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Transport and Compartmentation of 1-Aminocyclopropane-1-Carboxylic Acid and Its Structural Analog, α-Aminoisobutyric Acid, in Tomato Pericarp Slices

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ABSTRACT

The uptakes of 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor to ethylene, and its structural analog, α-aminoisobutyric acid (αAIB) by tomato pericarp slices were investigated. Both uptakes show a biphasic (saturable-linear) dependence on external concentration of the transported amino acid. At low concentrations, ACC uptake is competitively inhibited by αAIB and vice versa. Both uptakes also are inhibited by other neutral amino acids but not by acidic or basic amino acids. ACC and αAIB uptakes are metabolically dependent and are increased with time of tissue incubation. αAIB efflux patterns from pericarp slices indicated three distinct αAIB compartments having efflux kinetics consistent with those for cell wall, cytoplasm, and vacuole. The bulk of the αAIB taken up by pericarp tissue is sequestered into the vacuole. The ability of pericarp tissue to accumulate αAIB in the vacuole declines with fruit development.

Ethylene is a plant hormone usually associated with fruit ripening and senescence. Generally, an increase in ethylene production is accompanied by an increase in activity of ACC[1] synthase (6) which mediates the formation of ACC, the immediate precursor of ethylene. ACC synthase is a key enzyme in ethylene biosynthesis (18). In addition to ACC synthase activity, there also appears to be other regulation point(s) in the conversion of ACC to ethylene during ripening. For instance, tissue ACC levels sometimes are high relative to the rate of ethylene formation in the tissue (6, 18). This lack of correlation indicates that ethylene biosynthesis is limited by factors in addition to the availability of ACC. Ethylene biosynthesis may be regulated by the amount or activity of ACC synthase (6). Ethylene biosynthesis may also be regulated by the net transport of ACC from its site of synthesis in the cytoplasm (4) to its probable site of conversion into ethylene in the vacuole (4, 5) and/or other compartments of the cell (18). In this regard, we wanted to study ACC transport and allocation patterns in tomato pericarp tissue during development. However, the conversion of ACC to ethylene and to its malonyl conjugate (1) would severely complicate any study on ACC transport and compartmentation. The structural analog of ACC, αAIB, is an unnatural amino acid whose transport can be studied without interference from its metabolism in a variety of plant systems (3, 15). While neutral amino acid transport systems have not been very well characterized in higher plants, evidence is growing that there is a single transport system specific for neutral amino acids (17). Therefore, αAIB may offer a way to study ACC transport and compartmentation patterns. In this paper, we show that αAIB and ACC transport have a number of similar characteristics. We also show the allocation patterns of αAIB in tomato pericarp tissue during fruit development.

MATERIALS AND METHODS

Plant Material. Tomato (*Lycopersicon esculentum* Mill, cv Pik Red) plants were grown both in the field and in a greenhouse at the Beltsville Agricultural Research Center. Field plants were fertilized at a rate of 40 kg N per acre using a commercial 10-10-10 fertilizer. Greenhouse plants were grown in 20 cm pots using the same soil as that used for field plants and were fertilized as needed with a commercial 10-10-10 fertilizer. Fruits were harvested on the day of the experiment at the immature green, mature green, breaker, pink, and/or red stages of development. Immediately after harvesting, slices 1 mm thick were cut with a meat slicer, the skin removed, and the outer pericarp sectioned with a sharp razor blade into rectangular blocks 3 to 5 mm on a side. The tissue blocks were blotted gently with a Kimwipe,[2] weighed into 1 g lots and each lot equilibrated for 90 min at 23°C in 10 ml of aerated solution containing 150 mM sorbitol, 10 mM MES adjusted to pH 6.0 with 1.0 N KOH, and 1.0 mM $CaCl_2$. While calcium is not a necessary addition to the uptake medium, it may help to maintain membrane integrity during tissue incubations. In experiments where longer term preincubations occurred, 50 μg ml^{-1} chloramphenicol was added to this equilibration medium.

