amino Acids in the Root[1]

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SIMON W. J. BRIGHT, JOSEPH S. H. KUEH, AND SVEN E. ROGNES
Biochemistry Department, Rothamsted Experimental Station, Harpenden, Hertfordshire AL5 2JQ, United Kingdom (S. W. J. B., J. S. H. K.); and Department of Botany, University of Oslo, P.O. Box 1045, Blindern, Oslo 3, Norway (S. E. R.)

ABSTRACT

Amino acid uptake was examined in two barley (*Hordeum vulgare* L.) mutants R906 and R4402 which had been selected as resistant to the lysine analog *S*-(2-aminoethyl)-cysteine. The mutants were found to be allelic by crossing and examination of F_1 and F_2 progeny. The mutant genes were designated *aec1a* and *aec1b*, respectively. The uptake of the basic amino acids lysine, arginine, and ornithine from 50 micromolar solutions was strongly decreased in roots of the mutants, whereas uptake of neutral and acidic amino acids was unaffected. The pattern of uptake of lysine over the range 10^{-7} to 10^{-2} molar was consistent with there being, principally, two uptake systems operating for basic amino acids in roots and that a low-concentration, high-affinity system is reduced or lacking in the mutants. The residual transport activity in the mutants had a different relative affinity for lysine and arginine to the wild-type system. Uptake of lysine by leaf slices was unimpaired in the mutants suggesting that the leaf uptake system is unaffected by the *aec1* gene.

From screens for barley mutants with the potential to accumulate soluble lysine, we recovered two mutants resistant to the toxic effects of the lysine analog Aec[2]. Pure breeding lines of these mutants designated R(Rothamsted)906 (4) and R4402 (unpublished) were obtained. Examination of a number of aspects of lysine metabolism in R906 indicated that resistance in this mutant was due to reduced uptake of Aec in the roots (3). Lysine transport has been specifically studied in roots (15) and leaves (10, 11) of barley and in cultured cells of tobacco (6) and sugarcane (12). It is thought to be driven by the membrane potential (8, 11, 14). Mutants may help in elucidating the relationships between leaf and root uptake systems, in defining the number of carrier systems, and in establishing their relevance to multiple kinetic phases of uptake (2, 6, 10, 15, 16). We have therefore examined the transport of basic and other amino acids into roots and leaves of the mutants R906 and R4402 as well as establishing their genetic relationship.

MATERIALS AND METHODS

Plant Materials. Seed stocks of barley (*Hordeum vulgare* L.) cv Bomi and Maris Mink and their Aec-resistant mutants R906 (4) and R4402, respectively, were maintained by self-fertilization of glasshouse-grown plants. Flowers on selected female plants for crossing were emasculated and enclosed in cellophane bags for 2 to 4 d before pollination with anthers from selected male parents. Bags were kept over the ears until harvest. For uptake experiments, seeds were soaked in water at 4°C overnight and planted in seed trays with washed vermiculite in a growth chamber (20°/16°C, 12 h d, 180 μE m^{-2} s^{-1} from mixed fluorescent and tungsten lights). Trays were watered with tap water.

Aec resistance was tested by growing mature embryos (hand dissected and surface sterilized) under the conditions previously described (4) for 7 d on a medium containing 0.25 mM Aec and 0.6% (w/v) agar.

Uptake Experiments. Leaf uptake experiments were performed by a modification of the method of Lien and Rognes (10) using the primary leaves of 7-d-old plants. The terminal 1 cm of the leaf was discarded and up to 5 cm of the leaf was cut into approximately 1-mm-wide slices on graph paper. The slices were collected in buffer A (10 mM Tris-acetic acid [pH 6.0], 0.5 mM $CaCl_2$) at 4°C. About 30 mg fresh weight of leaf material was added to 5 ml buffer A containing ^{14}C-labeled amino acid and incubated for 60 min at 30°C on a reciprocal shaker (120 strokes/min). Uptake was terminated by washing the slices with 100 ml ice-cold distilled H_2O on a filter paper in a glass funnel. The slices were blotted, weighed, and incubated in a scintillation vial in 1 ml NCS solubilizer (Amersham) for 2 d. The sample was neutralized with 0.2 ml 25% (v/v) acetic acid and bleached with 0.3 ml of the supernatant of 7% (w/v) calcium hypochlorite before addition of 10 ml Tritosol scintillation cocktail (5).

