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Regulation of Development of Leucine Uptake Activity by Glutamine in the Scutellum of Germinating Barley Grain[1]

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ABSTRACT

Scutella from ungerminated grains of barley (*Hordeum vulgare* L. cv Pirkka) take up leucine at a slow rate, which increases rapidly during germination. When endosperms were removed from the grains after imbibition for 4 hours or after germination for 12 or 72 hours, the increase in the rate of leucine uptake was greatly accelerated during subsequent incubation of the embryos or scutella. These increases were rapidly inhibited by cordycepin and cycloheximide, suggesting that protein synthesis, probably synthesis of the carrier protein, was required for the development of the uptake activity.

In separated embryos or scutella, the increases in the leucine uptake activity were inhibited by glutamine. The inhibitions caused by glutamine and cycloheximide were not additive, suggesting that glutamine did not interfere with the function of the carrier but repressed its synthesis. Glutamine did not inhibit the simultaneous increase in peptide uptake; in this respect, its effect was specific for leucine uptake, which appears to be due to a general amino acid uptake system.

Some other protein amino acids also inhibited the increase in leucine uptake without inhibiting the increase in peptide uptake. However, these effects were smaller than that of glutamine.

These results suggest that the transfer of leucine (and other amino acids) from the endosperm to the seedling in a germinating barley grain is regulated at the uptake step by repression of the synthesis of the amino acid carrier protein by glutamine and—possibly to a lesser extent—by some other amino acids taken up from the endosperm.

The hydrolysis of reserve proteins in the starchy endosperm of a germinating barley grain results in a mixture of free amino acids and short oligopeptides (7, 13, 14, 20). The amino acids and peptides are taken up into the scutellum by separate transport systems (6, 11, 20–22). We have previously studied some properties of amino acid uptake into the scutellum using leucine as the substrate (24). Leucine is rapidly taken up by an active transport system(s), the rate of uptake being highest near pH 5, the pH of the starchy endosperm (14).

The uptake of leucine is slow in scutella separated from ungerminated grains, but increases about 10-fold during the first 3 d of germination of whole grains (24). This increase is followed by a lag phase of about 1 d, after which there is another increase.

Removal of the embryonic axis has no effect on the initial increase in the rate of uptake (24). On the other hand, removal of the endosperm either after imbibition for 4 h or after germination for 3 d enhances the rate of the increase during subsequent incubation. In both cases, the increase is completely prevented by CHI[2] and markedly slowed down by glutamine. It was therefore suggested that glutamine taken up from the endosperm might regulate the activity of the uptake system.

Strong evidence indicates that leucine and glutamine are taken up by a common uptake system(s), which is inhibited by all other protein amino acids and which therefore is likely to be a general amino acid uptake system (Väisänen and Sopanen, unpublished). The regulation of this system affects thus the uptake of all amino acids.

In the present study, experiments were carried out to investigate (a) whether glutamine exerts its effect by inhibiting the function of the carrier (trans-inhibition or allosteric inhibition by intracellular glutamine; see *e.g.* Ref. 5) or by preventing the synthesis of new carrier molecules; (b) whether other amino acids have regulatory effects similar to that of glutamine; and (c) whether this regulation is likely to have a role in an intact germinating grain.

Some preliminary results have already been reported (23).

MATERIALS AND METHODS

Plant Material. The Finnish six-row barley *Hordeum vulgare* L. cv Pirkka was used in all experiments and was obtained from Lahden Polttimo Oy, Lahti, Finland. This variety was preferred to the Himalaya variety used for the characterization of uptake because the embryos could be detached after a shorter imbibition time from Pirkka grains than from Himalaya grains. The grains were dehusked with 50% H_2SO_4 and surface-sterilized with NaOCl (18). To obtain ungerminated embryos, grains were allowed to imbibe at room temperature for 3.5 to 4.5 h in sterile distilled H_2O, whereafter the embryos were detached aseptically with the aid of a scalpel. Separated embryos or whole grains were allowed to germinate aseptically on agar gels in Petri dishes in the dark at 20°C, the time of imbibition always being included in the time of germination. With some exceptions, all liquids and agar gels were buffered to pH 5 with 2 mM sodium 3,3-dimethylglutarate buffer. After germination, the scutella were dissected out from the embryonic axis (about 3 mm long after 16 h of culture). The scutella were preserved in ice-cold water until the uptake of leucine was assayed (not more than 1 h).