Amino Acid Uptake Measurements. After equilibration, 1 g sets of tissue slices were blotted and transferred to 3 ml of uptake medium containing various μM concentrations of αAIB, ACC and/or other amino acids, 10 mM MES adjusted to pH 6.0 with 1.0 N KOH, 1.0 mM $CaCl_2$, and sufficient sorbitol to make the osmolarity of the uptake medium identical to that of the equilibration medium. The specific radioactivity and concentration of [3-^{14}C]αAIB in the incubation medium ranged between 19.9 and 0.2 μCi μmol^{-1} and 10 and 1000 μM αAIB, respectively. For ACC, the specific activity ranged between 80.0 and 0.2 μCi μmol^{-1}[2,3-^{14}C]ACC between 10 and 1000 μM ACC. The uptake

[1] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; αAIB, α-aminoisobutyric acid; SAM, S-adenosylmethionine; CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products which may also be available.

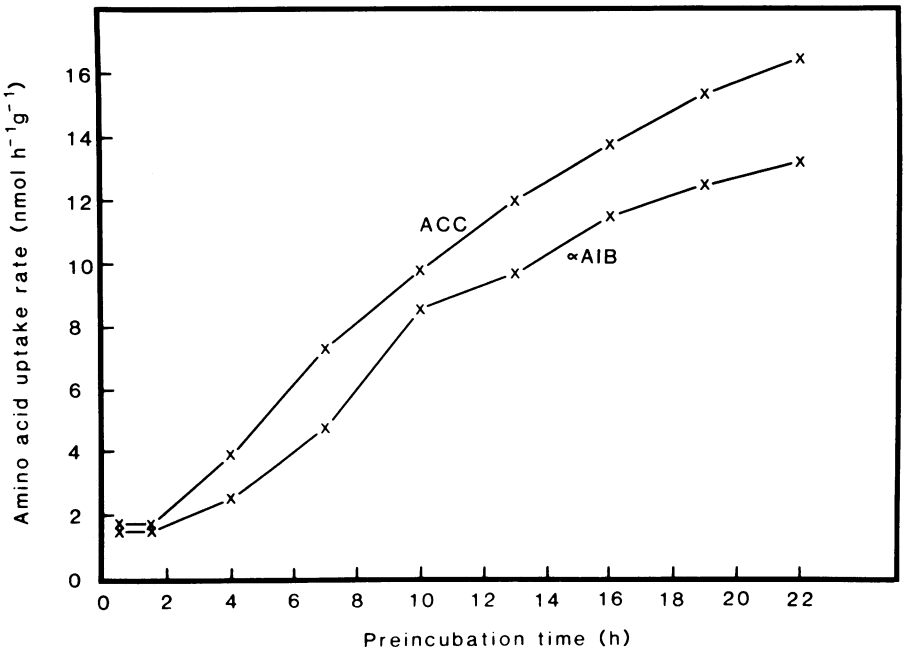

FIG. 1. Time course of ACC and αAIB uptake. Mature green pericarp slices were preincubated in equilibration solution for 0.5 to 22 h followed by incubation in labeled ACC or αAIB uptake medium containing 10 μM ACC or αAIB for 3 h.