For root uptake experiments, 3- to 4-d-old plants were used. Vermiculite was gently removed from the roots and the seedlings were collected in buffer A at 4°C. Incubations were started by cutting two entire root systems into 1- to 2-cm-long sections and placing the tissue in 3 ml (in some experiments, 5 ml) buffer A containing amino acids at the required concentration and specific radioactivity for 30 min at 30°C as above. Roots were collected, washed as for leaf slices, and incubated in 1 ml NCS solubilizer at 40°C overnight before neutralization with 0.2 ml 25% (v/v) acetic acid. Radioactivity was determined in 10 ml Tritosol. In some of the experiments in Table II and in Figure 2, the roots were held in empty tea bags, incubated together in 100 ml amino acid solution, and at the end of the experiment the bags were washed in six changes of ice-cold water. Although larger numbers of roots could be handled by this alternative method and similar results were obtained, the agreement between replicates was not as good.

Radioactive amino acids used were L-isomers and uniformly ^{14}C-labeled except for DL-[U-^{14}C]ornithine, all from Amersham International. Radioactive solutions were adjusted to give approximately 10^5 dpm per treatment where possible. Quench correction was by the channels ratio method in the range 78 to 86% counting efficiency.

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[2] Abbreviation: Aec, *S*-(2-aminoethyl)-L-cysteine.

Table I. *Inheritance of Resistance to Aec in Mutants R4402 and R906*

Embryos were germinated and grown for 7 d on medium containing 0.25 mM Aec. Plants were scored for the presence of roots penetrating the agar medium. F_2 seeds were obtained from at least three selfed F_1 plants.

Plant	No. of Plants with	
	Penetrating roots	Nonpenetrating roots
R906	21	0
R4402	41	0
Bomi	0	16
Maris Mink	0	20
Golden Promise	0	38
F_1 (R4402 × Golden Promise)	0	9
F_1 (R4402 × R906)	8	0
F_1 (R906 × R4402)	8	0
F_2 (R4402 × Golden Promise)	56	200
F_2 (Golden Promise × R4402)	27	89
F_2 (R4402 × R906)	121	0
F_2 (R906 × R4402)	109	0

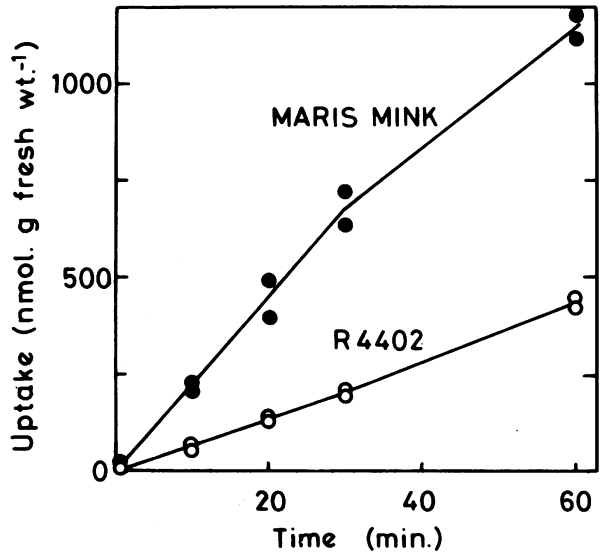

FIG. 1. Uptake of lysine by roots of 3-d-old Maris Mink (●) and R4402 (○) plants. The lysine concentration was 50 μM.

