Plant Physiol. (1978) 61, 933-937

Evidence for Amino Acid-H^+ Co-Transport in Oat Coleoptiles[1]

Received for publication August 3, 1977 and in revised form January 30, 1978

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ABSTRACT

Microelectrode and tracer techniques were used to test for possible amino acid-H^+ co-transport in coleoptiles of *Avena sativa* L. cv. "Garry." The amino acid analogue α-aminoisobutyric acid (AIB) caused transient depolarization of the membrane potential. The absolute magnitude of the maximum depolarization was affected by the same factors that affected AIB transport. Both increased with higher concentrations of AIB, increased with higher acidities in the medium, and were enhanced by indoleacetic acid (which hyperpolarized the membrane potential). AIB transport was reduced as K^+ concentrations in the medium were increased and by the metabolic inhibitor NaN_3, both of which reduce membrane potentials. Our data fit an amino acid-H^+ co-transport model in which transport is controlled by both the membrane potential and proton concentration components of the chemical potential difference of protons across the coleoptile cell membrane.

The cells of many organisms, from bacteria to mammals, accumulate high concentrations of amino acids and carbohydrates from surroundings in which these compounds are dilute (8, 11, 19, 21). Many workers propose that these energy-requiring transport processes are mediated by membrane carrier mechanisms that use the inwardly directed free energy (chemical potential) gradients of either sodium ions in animal cells (2, 12, 19) or hydrogen ions in bacteria, fungi, algae, and higher plants (6, 7, 8, 14, 22). This process, called co-transport (21) or symport (14), is essentially a means for coupling the active transport of one substrate to the passive movement of another substance—both moving in the same direction.

Theory. Figure 1 shows one of several possible models for co-transport (21). The model assumes a membrane carrier that combines with a substrate (an amino acid in our case) and a proton on one side of the membrane forming a ternary positively charged carrier-substrate-proton complex that moves to the inner membrane surface; at that point the substrate and proton are released and the carrier is allowed to recycle. The direction of the net transport of substrate and its final internal to external concentration ratio depend upon the cytoplasmic and medium substrate and hydrogen ion concentrations, as well as on the potential difference across the membrane. Presumably, the proton concentration on either side of the membrane would affect the rates of proton association or dissociation with the carrier. The membrane potential would act electrophoretically upon the charged membrane species.

In the model (Fig. 1), the normal negative membrane potential would promote movement of the carrier complex into the cell. The maximum amount of energy available for the transport of amino acids when both the proton concentration differences and electrical potential differences are coupled is given by equation I (3, 15):

$$\mu_{H_0^+} - \mu_{H_i^+} = RT \ln \frac{\alpha_{H_0^+}}{\alpha_{H_i^+}} - zF(E_i - E_0) \qquad (I)$$

$\mu_{H_0^+} - \mu_{H_i^+}$ is the difference in the chemical potential (partial molar free energy) of protons between the outside and inside of a cell. $\alpha_{H_0^+}$ and $\alpha_{H_0^+}$ are the activities (concentrations, approximately) of protons outside and inside the cell. R is the gas constant, T the absolute temperature, z the valence of the ion (+1 for H^+), F is the Faraday, and $E_i - E_0$ the electrical potential difference (E_m) between the inside and outside of the cell. Equation I is similar in concept to the one for proton motive force proposed by Mitchell (14) but, in his equation, both sides are multiplied by $\frac{1}{F}$. Note that a membrane potential of −59 mv contributes as much to the free energy of protons (outside relative to inside) as a proton concentration ratio of 10/1.

Several predictions follow from the co-transport model in Figure 1: (*a*) transport should behave like a carrier-mediated process: (*b*) membrane depolarizations should be related to substrate transport rates; (*c*) substrate transport rates and substrate-induced depolarizations should be directly related to proton chemical potential differences caused either by changes in membrane potentials, changes in external to internal proton concentration ratios, or combinations of both; and (*d*) transport of substrate into the cell with a proton should leave the external solution more alkaline. In the present study, the first three predictions were tested.

MATERIALS AND METHODS

AIB[2] Uptake Experiments. Seedlings of *Avena sativa* L. cv. "Garry" were grown for 84 to 94 hr at 23 C in complete darkness on Vermiculite moistened with tap water. Coleoptiles were then removed and two 5-mm sections were cut 5 mm from the apical end. The sections were randomized and preincubated in the dark for 2 to 4 hr by floating them on a nutrient solution containing 1 mM $CaCl_2$, 1 mM KCl, 0.25 mM $MgSO_4$, and 1 mM $NaPO_4$ buffer to give a final pH of 6.5 (except for the pH, this solution is the same as 1× used in the membrane potential experiments).