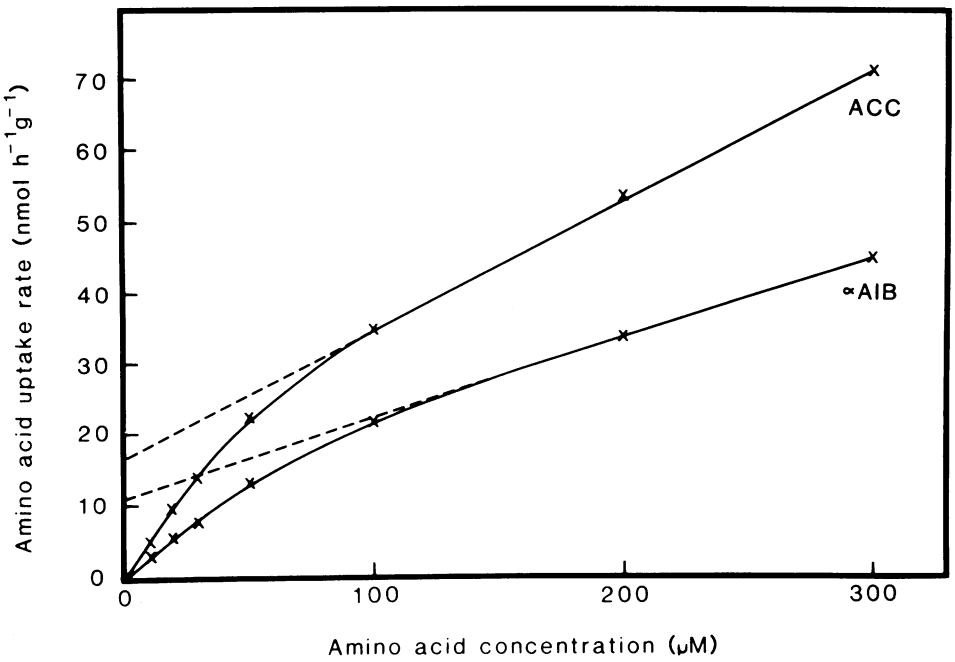

FIG. 2. Dependence of ACC and αAIB uptake upon external ACC and αAIB concentrations. Mature green pericarp slices of tomato were incubated in labeled ACC or αAIB uptake medium for 4 h at various amino acid concentrations.

medium was replaced as needed to keep the concentration of the amino acid from declining during the incubation period. Incubations were done at 23°C under illumination from laboratory fluorescent lights. Humidified laboratory air containing no detectable (<0.005 μl L^{-1}) ethylene was slowly bubbled through the uptake medium to prevent anaerobiosis. Inhibitors, when used, were present 15 min prior to the addition of the labeled uptake medium. When humidifed N_2 gas having no detectable ethylene was substituted for air to induce anaerobiosis, the N_2 treatment began 1 h prior to the addition of labeled uptake medium. After the uptake period (generally 4 h) each set of pericarp slices was removed from the medium by filtration and washed five times for 2 min each with 9 ml of unlabeled uptake medium to remove labeled amino acids from the cut cell surface and free space of the cell wall. Each set of discs was then prepared for liquid scintillation counting as previously described (14). In these and other experiments, three replications (*i.e.* three incubation vials) were run for each treatment. Each experiment was repeated at least twice.

Analysis of [3-^{14}C]αAIB Compartmentation. The technique of compartmental analysis of radioisotope elution from plant tissues was used to estimate the number of cellular compartments available for passive αAIB efflux and to estimate the αAIB content of each compartment (see Refs. 2 and 12 for additional information related to this technique). After incubation in [^{14}C] αAIB uptake medium, each set of tissue slices was removed from the labeled medium by filtration. Successive 2 ml volumes of efflux medium (unlabeled uptake medium) were added to each set of tissue slices for increasing time periods during a 5 h efflux period. The elution periods started at 0.5 min and gradually increased to 30 min. After the washout, each set of discs and each efflux medium was prepared for liquid scintillation counting

Table I. *Inhibition of ACC and αAIB Uptake by Various Amino Acids*

The uptake of labeled ACC and αAIB was tested at 10 μM in the absence and presence of 100 μM nonlabeled amino acid.

Nonlabeled Amino Acid Added (100 μM)	Percent of Control Uptake	
	[^{14}C]ACC	[^{14}C]αAIB
None	100	100
ACC		44
αAIB	53	
Met	24	18
Ala	61	50
Gly	80	80
Lys	95	100
Glu	101	97
Asp	103	96
SAM	93	90

(13). We determined that chloramphenicol at 50 μg ml^{-1} in the uptake and efflux media had no noticeable effect on uptake or passive efflux of αAIB, indicating that microbial contamination was not a problem in these relatively long-term experiments. As additional precautions, microscopic examinations of uptake and washout media indicated no evidence for a buildup of microorganisms and all incubation solutions were replaced every 1 to 3 h, washout solution every half-h or less.

Metabolism of Labeled αAIB. Pericarp slices at the mature green and pink stages of development were preincubated for 1.5 h in equilibration medium followed by incubation in 10, 100, and 300 μM labeled αAIB uptake media for 4 h, then rinsed as described for uptake measurements. The pericarp slices were extracted for 3 h in boiling 80% ethanol in a Soxhlet apparatus. The extracted tissue was ground in 5 ml of 10% TCA and aliquots were processed according to the method of Mans and Novelli (9) for estimation of labeled αAIB incorporation into protein. The ethanol extracts from above were evaporated *in vacuo* at 35°C to a residue which was dissolved in a minimal volume of 50% ethanol. An aliquot of each extract was chromatographed on paper using 1-butanol:acetic acid:water (4:1:1.5 by volume) (7). The radioactivity in the paper chromatogram was scanned using a Baird Atomic Radiochromatogram Scanner (Baird Atomic Co., Cambridge, MA). After scanning, labeled areas corresponding to αAIB and αAIB conjugate were eluted with water and the radioactivity determined.