RESULTS

Two pure breeding mutant lines of barley, R906 and R4402, were each derived from self-fertilization of single plants selected as resistant to Aec among screened M_2 embryo populations of cv Bomi (R906) or cv Maris Mink (R4402). It was known that Aec resistance in R906 was inherited as a single recessive nuclear gene (4). The relationship of this gene (*aec1*) to the gene for Aec resistance in R4402 was investigated by crossing R4402 with wild-type cv Golden Promise and with R906 plants (Table I). Inheritance of Aec-resistance was assessed by the penetration of the young roots into agar medium containing Aec (4). In the F_1 generation of crosses between R4402 and Golden Promise, all plants were sensitive and, in the F_2resistant plants (roots penetrating) and sensitive plants, were present in a ratio not significantly different from the 1:3 ratio (χ^2-values were 1.3 and 0.2 for the reciprocal crosses) expected for the segregation of a single recessive nuclear gene. In crosses between the two mutants, the F_1 and F_2 progeny were all resistant, suggesting that the resistance genes in R906 and R4402 are allelic.

In preliminary work, it had been shown that lysine and Aec uptake by intact roots from 2.5×10^{-4} M solutions was decreased

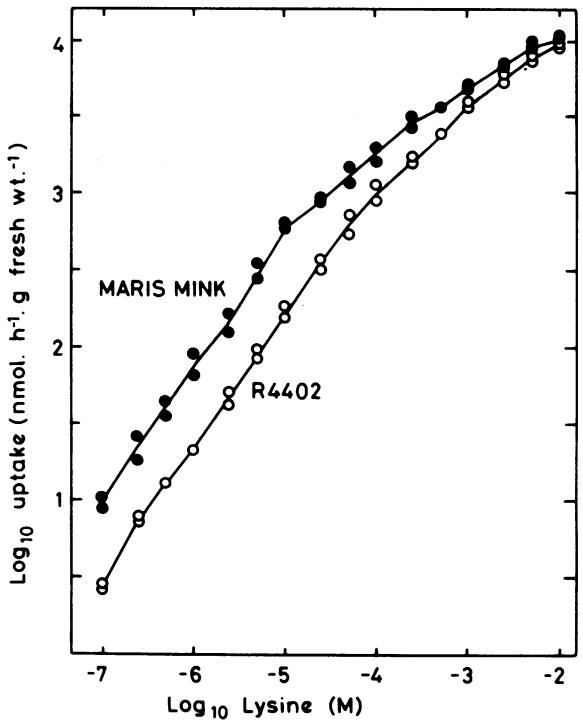

FIG. 2. Concentration dependence of lysine uptake in the range of 10^{-7} to 10^{-2} M external lysine by roots of 4-d-old Maris Mink (●) and R4402 (○) plants.

Table II. *Amino Acid Uptake by Roots of Maris Mink and R4402*

Results are expressed as means ± SD of triplicate assays using 4-d-old plants (Exp. I) or quadruplicate assays using 3-d-old plants (Exp. II). Amino acid concentration was 50 μM, except for DL-ornithine (100 μM). The right column (%) gives uptake by R4402 as percentage of uptake by Maris Mink roots.

Amino Acid	Uptake		
	Maris Mink	R4402	
	nmol/g fresh wt·h		%
Exp. I			
Leucine	457 ± 14	408 ± 17	89
Asparagine	222 ± 71	200 ± 14	90
Lysine	1013 ± 82	461 ± 35	45
Arginine	626 ± 46	333 ± 38	53
Exp. II			
Glutamic acid	47 ± 5	51 ± 6	108
Alanine	358 ± 41	376 ± 46	105
Lysine	1284 ± 152	455 ± 59	35
DL-Ornithine	658 ± 35	322 ± 30	49

in R906 (3). We used the two mutants and their parent cultivars to examine this phenomenon in more detail. Excised roots of Maris Mink and R4402 accumulated lysine from 5×10^{-5} M solution in a linear manner for 30 min (Fig. 1), with the mutant rate being 30% of the parent value. Lysine uptake by R4402 roots was compared with Maris Mink over the range 10^{-7} to 10^{-2} M lysine. Between 10^{-7} and 10^{-5} M, the mutant rate was roughly 30% of the control value, but above 10^{-5} M the two values approached each other until at 10^{-2} M the mutant rate was 90% of the control figure (Fig. 2).

