Phytochemistry, Vol. 21, No. 12, pp. 2831–2834, 1982.
Printed in Great Britain.

0031-9422/82/122831-04$03.00/0

UPTAKE OF ^{14}C-α-AMINOISOBUTYRIC ACID BY *PHASEOLUS VULGARIS*

LORETTA M. FINDYSZ, JOHN G. SEVERSON, JR. and RICHARD E. HILLMAN*

Department of Biology, Saint Louis University, Saint Louis, MO 63103, U.S.A.; *Department of Pediatrics, St. Louis Children's Hospital, and Washington University School of Medicine, Saint Louis, MO 63110, U.S.A.

(*Received* 20 *March* 1982)

Key Word Index—*Phaseolus vulgaris*; Leguminosae; bush beans; specific uptake; [^{14}C]AIB.

Abstract—Specific uptake (S.U.) of α-aminoisobutyric acid ([1 – ^{14}C]AIB), a non-metabolizable neutral amino acid analog, by dwarf bush bean plants (*Phaseolus vulgaris* cv Top Crop) demonstrated wide differences in active transport between various plant organs. The kinetic and timed uptake data reported were expressed as S.U. because this corrects for the diffusion of AIB which is part of the total AIB uptake process. Roots accumulated AIB to concentrations up to 18 times and leaf disks to twice those of the incubation medium. Stem tissue showed very little uptake, if any, that could not be accounted for by simple diffusion or water free space. Although initial rate kinetic studies demonstrated the presence of a normal transport system, timed uptake studies revealed greatly decreased transport by etiolated plants, suggesting a relationship between active transport and the lack of photosynthate. The reproducibility of the AIB uptake pattern by mature roots strongly supports the concept that the transport of neutral amino acids is biphasic and suggested one or more carrier systems are inducible by either low intracellular concentrations or repressed by high intracellular concentrations of the amino acid.

INTRODUCTION

The uptake of amino acids—the non-protein acid, α-aminoisobutyric acid (AIB), in particular—has been more widely studied in animals [1] than in plants. Although AIB uptake has been studied in bacteria [2] and yeast [3], it has only been in the past 10 years that AIB uptake has been studied in higher plants, viz. barley [4], tomato [5], oats [6], peas [7] and beans [8].

AIB is an alanine analog and because it is neither metabolized nor incorporated into protein of higher plants [9], it serves as an excellent compound for the investigation of events associated with uptake processes. It has been proposed that the uptake of some neutral amino acids, including AIB, by higher plants is mediated by a dual mechanism. A high affinity uptake mechanism has been assigned to the plasmalemma [10] and a lower affinity mechanism to the tonoplast [11]. Further, the AIB movement across the plasmalemma is fairly slow and the high affinity system predominant in its transport appears to be subject to adaptive and regulatory changes [12]. Multiphasic isotherms for the uptake of AIB have been reported [4].

To the best of our knowledge this is the first study of AIB transport in organs of the dwarf bush bean. The present paper demonstrates that AIB transport is an active, energy requiring process in bean plants and that differences in transport exist between various plant organs. More importantly, the experimental design is unique; it will simultaneously correct for AIB in water free space (WFS) and the diffusion of AIB. This approach mathematically separates specific uptake (S.U.) from total AIB uptake which includes the diffusion of AIB.

RESULTS AND DISCUSSION

Uptake of AIB by bean, root, hypocotyl, epicotyl and leaf disks

The distribution ratio (D.R.) for root tissue reached a value greater than one 15 min after the tissue was placed in the AIB incubation medium. (Fig. 1). The D.R. increased to its maximum after 4 hr. From a maximum value of 12.5 the D.R. dropped to 4.2 after 5 hr, but increased again to 8.2 at 7 hr. The S.U. trend followed the D.R., i.e. 243.1, 82.5, and 159.3 for the 4, 5 and 7 hr sampling times, respectively. While the D.R.s and the S.U.s for hypocotyls and epicotyls steadily increased during this experiment, the D.R.s never reached one. As one would expect, D.R.s for the two stem tissues were nearly the same. The S.U. for leaf tissue was much lower than for roots, but greater than for stem tissues. However, the D.R. values for leaf tissue were greater than one at both the 5 and 7 hr sampling times.