RESULTS AND DISCUSSION

Metabolism of Labeled αAIB. To verify that αAIB could be used in this study, the degree to which it is metabolized in tomato pericarp was checked. Of the total radioactivity taken up in any incubation, more than 98% was TCA soluble. More than 97% of the TCA soluble radioactivity cochromatographed with unmetabolized αAIB (R_F 0.37) and less than 1% cochromatographed with an αAIB metabolite (R_F 0.76) presumably N-malonyl αAIB (7). The αAIB metabolite level was lower in pericarp slices in the pink than in the mature green stage.

Characteristics of ACC and αAIB Uptake. Following a 1.5 h tissue equilibration, ACC and αAIB uptake into pericarp slices was approximately linear over a minimum of 4 h at external amino acid concentrations of 10 or 1000 μM. However, if the tissue was incubated for longer periods of time, the ACC and αAIB uptake rates were strongly enhanced (Fig. 1).

When pericarp slices were exposed to various concentrations of ACC or αAIB, the ACC and αAIB uptake rates appeared

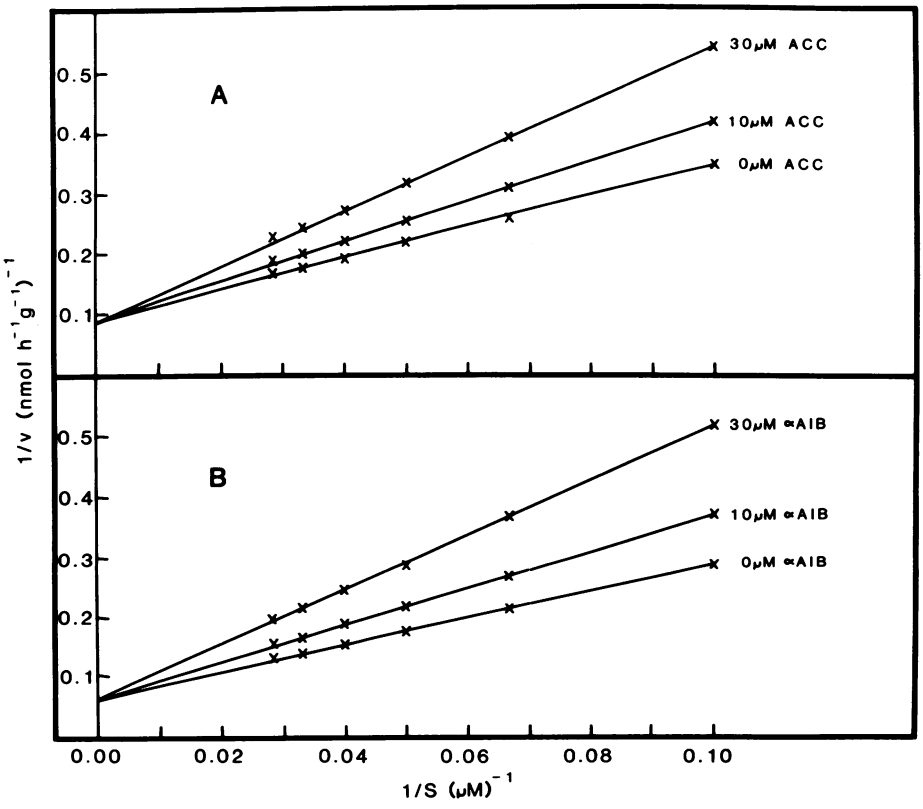

FIG. 3. Kinetics of the low concentration component of ACC and αAIB uptake. Data are presented as double reciprocal plots of uptake of labeled αAIB (part A) and ACC (part B) *versus* concentration. αAIB and ACC uptakes occurred in the absence and presence of 10 and 30 μM ACC (part A) and αAIB (part B), respectively.