Uptake Assays. The procedure was modified from the peptide and amino acid uptake assays described previously (22, 24). Four scutella were incubated in flasks containing 3 ml of 1 mM L-[U-^{14}C]leucine in 10 mM sodium 3,3-dimethylglutarate buffer (pH 5) in a shaking water bath at 30°C for 30 min. The scutella were then rinsed with water for 1 min and placed directly into 3 ml of the scintillation cocktail (26). The radioactivity was measured by liquid scintillation spectrometry after storage of 0.5 to 5 d at

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[2] Abbreviations: CHI, cycloheximide; Sar, sarcosine; COR, cordycepin; AIB, α-amino-iso-butyric acid.

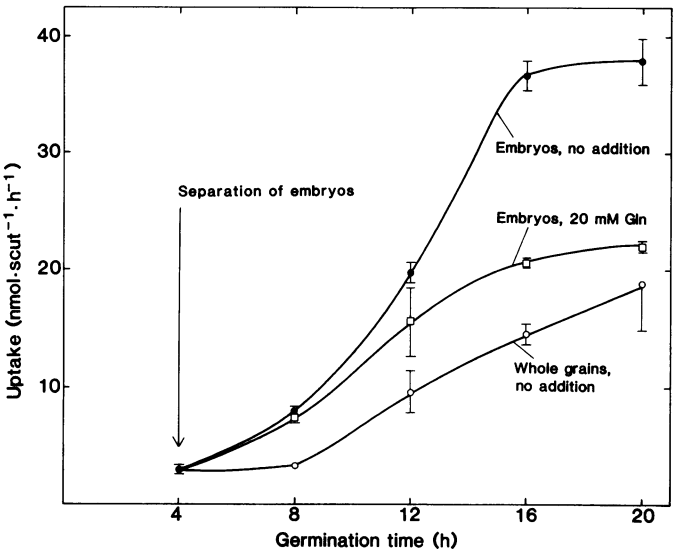

FIG. 1. Effect of removal of the endosperm and addition of glutamine on the increase of leucine uptake activity in the beginning of germination. Grains were imbibed for about 4 h in water. A sample of the grains was then allowed to germinate on buffered agar gel. From another sample, the embryos were detached and allowed to germinate, with the scutellum facing down, on buffered agar gel with or without 20 mM glutamine. Scutella were separated after every 4 h and the rate of leucine uptake was assayed.

room temperature.

The uptake of [^{14}C]Gly-Sar was assayed as described previously (19), with the exception of the incubation time (30 min) and the storage of the scintillation tubes, which was at room temperature.

The results are expressed as nmol of leucine or Gly-Sar taken up by one scutellum in 1 h ± SE. All values given are means of four determinations and virtually all of the experiments have been repeated.

Reagents. The reagents were purchased from the following sources: L-[U-^{14}C]leucine from the Radiochemical Centre, Amersham; glycylsarcosine from Bachem Feinchemikalien A.G.; amino acids from Fluka A.G., Merck A.G., or from Sigma Chemical Co., COR and CHI from Sigma Chemical Co.; [^{14}C] Gly-Sar was a gift from Prof. D. M. Matthews.

RESULTS

Time Course of the Effects of Removal of Endosperm and Addition of Glutamine in the Beginning of Germination. When whole barley grains were allowed to germinate, the rate of leucine uptake into the scutella began to increase after about 8 h (Fig. 1). In separated embryos, the corresponding increase began earlier and was much greater than in whole grains. This increase was greatly inhibited by 20 mM glutamine, especially after 12 h of germination. Apparently, glutamine inhibited the increase in leucine uptake in the same way as did the presence of the endosperm.