While there is a paucity of information which describes amino acid uptake kinetics in plant cells and tissues [13], several recent models have been proposed to explain the kinetics of amino acid uptake. It has been proposed that the uptake of some neutral amino acids is mediated by a dual uptake mechanism

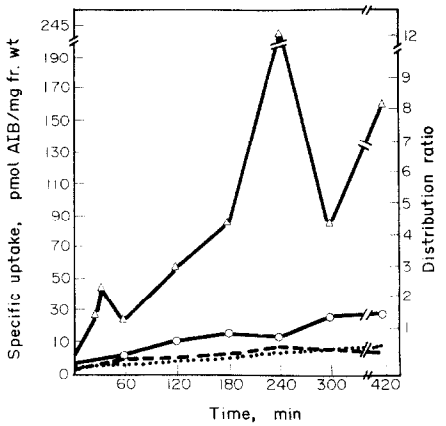

Fig. 1. Specific uptake of [^{14}C]AIB by organs of 17-day-old dwarf bush bean plants. Tissues were incubated in a medium which contained 0.022 mM AIB. Each point is the mean of five values. (△—△) Roots, (· · ·) hypocotyls, (---) epicotyls, (○—○) leaf discs.

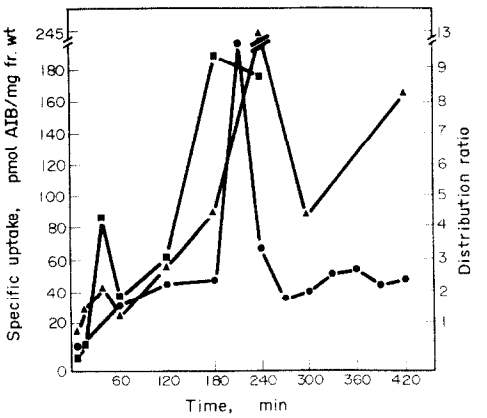

Fig. 2. Specific uptake of [^{14}C]AIB by bush bean roots. In experiments 1, 2 and 3 root tips were 16-, 17- and 21-days-old, respectively. Each point is the mean of five values. (■) Expt 1, (▲) expt 2, (●) expt 3.

which consists of a homogeneous high affinity mechanism at the plasmalemma and a second lower affinity uptake mechanism at the tonoplast [10, 11]. Further, multiphasic isotherms have been reported for AIB uptake [4].

With the stem's primary function being that of solution transport, it is not surprising that hypocotyl and epicotyl tissues absorbed low levels of AIB. Severson [14], using ^{32}P, reported that the majority of the labelled pulse fed to the roots of intact plants passed through stem tissues. However, the D.R. values for leaf tissue showed a gradual increase and finally exceeded one at the 5 hr sampling time, the uptake of AIB was quite low when compared with roots.

AIB uptake by roots

Because of the prodigious uptake of AIB by roots and the apparent reproducibility of this process, absorption of [1 − ^{14}C]AIB by root tissue was studied in two additional experiments and the results are shown in Fig. 2. The D.R.s obtained in each experiment exceeded one within the first 5–30 min. Uptake of AIB by roots showed an initial peak after 30 min followed by a larger peak after 180–240 min of exposure to [1 − ^{14}C]AIB. Although each of the experiments was conducted on a different day and using root tissues which were 16-, 17- and 21-days-old, the uptake patterns were remarkably similar and the D.R. maxima were nearly identical.