Uptake of a number of basic, neutral, and acidic amino acids were compared in R4402 and Maris Mink. The mutant took up near normal (89–108%) amounts of neutral (leucine, asparagine, alanine) and acidic (glutamic acid) amino acids, whereas the uptake of lysine, arginine, and ornithine were all impaired (Table

Table III. *Uptake of Lysine and Arginine by Roots of R4402, R906, and Their Parent Cultivars*

Results are mean values from duplicate assays using roots of 3-d-old plants.

Amino Acid	Concn.	Uptake					
		Maris Mink	R4402	Mutant *vs.* parent[a]	Bomi	R906	Mutant *vs.* parent[a]
	nM	*nmol/g fresh wt·h*		*%*	*nmol/g fresh wt·h*		*%*
Lysine	80	3.81	1.57	41	3.88	1.64	42
	750	34.2	13.1	38	30.9	11.8	38
	9430	361	117	32	351	113	32
Arginine	80	4.94	2.32	47	4.05	2.05	51
	790	42.5	17.3	41	35.9	15.4	43
	9470	264	170	64	266	131	49

Concn. (Arg–Lys)	Arginine Uptake/Lysine Uptake			
	Maris Mink	R4402	Bomi	R906
nM	*ratio*			
80–80	1.30	1.48	1.04	1.25
790–750	1.24	1.32	1.16	1.31
9470–9430	0.73	1.46	0.76	1.16

[a] Mutant value as percentage of its parent cultivar.

Table IV. *Effect of the Addition of Unlabeled Arginine, Lysine, and Aec on the Uptake of Radioactive Lysine*

Values for each treatment are means of duplicate assays with roots of 3-d-old plants. Figures in brackets are percentage of control value (no unlabeled competitor added). Results are calculated for lysine with constant specific radioactivity as supplied, thus treating unlabeled lysine as a competitor in the same way as the other amino acids. The concentration of labeled lysine was 2 μM in all assays.

^{12}C-Amino Acid Added	Concn.	Uptake of [^{14}C]Lysine		
		Maris Mink	R4402	R4402/Maris Mink
	μM	*nmol/g fresh wt·h*		*ratio*
None (control)	0	100 (100)	25 (100)	0.25
Arginine	5	47 (47)	21 (85)	0.46
	50	14 (14)	8.9 (35)	0.62
	500	4 (4)	3.7 (15)	0.91
Lysine	3	76 (76)	27 (106)	0.35
	48	42 (42)	18 (74)	0.44
	500	12 (12)	8 (34)	0.70
Aec	5	82 (82)	22 (89)	0.27
	50	54 (54)	23 (90)	0.42
	500	22 (22)	13 (52)	0.59

Table V. *Uptake of Lysine and Leucine by Leaf Slices*

Results are mean values ± SD from triplicate assays. Bracketed figures are the mutant values as percentage of the parent cultivar. Amino acids were supplied at 50 μM.

Plant	Uptake	
	Lysine	Leucine
	nmol/g fresh wt·h	
Maris Mink	464 ± 38	359 ± 28
R4402	420 ± 36 (90%)	339 ± 21 (94%)
Bomi	716 ± 75	551 ± 70
R906	612 ± 112 (86%)	460 ± 12 (83%)

II), suggesting that there is a specific deficiency in a basic amino acid transport system. The two mutants R906 and R4402 were compared at three lysine and arginine concentrations in the range 8×10^{-8} to 9.5×10^{-6} M to see if it was possible to distinguish between them (Table III). In all treatments, the behavior of R906 and R4402 was similar. The ratio of arginine to lysine uptake at the three concentrations was always higher in the mutants than in their parent cultivar, this effect being most marked at the 9.5×10^{-6} M concentration. This was investigated further in an experiment in which unlabeled arginine, lysine, or Aec was added to compete with the uptake of labeled lysine (Table IV). Arginine, lysine, and Aec (in order of decreasing efficiency) reduced the uptake of ^{14}C from labeled lysine in Maris Mink and, less effectively, in R4402, such that the ratio of uptake by the mutant relative to Maris Mink was increased from 0.25 up to 0.91.