Table II. *Rate Constants and Half-time Values for αAIB Efflux*

Stage of Fruit and Compartment	Rate constant	Half-time
	min^{-1}	*min*
Immature green		
Vacuole	3.30×10^{-4}	2101
Cytoplasm	3.63×10^{-2}	19.1
Free space	0.36	1.9
Mature green		
Vacuole	3.64×10^{-4}	1903
Cytoplasm	3.67×10^{-2}	18.9
Free space	0.35	2.0
Breaker		
Vacuole	4.02×10^{-4}	1725
Cytoplasm	3.67×10^{-2}	18.9
Free space	0.35	2.0
Pink		
Vacuole	9.18×10^{-4}	755
Cytoplasm	3.87×10^{-2}	17.9
Free space	0.33	2.1
Red		
Vacuole	1.30×10^{-3}	532
Cytoplasm	4.28×10^{-2}	16.2
Free space	0.34	2.0
Late red		
Vacuole	5.25×10^{-3}	132
Cytoplasm	—[a]	—
Free space	—	—

[a] Linear regression lines for the cytoplasmic and free space compartments were not present in plots of efflux elution data from tissue at this stage of development.

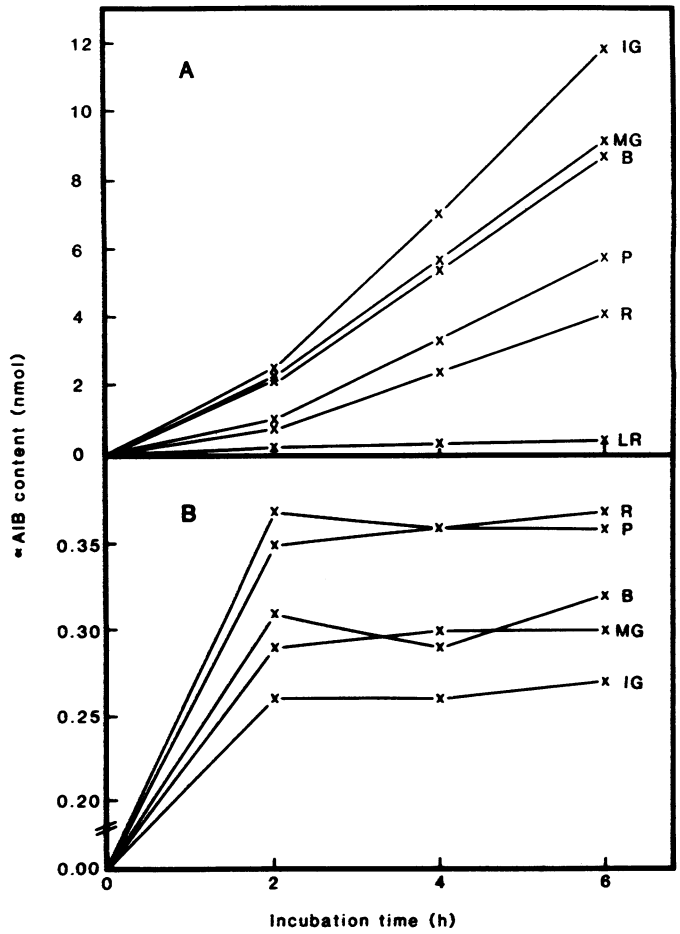

FIG. 4. αAIB content in the vacuolar and cytoplasmic compartments of tomato pericarp tissue as a function of incubation time. Pericarp slices at six developmental stages were incubated 2, 4, or 6 h in labeled αAIB uptake medium followed by a 5 h compartmental analysis washout. The αAIB content in the vacuolar (part A) and cytoplasmic (part B) compartments is shown as a function of incubation time. IG, immature green; MG, mature green; B, breaker; P, pink; R, red; LR, late red.

biphasic (Fig. 2), the same kinetic pattern found previously for αAIB and other amino acids in a variety of plant tissues (reviewed in Reinhold and Kaplan [11]). ACC uptake was generally faster (up to two times) than αAIB uptake. The faster uptake of ACC may be due to a greater cellular utilization of ACC resulting in a more favorable chemical potential gradient for ACC uptake. Figure 2 further shows a linear dependence of ACC and αAIB uptake rate *versus* ACC and αAIB concentration at concentrations above 100μM. The dashed lines indicate the extrapolation of the superimposed high amino acid concentration uptake component. Subtracting this low affinity component from the original uptake curve reveals a second lower amino acid concentration component having curvilinear kinetics. In many cases, amino acid uptake has been described as a Michaelis-Menten component and a linear component. Described in this way, the low concentration components of ACC and αAIB uptake have apparent K_ms between 25 and 40 μM and V_{max}s between 10 and 45 nmol h^{-1} g^{-1}. ACC had a higher apparent K_m and V_{max} than αAIB. However, the accuracy of the kinetic values is dependent upon the specific activity of the amino acid at the uptake site being similar to that in the external solution. For αAIB, an unnatural amino acid, all αAIB specific activities in the tissue would be identical and thus the kinetic values for αAIB are deemed accurate. The kinetic values for ACC could be in considerable error if its transport through cellular ACC compartments and/or metabolism significantly modified its specific activity at its uptake site(s). However, the overall ACC and αAIB transport patterns are still quite similar. Besides enhanced uptake with time and the biphasic nature of uptake, both ACC and αAIB uptake were very sensitive to physiological temperature changes (Q_{10} between 4.0 and 5.1 depending on temperature regimes). Uptake of both amino acids was optimal at pH 5.5 to 6.0. The characteristics of ACC and αAIB uptake described above were demonstrated in pericarp tissue at all stages of fruit development.