In contrast to the uptake of amino acids by animal cells, where the incubation medium and cellular amino acid concentrations reached an equilibrium [15], the uptake process by roots was continuous throughout the 7 hr incubation period. This concurs with the results presented by Birt and Hird [16] who studied amino acid uptake by carrot tissue. The continuous AIB absorption occurs in spite of the fact that AIB is neither being metabolized nor incorporated into protein. Kotyk and Rihova [3] also noted that AIB uptake by baker's yeast required metabolic energy and even after a 10 hr incubation did not reach a steady-state level, as long as a source of energy and external AIB were present. More recent evidence for the unlimited uptake of neutral amino acids by higher plants is given by Borstlap [17]. There have been several interpretations given for the multiphasic uptake kinetic mechanism: (a) the rate-limiting step shifts from the plasmalemma to other membranes, e.g. the tonoplast [18]; (b) excess substrate affects non-interacting uptake sites—each displaying Michaelis–Menten kinetics and acting in parallel [9]; (c) the uptake site exhibits negative cooperativity [19]; (d) all-or-none transitions occur in the transport systems [20]; and (e) the indication of an intrinsic property of the transport system may not be of functional significance [21].

Kinetic studies of AIB uptake by roots and leaf disks

Six sets of 22-day-old root tissues were exposed to varying concentrations of [^{14}C]AIB for 10 min. The Lineweaver–Burk plot showed that the apparent K_m value for AIB uptake was 0.071 mM. The V_{max} was 0.045 nmol AIB/mg fr. wt/min. When the same experimental design was employed for leaf disks, the K_m value for AIB uptake was 0.1 mM and the V_{max} was 0.003 nmol AIB/mg fr. wt/min.

The lower K_m value for root tissue indicated that roots have a greater affinity for AIB than do leaf cells. While leaf cells have retained their ability to absorb foliarly applied compounds, it was not surprising that roots had a greater absorptive capacity, even when exposed to a non-naturally occurring amino acid.

Uptake of AIB by roots and hypocotyls of etiolated bean seedlings

For root and hypocotyl tissue of 8-day-old etiolated bean seedlings, the S.U. values were 5 and 1, 6 and 2, and 7 and 2 pmol AIB/mg fr. wt at the 1, 4 and 7 hr sampling times, respectively. At all sampling times the D.R.'s for roots tissue exceeded those obtained for hypocotyl tissue. The S.U. and D.R. values for etiolated tissue were much less than the values obtained for the same tissues of plants grown in the light (Fig. 1).

Because AIB uptake is an energy requiring process,

the reduction of AIB uptake by etiolated plants is probably related to the limited amount of ATP and other energy containing compounds. This, of course, is ultimately related to the absence of photosynthate. Van Bel and Van Der Shoot [22] recently demonstrated that light stimulated the uptake of alanine by xylem parenchyma cells of tomato. The uptake of alanine showed a biphasic pattern and the stimulating effect of light which affected both uptake mechanisms.

When root tissue from 11-day-old etiolated bean seedlings was exposed to different concentrations of AIB for 10 min, the Lineweaver–Burk data gave a K_m of 0.043 mM and a V_{max} of 0.04 nmol AIB/mg fr. wt/min. Because initial rate kinetics are similar for roots of mature and etiolated plants, the data suggested that the transport system for AIB was intact. These data further supported the concept that the decreased uptake seen in the timed uptake study was related to a lack of photosynthate and not to a specific change in the transport process.

Because photosynthesis is the main source of energy for green plant metabolism, an etiolated bean seedling must rely on the compounds stored in the cotyledons of the seed as its source of energy. The catabolism of these reserve compounds results in the generation of ATP and the substrates necessary for subsequent plant growth. To a lesser degree, substrates and energy containing compounds can be derived from the dark fixation of carbon dioxide. The rate of this process is only *ca.* 0.4% of the photosynthetic rate [23].

In conclusion, mature bean plants exhibited vigorous uptake of the neutral amino acid analog, AIB. Roots accumulated AIB to a great extent and leaves to a lesser degree. Stem tissues showed very little transport that could not be accounted for by simple diffusion of AIB and that found in WFS. These studies demonstrate the absorptive and storage roles of roots, the conduit-like characteristics of stem, and the ability of the leaf to utilize amino acids stored by the roots or produced in other leaf tissues.

The active nature of the uptake process is clearly shown by the high D.R.s and the kinetic studies. The energy requirements for transport are supported by the relatively low uptake of AIB in etiolated plants when compared to that of photosynthesizing plants. Despite the low uptake by etiolated plants, the initial rate kinetic studies indicate that a normal transport system is present.