Lysine uptake by leaf slices of mutant and parent plants was compared to establish whether there was a loss of uptake capacity in leaf cells parallel with the loss in the roots. No significant differences in lysine or leucine uptake rates between mutants and parents were observed (Table V). In this experiment, but not another, a difference between the two cultivars used was observed, most probably due to differences in leaf slice thickness.

DISCUSSION

In the mutant R4402, the gene conferring Aec resistance behaved as a single recessive nuclear gene (Table I) allelic to the gene designated *aec1* in R906 (4). It is appropriate therefore to distinguish the genes in these mutants (which arose as separate mutational events in different cultivars) as *aec1a* in R906 and *aec1b* in R4402.

Both mutants have a similar phenotype, being able to grow in the presence of aminoethylcysteine and having reduced uptake of lysine into the roots. Previous work with R906 (3) had established (a) that the synthesis of lysine and threonine from [^{14}C]acetate was

regulated in a normal manner, (b) that the free amino acid pools of young mutant plants growing in the presence of Aec contained less soluble Aec, and (c) that young mutant plants had similar contents of soluble lysine and threonine to the parent when grown in the absence of Aec. These results, together with our present studies, strongly suggest that decreased uptake is the primary lesion in the mutants rather than being a secondary effect of some other metabolic alteration. A carrot cell line resistant to Aec was also found to have decreased lysine uptake, but no further characterization of uptake in the variant line was reported (13).

A comprehensive analysis of data on amino acid transport in higher plants has led Kinraide (9) to propose that two carrier systems are normally present, a general amino acid transport channel of low specificity (10) and a more specific basic amino acid transport system (1, 6, 12). The decreased uptake of lysine observed in the present study (Fig. 2) was most apparent at concentrations below 10^{-5} M; at higher concentrations, the difference between mutant and parent decreased progressively. In accordance with the above two-system hypothesis, the simplest interpretation of our results is that two carrier systems for lysine exist in the barley root cell membrane and that one of these, a high-affinity system, is altered or lacking in the mutants. Multiphasic uptake kinetics with four or five phases of lysine uptake have been reported for barley roots (15). Double-reciprocal or Eadie-Hofstee plots of our data (Fig. 2) over the lower concentration ranges also indicated multiple phases. The mutants described here have significantly reduced uptake over a range of at least three of these phases ($\leq 2.5 \times 10^{-4}$ M lysine) (15). It is clear therefore that these phases, if they represent a valid interpretation of the data (2, 16), are operating through or regulated by a single gene product as they are all altered by a single mutation.

We have further defined the characteristics of the altered system in the mutants by investigating which amino acids were transported to a lesser extent. This approach allows a distinction between amino acids which are taken up by a particular system from those which may only compete with the uptake of a given amino acid (7, 9). Only the uptake of the basic amino acids lysine, arginine, and ornithine was decreased in the mutants (Table II), several neutral or acidic amino acids being relatively unaffected. We conclude that the altered or missing system is one which specifically transports basic amino acids into the root cells.

Lysine uptake into barley leaf slices is an active accumulation process whose kinetics, specificity, and energetics have been described. It is linear for up to 5 h with little metabolism of lysine taking place in the 1st h (10, 11). Our experiments have examined the effect of the *aec1* gene on leaf uptake. In mutant roots, lysine uptake was reduced while leucine uptake was relatively unaffected. In leaves, however, neither lysine nor leucine uptake was significantly affected in mutant leaves at the concentration tested (Table V). It seems that the uptake of lysine in leaves, as in the scutellum (3), is unaffected by the *aec1* gene.