Specificity of ACC and αAIB Uptake. Since ACC and αAIB show similar uptake characteristics, we did inhibition and competition experiments to see if ACC and αAIB might share a common transport system. Present evidence indicates the presence of a single transport system specific for neutral amino acids in higher plants (17). In a screening experiment, the uptake of labeled ACC and αAIB were determined in the presence of neutral, acidic, and basic amino acids. The uptake of 10 μM [^{14}C] ACC was inhibited in the presence of 100 μM concentrations of αAIB and a number of other neutral amino acids but not by the basic amino acid, lysine, the bulky charged amino acid, SAM, or the acidic amino acids, aspartic and glutamic acid (Table I). Reciprocally, αAIB uptake was inhibited by ACC and the same amino acids that inhibited ACC uptake (Table I; [13]). Of the neutral amino acids, methionine was always the strongest inhibitor of both ACC and αAIB uptake. This pattern of amino acid

Table III. *Concentrations of αAIB in Tomato Pericarp Compartments after Incubation in 10 μM αAIB Uptake Medium for Various Periods of Time*

Stage of Fruit	Incubation Time (h)		
	2	4	6
	Vacuolar concentration (μM)		
Immature green	3.27	9.09	15.40
Mature green	2.94	7.35	11.90
Breaker	2.81	7.03	11.33
Pink	1.31	4.31	7.45
Red	0.96	3.04	5.30
Late red	0.25	0.37	0.51
	Cytoplasmic concentration (μM)		
Immature green	7.3	7.2	7.5
Mature green	8.1	8.4	8.3
Breaker	8.6	8.2	8.8
Pink	9.8	9.9	9.9
Red	10.3	10.0	10.1
Late red	—[a]	—	—
	Free space concentration (μM)		
Immature green	6.4	7.0	6.6
Mature green	7.5	7.7	7.8
Breaker	7.7	7.7	7.7
Pink	9.4	9.9	9.5
Red	10.0	9.9	9.9
Late red	—	—	—

[a] Linear regression lines for the cytoplasmic and free space compartments were not present in plots of the efflux elution data from tissue at this stage of development.

inhibition of ACC and αAIB uptake in tomato pericarp slices is very similar to the pattern Lürssen (8) observed for amino acid inhibition of ACC-dependent ethylene production in soybean leaf discs.

The concentration dependence of the low concentration component of αAIB uptake in the absence and presence of 10 and 30 μM ACC is shown in Figure 3A as double reciprocal plots. The three curves intercept the ordinate at the same point. The slopes of the three curves are different, which is often interpreted as indicating competitive inhibition (10). For the experiment shown in Figure 3A, the apparent K_m for αAIB uptake is 28.3 μM and the K_i for ACC inhibition was 35.0 and 38.0 μM at ACC concentrations of 10 and 30 μM, respectively. αAIB has a similar inhibitory effect on the low concentration component of ACC uptake (Fig. 3B). In this case, the apparent K_m for ACC uptake was 35.5 μM and the K_i for αAIB was 26.4 and 29.0 μM at αAIB concentrations of 10 and 30 μM, respectively. For two substances to competitively inhibit each other, the K_i values for one substance A inhibiting the uptake of another substance B should be equal to the apparent K_m value of substance A and vice versa. For the saturable component of ACC and αAIB uptake and at low inhibitor concentrations, this criterion is met. When uptake was studied at concentrations above 100 μM where the high concentration, linear component of amino acid uptake is apparent, αAIB strongly inhibited ACC uptake and vice versa. Since the high concentration component of ACC and αAIB uptake was linear, the nature of the inhibition could not be analyzed in the same manner as the low concentration component. Nonetheless, all of our data are consistent with one biphasic carrier system responsible for the uptake of ACC and αAIB in tomato pericarp. Methionine also seemed to competitively inhibit the low concentration component of both ACC and αAIB uptake and vice versa (data not shown).