Effects of CHI, COR, and Glutamine. There are two main types of mechanism which could explain the inhibition caused by glutamine or by the presence of the endosperm in the increase of leucine uptake activity (see *e.g.* 5): direct inhibition of the function of the carrier or prevention of the synthesis of new carrier molecules. Because the triggering of germination is a very complex process, the mode of inhibition was first studied when the germination had already started and the rate of uptake was increasing. This allowed the use of relatively short times of treatment (1–4 h), which decreased the possibility of nonspecific effects.

In the first experimental system, separated embryos were cultivated on plain agar gels for 12 h, at which time the rate of uptake was increasing rapidly. Thereafter the 'derepressed' embryos were transferred onto other agar gels containing the compounds tested and changes in the rate of leucine uptake were followed for 4 h (Fig. 2, A and B). When the embryos were transferred on agars containing CHI, the increase in the rate of leucine uptake stopped immediately. The presence of 0.3 mM CHI during the uptake assay with 3-d scutella had no effect on the uptake, which rules out the possibility of a direct effect of CHI on the uptake. With COR, the increase stopped after 1 h of treatment. These results indicate that the increase in the rate of uptake was due to synthesis of a protein(s), probably the carrier protein, and that mRNA synthesis was also needed. The rapidity of the effect of COR indicates that the mRNA coding for the protein synthesized had a short half-life.

In the presence of 20 mM glutamine, the rate of leucine uptake increased during the first hour, but thereafter it began to decrease rapidly (Fig. 2A). When 20 mM glutamine was added together with CHI, the rate remained at about the same level as with CHI alone (Fig. 2B). This nonadditivity of the effects of glutamine and CHI indicates that glutamine also inhibits only the synthesis and not the function of the carrier. However, at a concentration of 50 mM, glutamine caused a decrease in the rate of uptake even in the presence of CHI. This suggests that very high glutamine concentrations may cause direct inhibition of the function of the carrier or that glutamine somehow accelerates the degradation of the carrier (4).

In the second experimental system, whole grains were allowed to germinate for 12 h, and only after this the embryos were separated and transferred onto different agars (Fig. 2C). In this case, the rate of leucine uptake at the time of the transfer was increasing more slowly than in the experiments described above, due to the inhibition by the endosperm (compare whole grains and embryos in Fig. 1). When these 'partly repressed' embryos were incubated on plain agar gels, the rate of uptake began to increase much more steeply than in the embryos of whole grains (Fig. 2C). Apparently, the inhibitory compound(s) coming from the endosperm was rapidly eliminated from the epithelial cells (*e.g.* transported to other cells). Again, the increase was completely arrested by CHI. CHI is not likely to prevent the rapid elimination of the inhibitory compound from the epithelial cells; the absence of any increase in the rate of uptake in the presence of CHI therefore indicates that the inhibition caused by the presence of the endosperm is probably due to inhibition of protein synthesis (synthesis of the carrier) and not due to direct inhibition of the function of the carrier. In this case, also, COR as well as glutamine stopped the increase in leucine uptake.

In the third experimental system, whole grains were allowed to germinate for 3 d, and only then were the scutella separated. At this time, the rate of leucine uptake was not increasing at all in the intact grain (24). When the separated, 'completely repressed' scutella were incubated in plain water, the rate of leucine uptake increased by about 70% in 3 h (Table I). Again, CHI as well as glutamine prevented the increase, and when they were added together, the activity was the same as with glutamine alone. These results confirm those above and show that the regulatory effects of the endosperm and glutamine at a later stage of germination are qualitatively similar to those in the beginning of the germination.

Effects of Other Amino Acids on the Development of Leucine or Glycylsarcosine Uptake Activities. In order to understand the regulation of the amino acid uptake system *in vivo*, it was necessary to know whether other amino acids in addition to glutamine inhibit the increase in the rate of leucine uptake. The presence of a single amino acid might lead to many changes in the metabolism and affect *e.g.* protein synthesis in general;