Estimates of K_m and V_{max} values for lysine uptake by R4402 and Maris Mink roots between 10^{-7} and 10^{-5} M external concentration were quite variable both between experiments and with different methods of data analysis as had been previously observed (15). The apparent V_{max} values were, however, always lower in the mutants by a factor of three or four. Two pieces of evidence suggest that the residual transport activity in the mutants has different characteristics to the parent system. First, there was a greater loss of uptake capacity for lysine (and presumably Aec) than for arginine (Tables II and III) and ornithine (Table II). Consequently, the ratio of arginine to lysine taken up by the mutants was higher than in their parents (Table III). Second, the residual lysine transport activity in R4402 at 2×10^{-6} M was competitively inhibited by arginine, lysine, and Aec in the same order as in the parent, but much less effectively, Aec being particularly ineffective (Table IV). Two explanations are possible. First, the residual transport activity in the mutants could represent a changed form of the wild-type basic amino acid carrier system, with altered relative affinities for arginine, lysine, Aec, and ornithine. Alternatively, there may be in the wild type two carrier systems operating at low concentrations with some discrimination between lysine and arginine, with the mutants having lost one of them. A specific arginine-transporting system and a system transporting both arginine and lysine have been suggested for sugarcane cells (12) and perhaps for tobacco cells (1).

To our knowledge, the R906 and R4402 mutants are the first in higher plants which have been used in a combined biochemical and genetic study to examine aspects of amino acid uptake. We believe that the results justify further work in this area.

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LITERATURE CITED

1. BERRY SL, HM HARRINGTON, RL BERNSTEIN, RR HENKE 1981 Amino-acid transport into cultured tobacco cells. III. Arginine transport. Planta 153: 511–518
2. BORSTLAP AC 1981 Invalidity of the multiphasic concept of ion absorption in plants. Plant Cell Environ 4: 189–195
3. BRIGHT SWJ, LC FEATHERSTONE, BJ MIFLIN 1979 Lysine metabolism in a barley mutant resistant to S(2-aminoethyl)cysteine. Planta 146: 629–633
4. BRIGHT SWJ, PB NORBURY, BJ MIFLIN 1979 Isolation of a recessive barley mutant resistant to S-(2-aminoethyl)L-cysteine. Theor Appl Genet 55: 1–4
5. FRICKE U 1975 Tritosol: a new scintillation cocktail based on Triton X-100. Anal Biochem 63: 555–558
6. HARRINGTON HM, RR HENKE 1981 Amino acid transport into cultured tobacco cells. I. Lysine transport. Plant Physiol 67: 373–378
7. KING J, R HIRJI 1975 Amino acid transport systems of cultured soybean root cells. Can J Bot 53: 2088–2091
8. KINRAIDE TB, B ETHERTON 1980 Electrical evidence for different mechanisms of uptake for basic, neutral, and acidic amino acids in oat coleoptiles. Plant Physiol 65: 1085–1089
9. KINRAIDE TB 1981 Interamino acid inhibition of transport in higher plants. Evidence for two transport channels with ascertainable affinities for amino acids. Plant Physiol 68: 1327–1333
10. LIEN R, SE ROGNES 1977 Uptake of amino acids by barley leaf slices: kinetics, specificity, and energetics. Physiol Plant 41: 175–183
11. LIEN R, SE ROGNES 1982 Salt effects on amino acid transport into barley leaf cells. Biochem Physiol Pflanzen 177: 54–60
12. MARETZI A, M THOM 1970 Arginine and lysine transport in sugarcane cell suspension cultures. Biochemistry 9: 2731–2736
13. MATTHEWS BF, SCH SHYE, JM WIDHOLM 1980 Mechanism of resistance of a selected carrot cell suspension culture to S(2-aminoethyl)-L-cysteine. Z Pflanzenphysiol 96: 453–463
14. NIVEN DF, RE JEACOCKE, WA HAMILTON 1973 The membrane potential as the driving force for the accumulation of lysine by *Staphylococcus aureus*. FEBS Lett 29: 248–252
15. SOLDAL T, P NISSEN 1978 Multiphasic uptake of amino acids by barley roots. Physiol Plant 43: 181–188
16. VAN BEL AJE, AC BORSTLAP, A VAN PINXTEREN-BAZUINE, A AMMERLAAN 1982 Analysis of valine uptake by *Commelina* mesophyll cells in a biphasic active and a diffusional component. Planta 155: 335–341