In separate experiments AIB transport by root tissue showed a remarkably reproducible pattern. These findings suggested that the neutral amino acid system in bean roots is inducible or that transport can be repressed by high concentrations of amino acids. This uptake pattern is unique to plant tissue and has not been described for animal or bacterial systems. AIB transport is a clearly definable phenomenon of nutrient uptake. This system should be quite useful for the study of the effects of environmental perturbations on the active transport process.

EXPERIMENTAL

Timed and concn or kinetic uptake expts were conducted using leaf disks, hypocotyls, epicotyls and roots of 16–22-day-old bean plants; and roots and hypocotyls of 8–11-day-old etiolated bean seedlings.

Growth of bean plants. Uniform fungicide-treated seeds of the dwarf bush bean, *Phaseolus vulgaris* L. cv Top Crop, were sown in pots containing a mixture of composed soil, peat, and Vermiculite. The plants were grown in a room which provided 3.7 klx at soil level, a 14 hr photoperiod, a day/night r.h. of 25/40. The etiolated bean seedlings were grown in Vermiculite in a chamber which provided a constant temp. of 23° and a r.h. of 25–30.

Incubation: timed uptakes. Leaf disks (1 cm) from the first set of trifoliate leaves, epicotyl and hypocotyl segments (1 cm) and root tip segments (3.5 cm) from green plants; and hypocotyl segments (2 cm) and roots from etiolated seedlings were placed into incubation media which consisted of 50 ml Murashige–Skoog (M–S) soln (without sucrose) at pH 5 [24], 100 μl 10 mM AIB, and 50 μl [1 – ^{14}C]AIB at 27°. The sp. act. of AIB was 5 μCi/μmol and the final AIB concn in each incubation medium was 0.022 mM. The sampling times were from 5 to 450 min for leaf disks, stem tissue and roots. Five independent measurements were made at each sampling time.

At each sampling time, tissue samples were removed and blotted. The tissue was rinsed twice with M–S soln (pH 5 at 0°) to remove [^{14}C]AIB from apoplastic spaces and to stop the uptake process. Fr. wts of the tissue samples were then determined and each sample was placed into a vial which contained 1 ml of tissue dissolving 0.6 N NCS soln (Amersham Corp. Arlington Heights, IL). Each vial was capped and warmed to 55°. After 2 hr, 9 ml of a POP–POPOP–toluene scintillation fluor was added to each cooled vial. Radioactivity was determined using a scintillation spectrometer. The [^{14}C]AIB content of hypocotyl tissue of etiolated seedlings was determined using 0.1 ml H_2O and 9 ml of New England Nuclear-963 aq. counting fluid. These methods gave the best counting efficiencies for the various tissues. The level of [^{14}C]AIB and μmol AIB in the incubation medium prior to incubation and at each sampling time were determined. All counts were corr. for quenching. Five independent dererminations were made for the four different tissues at each sampling time.

Incubation: kinetic studies. The incubation of root and leaf disk tissue from green plants and roots of etiolated seelings, rinsing and determination of [^{14}C]AIB levels were described above. Leaf disk and root tissues were incubated for 5 and 10 min, respectively, in media with AIB concns ranging from 0.004 to 0.302 mM. Ten independent determinations were made at each concn in each of several separate expts.

Measuring AIB uptake: calculation of S.U. and D.R. Uptake of AIB is expressed in two different ways: S.U. and D.R. The S.U. represents the total apparent AIB uptake which has been corr. for AIB in WFS and non-specific AIB diffusion. The D.R. represents the ratio of the concn of AIB inside the tissue to the media concn. The tissue concn of AIB was calculated from the uptake and the total tissue H_2O corr. for WFS. A D.R. greater than one is evidence that a compound is transported against the concn gradient.