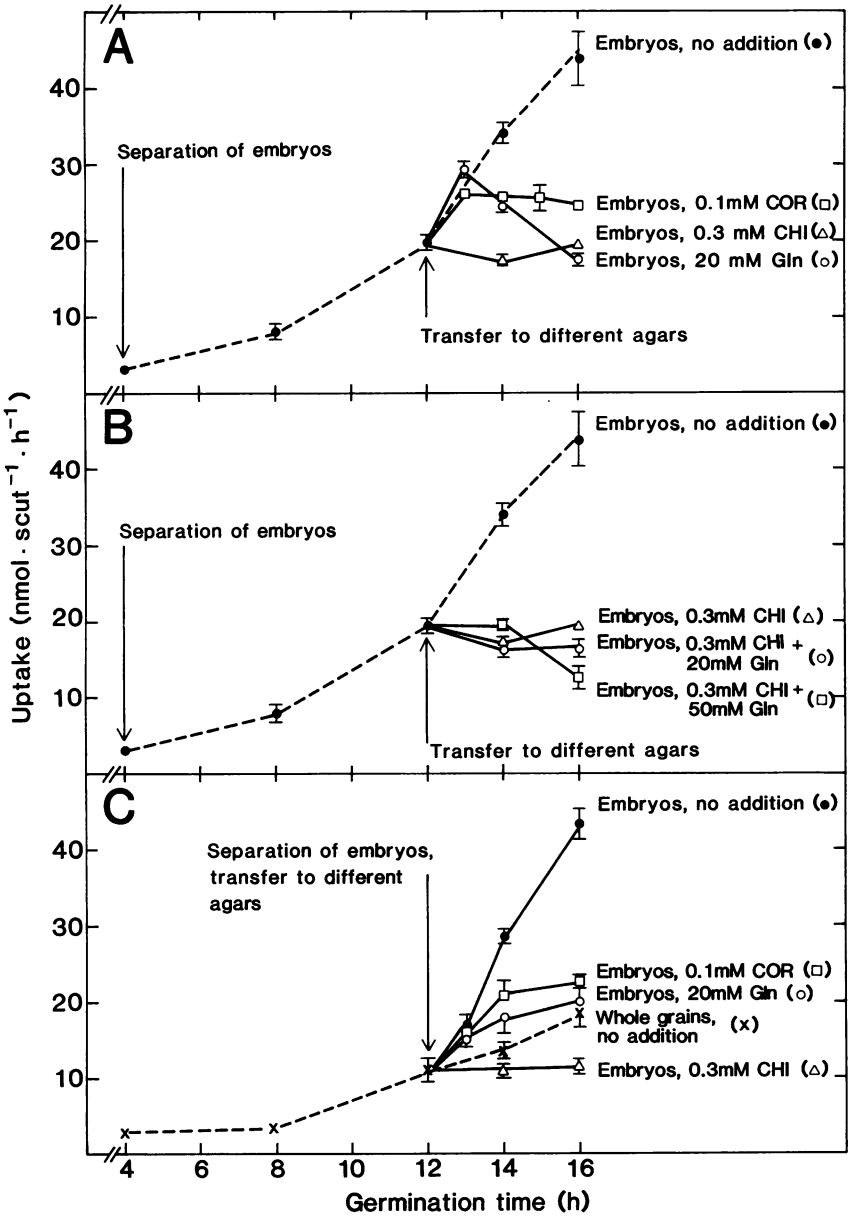

FIG. 2. Effect of CHI, COR, and glutamine on the increase in the leucine uptake activity in separated embryos. A and B: Grains were imbibed for about 4 h in water, whereafter the embryos were separated and placed on unbuffered agar gels. After 12 h from the beginning of the imbibition, the embryos were placed on buffered agar gels containing either 0.1 mM cordycepin, 0.3 mM CHI, 20 mM glutamine, or buffer only (A) or 0.3 mM CHI, 0.3 mM CHI + 20 mM glutamine, or 0.3 mM CHI + 50 mM glutamine (B). After 1 to 4 h of further incubation, the scutella were separated and the rate of leucine uptake was assayed. C: Whole grains were allowed to germinate on agar gels for 12 h, and only after this were the embryos separated and placed on agar gels containing either 0.3 mM CHI, 0.1 mM COR, 20 mM glutamine, or buffer only. The changes in the rate of leucine uptake were followed for 4 h.

therefore, the effects of various amino acids on the increase in the rate of leucine uptake were compared to their effects on the development of the peptide uptake system. The activity of peptide uptake increases rapidly in the beginning of the germination. This increase is prevented by CHI and COR, but it is not affected by removal of the endosperm (18) and therefore appears to be a good control system.