Compartmentation of αAIB. Based on the results of the previous studies, αAIB was used to study the compartmentation of ACC. A semilog plot of the labeled αAIB content in the tissue as a function of time revealed three distinct compartments believed to be the cell wall free space, the cytoplasm, and the vacuole (2, 12). The vacuole would be the slowest releasing compartment. The apparent αAIB content at the beginning of the washout period, the rate constant, and the half-time for passive αAIB efflux, were calculated (2, 12) for each compartment. The results from one experiment are presented in Table II. Other experiments revealed similar results. For the cytoplasmic compartment, the rate constant always increased and the half-time value always decreased slightly between the immature green and the pink stages of development but more noticeably between the pink and red stages of development. Such changes may indicate an increased passive permeability of the plasma membrane for αAIB, at least during the latter stages of ripening. The data in Table II also shows that the rate constant for αAIB loss from the vacuolar compartment increased from 3.30×10^{-4} to 5.25×10^{-3} and the half-time values decreased from 2101 to 132 min during fruit development. The vacuolar rate constant and half-time value changes are larger than corresponding changes for the cytoplasmic compartment during fruit development except in going from the pink to red stages of development. Vickery and Bruinsma (16) suggested that the vacuolar rate constant in tomato pericarp can be used as a measure of the passive permea-

Table IV. *Concentrations of αAIB in Tomato Pericarp Compartments after Incubation in αAIB Uptake Media for 4 h*

αAIB was supplied at a concentration of 10 μM to green tissue and at a concentration of 40 μM to ripening tissue.

Stage of Fruit	αAIB Concentration Supplied	Compartment Concentration		
		Vacuole	Cytoplasm	Free space
	μM		μM	
Immature green	10	8.50	6.5	7.4
Mature green	10	7.40	7.4	7.8
Breaker	40	25.55	30.8	35.6
Pink	40	15.28	34.2	36.8
Red	40	10.14	38.5	39.0
Late red	40	3.10	—[a]	—

[a] Linear regression lines for the cytoplasmic and free space compartments were not present in plots of the efflux elution data from tissue at this stage of development.

bilities of the tonoplast and plasma membrane and that the cytoplasmic rate constant is a measure of the passive permeability of the plasma membrane alone. If this is true, the passive permeability of the tonoplast for αAIB may increase throughout development to the red stage. The rate constants and half-time values shown in Table II are representative of pericarp slices incubated in equilibration medium for 1.5 h and in uptake medium for 2, 4, or 6 h. During longer incubations where the rate of αAIB (and ACC) uptake is enhanced (Fig. 1), the vacuolar rate constants generally decrease (and the half-times increase). The αAIB efflux rate from the vacuolar compartment declines with tissue ageing beyond 8 h.

From tissue equilibrated 1.5 h and incubated in labeled αAIB uptake medium for 2 to 6 h, the vacuolar content of αAIB increased linearly with incubation time for each stage of development (Fig. 4A). However, the net uptake rate into the vacuole declined throughout fruit development. In addition, more than 95% of the net uptake into the vacuole is inhibited by addition of 1.0 μM CCCP to the αAIB uptake medium or by substitution of N_2 for air in the bubbling system of incubating tissue slices. During the same incubation period, the cytoplasmic content of αAIB increased during the first 2 h of αAIB uptake, then stabilized (Fig. 4B). However, the cytoplasmic content of αAIB increased throughout fruit development (Fig. 4B). Adding 1.0 μM CCCP or N_2 to the incubation medium invariably raised the cytoplasmic content of αAIB at all stages of fruit development, sometimes dramatically (about 4-fold).