Determination of WFS was done using [^{14}C]inulin and the procedure of ref. [25]. The WFS values for determining S.U. and D.R. were 0.028, 0.016, 0.015 and 0.013 μl/mg tissue for bean roots, hypocotyls, epicotyls and leaf disks, respectively. For roots and hypocotyls of etiolated bean seedlings, WFS values were 0.014 and 0.001 μl/mg. Tissues were treated as described in the timed uptake section, and the inulin factor of WFS for each tissue is the average of five pieces per sampling time.

Total tissue H_2O was determined by comparing the fr. and dry wts of the tissues used in this study. The average total tissue H_2O for bean roots, hypocotyls, epicotyls and leaf disks were 87.8, 93.2, 92.7 and 79.3%, respectively; for etiolated seedlings, the values for hypocotyl and root tissues were 91.4 and 93.9%. Five samples of each tissue were used to determine the dry wt at each sampling time. For each tissue, the sampling time means were then averaged to yield total average H_2O content.

The apparent diffusion constant (K_D) was calculated by the procedure of ref. [26]. The K_D estimates the net amount of AIB which diffuses into the cell, and for a 10 min incubation period the K_D for root tissue was 0.076 μl/mg fr. wt. After a 5 min incubation period for leaf tissue, the K_D was 0.016.

Acknowledgement—This research was supported in part by a U.S. Public Health Service Grant AM 15531.

REFERENCES

1. Martin, M. and Pohl, S. (1979) *J. Biol. Chem.* **254**, 9976.
2. Langheinrich, W. and Ring, K. (1976) *Arch. Microbiol.* **109**, 227.
3. Kotyk, A. and Rihova, L. (1972) *Biochim. Biophys. Acta.* **288**, 380.
4. Shtarkshall, R. and Reinhold, L. (1974) *Membrane Transport in Plants* (Zimmermann, V. and Dainty, J., eds.) p. 338. Springer New York.
5. Van Bel, A. (1978) Proc. FESPP Meeting, Edinburgh, p. 536.
6. Rubinstein, B. and Tattar, T. (1980) *J. Exp. Botany* **31**, 269.
7. Cheung, Y-S. and Nobel, P. (1973) *Plant Physiol.* **52**, 633.
8. Amar, L. and Reinhold, L. (1973) *Plant Physiol.* **51**, 620.
9. Shtarkshall, R., Reinhold, L. and Harel, H. (1970) *J. Exp. Botany* **21**, 915.
10. Eptstein, E. (1973) *Int. Rev. Cytol.* **34**, 123.
11. Torii, K. and Laties, G. (1966) *Plant Physiol.* **41**, 863.
12. Christensen, H. (1975) *Biological Transport*, 2nd edn. pp. 435–436. W. A. Benjamin, Reading.
13. Blackman, M. and McDaniel, C. (1980) *Plant Physiol.* **66**, 261.
14. Severson, J. (1974) *Phyton (Buenos Aires)* **32**, 81.
15. Hillman, R., Albrecht, I. and Rosenberg, L. (1968) *Biochim. Biophys. Acta* **150**, 528.
16. Birt, L. and Hird, F. (1958) *Biochem.* J. **70**, 286.
17. Borstlap, A. (1977) *Acta Bot. Neerl.* **26**, 115.
18. Laties, G. (1969) *Annu. Rev. Plant Physiol.* **20**, 89.
19. Hodges, T. (1973) *Adv. Agron.* **25**, 163.
20. Nissen, P. (1974) *Annu. Rev. Plant Physiol.* **25**, 53.
21. Bange, G. (1979) *Z. Pflanzenphysiol.* **91**, 75.
22. Van Bel, A. and Van Der Shoot, C. (1980) *Plant Sci. Letters* **19**, 101.
23. Devlin, R. and Barker, A. (1971) in *Photosynthesis.* Van Nostrand Reinhold, New York.
24. Murashige, T. and Skoog, F. (1962) *Plant Physiol.* **15**, 473.
25. Rosenberg, L., Downing, S. and Segal, S. (1962) *Am. J. Physiol.* **202**, 800.
26. Akedo, H. and Christensen, H. (1962) *J. Biol. Chem.* **237**, 113.