In the first experimental system (similar to that in Fig. 1), separated embryos were placed on agar gels containing the amino acid tested at 5 mM concentration. After 16 h, the rate of leucine or Gly-Sar uptake by the scutella was assayed. Glutamine did not inhibit the development of the peptide uptake system, although it strongly slowed down the increase in the rate of leucine uptake (Table II); therefore, the effect of glutamine seems to be specific for the amino acid uptake system. In addition to glutamine, glycine, arginine, alanine, histidine, proline, threonine, and tryptophan slowed down the increase in leucine uptake without affecting the increase in Gly-Sar uptake.

Five other amino acids slowed down the increase in leucine uptake, but inasmuch as they also slowed down the increase in Gly-Sar uptake, their effects seem to be less specific. The remaining amino acids did not affect the increase in leucine uptake, but three of them decreased the development of the peptide uptake system.

Most of the amino acids retarded the growth of the embryos during 2 d of cultivation, indicating that they had other effects

Table I. *Effect of CHI and Glutamine on the Increase of Leucine Uptake Activity in Scutella Separated after 3 Days of Germination*

Grains were allowed to germinate for 3 d and the scutella were dissected out. Samples of 20 scutella were placed in 100 ml of water or in 100 ml of solutions containing either 0.3 mM CHI, 20 mM glutamine, or both. In order to stir the solutions, air was bubbled through. The solutions were kept at room temperature. After 3 h, rate of leucine uptake was assayed.

Treatment	Rate of Uptake	Change
	$nmol \cdot scut^{-1} \cdot h^{-1}$	$nmol \cdot scut^{-1} \cdot h^{-1}$ (%)
Before incubation	40.3	
After incubation in:		
Water	66.3	+26.3 (+65)
CHI, 0.3 mM	42.7	+2.4 (+6)
Gln, 20 mM	30.6	−9.7 (−24)
CHI, 0.3 mM + Gln, 20 mM	33.1	−7.2 (−18)

as well. These effects on growth were similar to those reported earlier by Miflin (12).

With glutamine, the maximal inhibition (about 50%) of the increase in the rate of leucine uptake was obtained at about 5 mM concentration (Fig. 3A). Two other specific inhibitors, alanine and proline, had qualitatively similar but much weaker effects than glutamine. The curve for methionine, which inhibits the increase in leucine as well as in peptide uptake, indicated no saturation.

In the second experimental system (corresponding to that in Fig. 2, A and B), embryos were first cultivated on plain agar gels and transferred after 12 h to agar gels containing the amino acid tested (Table III). In this case, as above, glutamine was a strong inhibitor; the rate of leucine uptake after 4 h incubation had decreased to below the initial 12-h value. The time course of this process is shown in Figure 2A. Arginine, threonine, alanine, histidine, and proline also slowed down the increase in leucine uptake; these amino acids were among the specific inhibitors in the previous experiment (Table II). No significant effect was caused by glutamic acid, asparagine, leucine, and AIB which had also been without effect in the other experimental system (Table II).

There were some differences in the rate of Gly-Sar uptake after incubation on different amino acids, but the values did not differ significantly from the control value.

In this experimental system, too, strong inhibition was already obtained at a glutamine concentration of 2 mM, and the maximal effect was reached at 5 mM glutamine (Fig. 3B). Proline and arginine appeared to give qualitatively similar but weaker effects than glutamine. Cysteine, which decreased both uptake activities (Table II) had a strong, probably toxic effect at concentrations above 5 mM.