For uptake measurements, 10 sections were transferred to a 20-ml beaker containing 2.5 ml of the nutrient solution; the solution was then aspirated and replaced with 2.3 ml of nutrient solution containing 0.4 μM ^{3}H-α-aminoisobutyric acid (2.5 Ci/mmol) and enough carrier AIB for the desired final amino acid concentration (usually 40 μM). When necessary, the pH was reduced by addition of HCl. After an uptake period of 15 min, the radioactive solution was aspirated and cold distilled H_2O or 0.1 M AIB was added for

[1] Research supported by Vermont Agricultural Experiment Station and by National Science Foundation Grant PCM 76-00439 to B. R. Vermont Agricultural Experiment Station Journal Article No. 380.

[2] Abbreviations: AIB: α-aminoisobutyric acid; FC: fusicoccin.

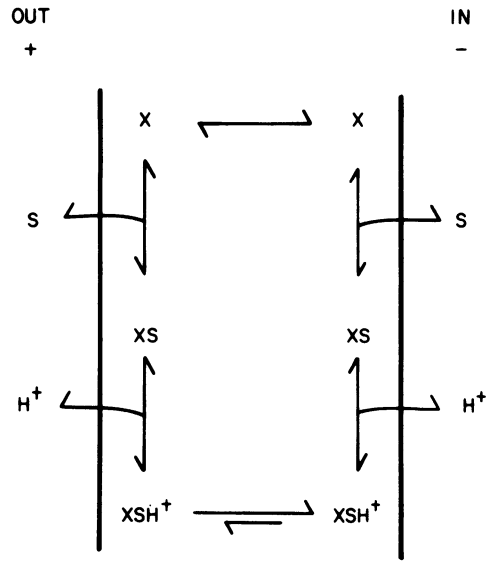

FIG. 1. Model for a proton-dependent co-transport system as proposed by Slayman (21). The membrane carrier is represented by X and the substrate by S.

10 to 15 min. The sections were then placed into 4 ml of Multisol (Interex Corp.) and counted with a Beckman LS-100 scintillation counter. While somewhat more counts appeared if the tissues were first homogenized, the relationship between treatments was not affected. Treatments were done in duplicate and experiments repeated on at least 2 different days.

Membrane Potential Experiments. The oat seedlings were grown in the dark at 25 C in Vermiculite saturated with a 10× solution having the following composition, in mM: 10 $Ca(NO_3)_2$; 10 KCl; 9.1 NaH_2PO_4; 0.5 Na_2HPO_4; 2.5 $MgSO_4$; pH 5.4 (4). After about 96 hr, coleoptiles were isolated under regular laboratory light (cool-white fluorescent) and a section 1 cm long was removed 5 mm below the tip. To facilitate microscopic observation of cells, a segment about 2 × 1 mm was cut away from the top of the coleoptile cylinder and discarded. The remaining notched coleoptile segment was mounted vertically in a Lucite chamber and aerated for 3 to 4 hr in the dark at 25 C in a solution that was 0.1 times as concentrated as the above solution (1×).

To measure membrane potentials, the Lucite chamber was attached to a modified microscope stage and perfused with the desired solution. A glass capillary microelectrode with less than 1-μm tip diameter and filled with 2 M KCl was inserted into the vacuole of a cortical cell close to the upper surface of the coleoptile. The insertion was with a hand-operated micromanipulator. All operations were observed under 100× magnification. Membrane potentials were measured between an external electrode (filled with 2 M KCl in 2% agar) and the microelectrode in the cell. The two electrodes were connected by Ag-AgCl wires to an amplifier (WP Instruments model 4A) and the membrane potentials were recorded on a chart recorder.

The standard solution (S1) used for all tests with the amino acid was essentially the same as 1× except that the Na^+ concentration was increased to 4 mM with added NaCl. This permitted adjusting the pH between 4 and 6.5 while maintaining the Na^+ concentration constant. Changes in the chloride concentration were minimal.

RESULTS

AIB Uptake. The uptake of AIB over a 30-min time period is shown in Figure 2. The rate remains constant but does not extrapolate to zero, indicating that about 8% of the label taken up after 15 min is not removed by the washing procedure. The data presented in Figure 2 were for pH 4 and 4 mM AIB, but a linear uptake was also seen at pH 6.5 and 40 μM AIB.