The Q_{10} for uptake into the vacuolar compartment is greater than four, an indication that the vacuolar uptake of αAIB is probably dependent on metabolic energy. On the other hand, αAIB uptake into the cytoplasmic compartment reaches a near steady state condition within 2 h (Fig. 4B). Because of the rapid onset of vacuolar uptake, the nature of the αAIB uptake into the cytoplasm could not be studied. The bulk of the αAIB entering tomato pericarp tissue is sequestered into the vacuolar compartment. This finding agrees with the finding in pea and broad bean protoplasts that the bulk of the cell's ACC is localized in the vacuole (5).

To express the data in another way, we calculated the concentration of αAIB in each compartment at each stage of development by dividing the content of αAIB in the compartment by the compartment volume. The vacuole occupies about 82% of the tissue volume and the cytoplasm about 3.8% (2) in tomato pericarp tissue at all stages of development. The cell wall free space volume is about 7.7% of the tissue volume at the immature green, mature green, and breaker stages of development and about 8.8% in pink and red pericarp tissue (2). From fresh weight/dry weight measurements, the water content of 1 g of pericarp slices is 0.943 ml in immature green, mature green, and breaker stages, 0.948 ml at the pink stage, and 0.958 ml at the red stage of development. Using these values and the αAIB content of the cellular compartments, the concentration of αAIB in each compartment at each stage of development was determined (Table III). At all incubation times, the vacuolar concentration of αAIB decreases throughout fruit development. In contrast, the cytoplasmic concentration increases during pericarp development at least until the red stage of development. The αAIB concentration in the free space was lower than that of the external medium and increases during pericarp development. Given sufficient time, the vacuolar concentration goes higher than that of the cytoplasm and exterior of the cell at all stages of fruit development with the possible exception of the red stage. The generation of a higher vacuolar to cytoplasmic concentration of αAIB is yet another indication that the uptake of αAIB into the vacuole is dependent on metabolic energy. Incubations at 1000 μM αAIB showed the same trends for the cytoplasmic and vacuolar compartments as that shown in Table III for 10 μM αAIB incubations but the concentrations in the compartments were about 100-fold higher.

During pericarp development, ACC synthase activity is 2 to 10 times higher in ripening pericarp slices than it is in green pericarp slices (6). Furthermore, we found that the ACC content was about 4 times higher in ripening tissue than it was in green tissue (JE Baker, RA Saftner, unpublished results). To roughly mimic probable differences in ACC levels during development, experiments were done where green pericarp tissue was incubated in 10 μM αAIB uptake medium and ripening tissue was incubated in 40 μM αAIB medium. When this is done, the vacuolar concentration of αAIB peaks at the beginning of ripening and declines as ripening progresses (Table IV). The cytoplasmic and free space concentrations rise throughout fruit development (Table IV). When αAIB concentrations other than 10 μM for green pericarp slices and 40 μM for ripening pericarp slices are used, the same compartmentation pattern is observed, provided the αAIB concentration supplied to ripening pericarp slices is at least 2.5-fold higher than that supplied to green pericarp slices.

In tomato pericarp tissue, the rate of ethylene production increases during development, reaches a peak at or near the beginning of ripening, then declines as ripening progresses (6). Despite the increasing αAIB concentration in the cytoplasm during development, the αAIB concentration (content) in the vacuole declines steadily during development (Table III; Fig. 4A). Therefore, the relative ability of the tissue to transport and accumulate αAIB in the cytoplasm or the vacuole is not correlated, in itself, to the rate of ethylene biosynthesis during fruit development. However, when αAIB is supplied to the pericarp tissue at concentrations that would roughly mimic the relative ACC content of the tissue during development (Table IV), the vacuolar αAIB concentration (content) shows a similar pattern to that observed for ethylene biosynthesis during pericarp development. Thus, ethylene biosynthesis may be coordinately dependent upon ACC production, *i.e.* ACC synthase activity, and upon ACC transport into and sequestration in the vacuole. If αAIB mimics ACC transport and compartmentation and if ethylene biosynthesis occurs inside the vacuole (or tonoplast), then the decline in ethylene biosynthesis that occurs during pericarp ripening may be due primarily to decreased net uptake of ACC into the vacuole. Nevertheless, until the exact site(s) of ethylene biosynthesis is (are) known, the importance of ACC transport and compartmentation in the vacuole and the passive permeability of the tonoplast in relationship to ethylene biosynthesis will remain unclear. However, our data indicate that ethylene biosynthesis may be limited, in part, by compartmentation of ACC, *i.e.* by factors affecting the transport and allocation of ACC in tomato pericarp tissue during fruit development.

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