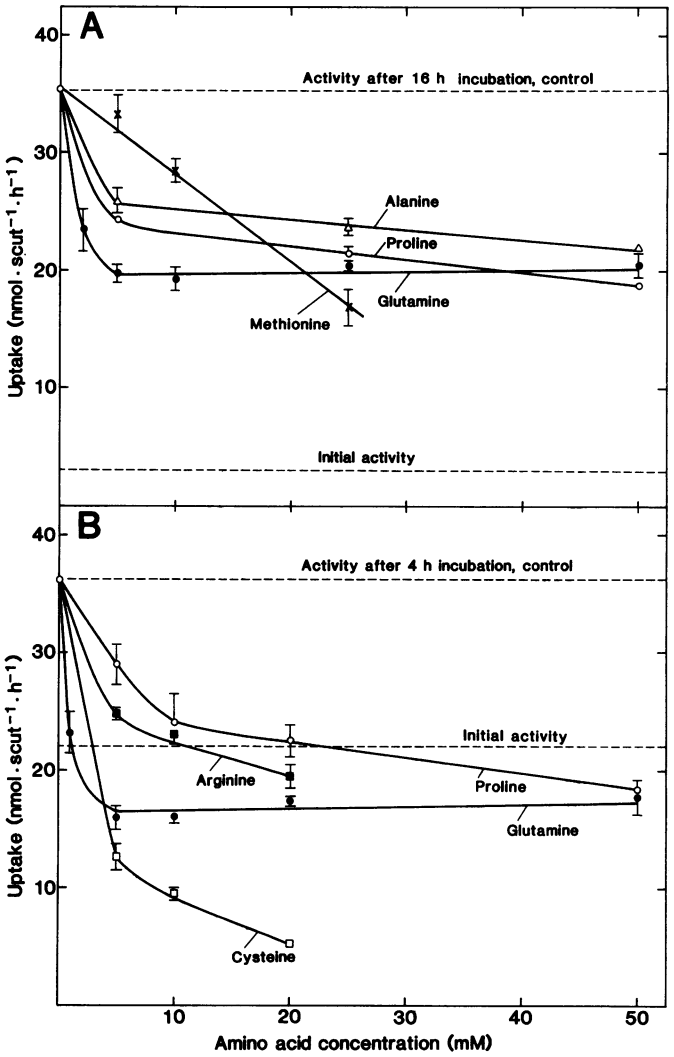

FIG. 3. Effect of amino acid concentration on the increase of leucine uptake activity. A: Grains were allowed to imbibe for about 4 h, after which the embryos were separated and placed on buffered agar gels which contained 0 to 50 mM glutamine, proline, alanine, or methionine. After 16 h from the beginning of the imbibition, the scutella were separated and their activity in leucine uptake was assayed. B: Grains were allowed to imbibe for about 4 h, whereafter the embryos were detached and placed on unbuffered agar gels. After 12 h from the beginning of the imbibition, the embryos were transferred to buffered agar gels containing 0 to 20 or 50 mM glutamine, proline, arginine, or cysteine. After 4 h, the scutella were cut out and their activity in leucine uptake was assayed.

DISCUSSION

On the basis of the results presented above, it is likely that the increase in the rate of leucine uptake in the scutellum during germination is due to protein synthesis. It is also likely that glutamine inhibits this increase at least mainly through inhibition of protein synthesis, probably at the level of transcription. Glutamine may, however, have other effects as well: it seemed to accelerate the degradation of the leucine carrier (*e.g.* Fig. 2A; Table I) and, at high concentration, it seemingly caused direct inhibition of the uptake. Interestingly, similar complex effects of amino acids on amino acid uptake have also been found, *e.g.* in cultured human fibroblasts (4). Because glutamine slows down the increase in the rate of leucine uptake without affecting the increase in peptide uptake, glutamine is likely to act specifically on the synthesis of the amino acid carrier. The inhibition caused by glutamine appears therefore to be repression-like.

In both of the experimental systems used, glutamine caused the strongest inhibition (except for 2 mM tyrosine, Table II). Also, some other amino acids appeared to inhibit specifically the increase in leucine uptake. These amino acids are structurally very different from each other, and therefore it is possible that they are not acting *per se* but only after catabolism. One possibility is that the amino groups of these amino acids are used to synthesize glutamine, which is commonly used for 'long distance' translocation of nitrogen in cereals (17, 25), and might be the primary 'co-repressor'.

The inhibitory effect of the endosperm on the increase in leucine uptake activity *in vivo* appears to be mainly due to glutamine taken up from the starchy endosperm into the epithelial cells of the scutellum. (a) The endosperm, like glutamine,

Table II. *Effect of Amino Acids on the Development of Leucine and Gly-Sar Uptake Activities and on the Growth on Separated Embryos*

Embryos were separated from imbibed grains and allowed to germinate on buffered agar gels containing different amino acids (5 mM). After germination for 16 h, the scutella were separated and their rate of leucine or Gly-Sar uptake was assayed. The growth of the embryos was visually estimated after 2 d of germination and compared to that of controls grown on buffered agar gels. Symbols: +, no effect or small stimulation; −, growth retarded; =, growth strongly retarded.

Amino Acid (5 mM)	Leucine Uptake		Gly-Sar Uptake		Effect on the Growth
	Uptake after 16 h	Increase	Uptake after 16 h	Increase	
	nmol·scut^{-1}·h^{-1}	*% of increase in control*[a]	*nmol·scut^{-1}·h^{-1}*	*% of increase in control*[a]	
Control, no addition	35.3 ± 1.4	100	15.3 ± 0.6	100	+
Effect on leucine uptake:					
Gln	19.9 ± 0.8***[b]	52	14.3 ± 0.9	92	−
Arg	23.0 ± 0.3***	62	15.9 ± 0.8	105	−
Gly	24.0 ± 0.5***	65	17.9 ± 0.7	120	=
Ala	24.9 ± 1.1**	69	15.3 ± 0.5	100	+
Pro	26.4 ± 2.5*	72	15.4 ± 0.9	101	=
Thr	26.6 ± 1.3**	73	15.3 ± 0.5	100	−
His	26.8 ± 1.3**	74	13.9 ± 0.6	89	−
Try	29.8 ± 0.9*	83	13.3 ± 1.1	85	=
Effect on both systems:					
Tyr (2 mM)	19.6 ± 1.0***	51	11.5 ± 0.5**	71	=
Lys	20.2 ± 0.3***	53	12.2 ± 1.0*	77	=
Cys	20.7 ± 0.6***	55	10.9 ± 0.3***	67	=
Met	24.1 ± 1.0***	65	11.0 ± 0.6**	68	−
Ser	27.5 ± 1.5**	76	11.8 ± 1.1*	74	=
Effect on Gly-Sar uptake:					
Leu	31.4 ± 1.4	88	10.2 ± 0.7**	62	=
Glu	32.2 ± 1.8	90	11.0 ± 0.1***	68	
Val	35.5 ± 1.5	101	12.0 ± 0.5**	75	
No effect on either system:					
Ile	36.4 ± 0.4	103	15.8 ± 1.0	104	=
Asp	35.8 ± 2.1	102	16.4 ± 1.1	108	−
Asn	33.6 ± 0.3	95	15.2 ± 0.7	99	−
Phe	33.5 ± 2.1	94	16.4 ± 1.0	108	+
AIB	31.4 ± 1.4	88	15.0 ± 1.0	98	=

[a] Uptake of leucine by scutella from ungerminated grains was 3 nmol·scut^{-1}·h^{-1} and that of Gly-Sar was 2 nmol·scut^{-1}·h^{-1}.

[b] The asterisks indicate differences from the control at the 5% (*), 1% (**), or 0.1% (***) significance level (Students *t* test).

acts by inhibiting protein synthesis. (b) Glutamine is the most abundant amino acid in the reserve proteins of barley (3) and the average concentration of free glutamine in the starchy endosperm is about 4 mM, which is higher than that of most other amino acids (our unpublished results, based on automatic amino acid analysis of the starchy endosperm of Himalaya barley after 4 d of germination; the corresponding value for glutamine in the starchy endosperm of wheat is about 8 mM, calculated from the data of Chittenden *et al.* [1]). (c) Inasmuch as glutamine strongly inhibits the increase in leucine uptake activity at a concentration as low as 2 mM, it could by itself explain the inhibition caused by the endosperm *in vivo*. The other apparently specific amino acids are inhibitory at higher concentrations than glutamine and—with the exception of proline (about 13 mM)—occur in the starchy endosperm at lower concentrations (roughly 1.5–2.5 mM) than glutamine. Each of them appears therefore to be individually less important than glutamine in the regulation of amino acid uptake, but together they may play a considerable role.

In many bacteria, fungi, and animal tissues, amino acid or nitrogen starvation leads to an increase in the activity of some amino acid uptake systems (*e.g.* 5, 16, 27). Generally, this appears to be due to derepression of the synthesis of the uptake system (4, 8, 28). Often amino acid uptake is also regulated by transinhibition by intracellular amino acids (8, 15, 28). At least in *Aspergillus nidulans*, glutamine appears to be a specific corepressor (2).

In higher plants, regulation of amino acid uptake by amino acids has not been reported, as far as we know. However, when rapeseed or soybean cells are cultured in suspension in the presence of NH_4NO_3, they take up amino acids, *e.g.* glutamine and leucine, very slowly (9, 10). When the cells are transferred to a medium without nitrogen nutrients, their capacity for uptake of many amino acids increases several-fold in 24 h. The authors suggested that one possible explanation for the inhibition caused by NH_4NO_3 is that NH_4^+ increases the intracellular concentration of glutamine, which would then inhibit amino acid uptake

Table III. *Effect of Various Amino Acids Added after 12 Hours of Germination of Separated Embryos on the Increase in the Uptake of Leucine or Gly-Sar*

Embryos were separated after imbibition of the grains. They were allowed to germinate for 12 h on buffered agar gels and transferred thereafter to agar gels containing buffer only or 5 mM amino acids in buffer. After 4 h of further incubation, scutella were separated and their capacity to take up leucine or Gly-Sar was assayed.

Amino Acid (5 mM)	Leucine Uptake		Gly-Sar Uptake	
	Uptake after 4 h incubation	Increase	Uptake after 4 h incubation	Increase
	$nmol \cdot scut^{-1} \cdot h^{-1}$	*% of increase in control*[a]	$nmol \cdot scut^{-1} \cdot h^{-1}$	*% of increase in control*[a]
Control, no addition	36.2 ± 1.7	100	15.6 ± 1.5	100
Gln	14.2 ± 0.9***[b]	−55	16.5 ± 0.8	116
Arg	24.4 ± 1.4**	17	17.0 ± 2.6	125
Thr	26.3 ± 1.5**	30	13.3 ± 1.2	58
Ala	28.3 ± 1.3*	44	15.7 ± 0.6	102
Pro	28.9 ± 1.5*	49	14.7 ± 1.2	84
His	29.0 ± 1.4*	49	15.3 ± 2.0	95
Glu	32.7 ± 1.7	74	13.9 ± 1.1	69
Asn	33.5 ± 1.0	81	16.9 ± 2.9	124
AIB	35.3 ± 0.5	94	16.3 ± 1.8	113
Leu	38.4 ± 2.3	115	14.0 ± 1.0	71

[a] Uptake of leucine after 12 h of germination on unbuffered agar was 22.0 ± 1.3 $nmol \cdot scut^{-1} \cdot h^{-1}$ and that of Gly-Sar was 10.1 ± 1.2 $nmol \cdot scut^{-1} \cdot h^{-1}$.

[b] The asterisks indicate differences from the control at the 5% (*), 1% (**), or 0.1% (***) significance level (Students *t* test).

through transinhibition (9). It is quite possible, however, that amino acid uptake in these cells is regulated by a repression-like mechanism similar to that in the barley scutellum.

The physiological function of the regulation of amino acid uptake in the scutellum seems obvious. Without regulation, the uptake would be very fast (Figs. 1 and 2), probably exceeding the needs and capacity for use of the growing embryo or seedling. The regulation could adjust the uptake by the scutellum to match the needs of the seedling throughout the protein mobilization process in the endosperm. This line of thought fits well with the two-phase development of leucine uptake activity during germination (24). The plateau in the development at days 3 to 4 coincides with the time when the carboxypeptidases—the only enzymes liberating amino acids in the starchy endosperm (13, 14)—have reached their maximal activities and abundant substrate is still available (14, 20); the final increase at days 5 to 6 corresponds to the period when the proteins in the starchy endosperm begin to be depleted and the amino acid concentrations are likely to decrease.

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