The effect of the external AIB concentration on AIB uptake for a 15-min period is presented in Figure 3. The curve generally resembles curves reported earlier for leaf tissues (17). We often observed inflections in the concentration curves at about 1 to 3 mM AIB, which could be interpreted as multiphasic kinetics; saturation occurred at concentrations higher than 8 mM (data not shown).

Table I shows the effect of two external proton concentrations on AIB uptake. The lower pH does not stimulate uptake unless the nutrient solution is supplemented with K^+ at about 10 mM or above, the effect of K^+ being to depress uptake at pH 6.5 more markedly than uptake at pH 4. Similar inhibitory effects were observed when KNO_3 or K_2SO_4 was substituted for KCl although

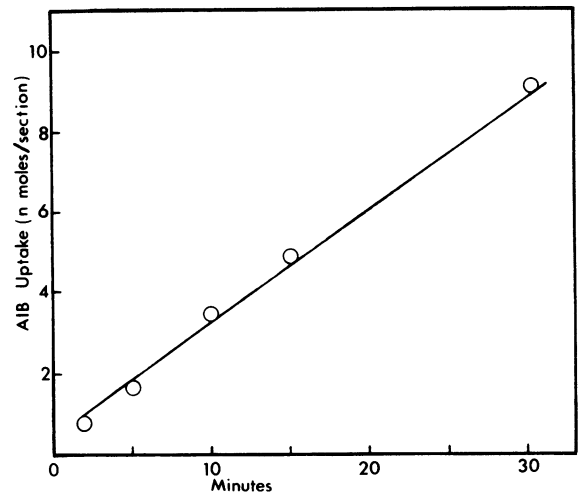

FIG. 2. Time course of AIB uptake into *Avena* coleoptile sections. After preincubation in nutrient solution for 3 hr, the tissue was transferred to nutrient solution at pH 4 containing 0.4 μM ^{3}H-AIB and 4 mM unlabeled AIB. Washing period was 15 min for all uptake times.

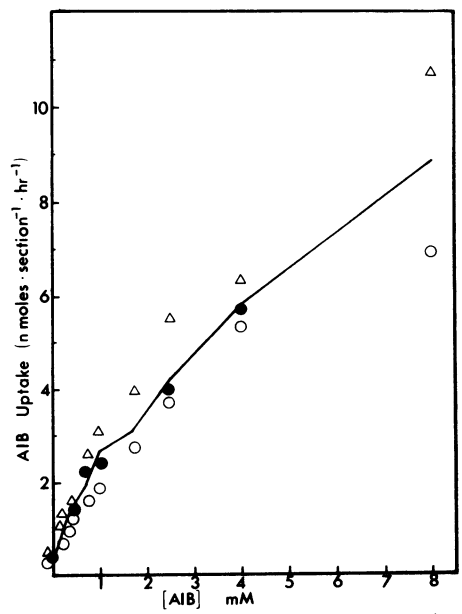

FIG. 3. Effect of AIB concentration on AIB uptake. Coleoptile sections were transferred after preincubation to nutrient solution with 0.4 μM ^{3}H-AIB and the concentration of AIB indicated on the abscissa. Uptake periods were 15 min. Each class of symbols represents an experiment performed on a different day. The line is drawn through the average of all of the points for each concentration.

K_2SO_4 was somewhat more inhibitory (Table I). Values for the two controls at pH 6.5 with no added K^+ salts show typical day to day variation. Each experiment was internally consistent, however. Na^+ affected uptake in a manner similar to K^+ and the acid stimulation in the presence of elevated K^+ or Na^+ levels was seen at .04, 0.4, and 4 mM AIB (data not shown).

Because IAA and FC stimulated proton extrusion from coleoptile tissue, the effect of these substances on AIB uptake was compared with uptake into coleoptiles floated on low pH. As seen in Table II, both IAA and FC stimulate the uptake of AIB but only at the higher concentration of Na^+. A similar requirement for a high concentration of a monovalent cation was seen for promotion of AIB uptake by low pH (Table I). Furthermore, AIB uptake stimulations by IAA, and low pH are eliminated by 2 mM NaN_3.

Membrane Potential Data. Figure 4 shows how membrane potentials of oat coleoptile cells changed after the tissues were exposed to AIB. In general, AIB induced a rapid depolarization (E1), which attained a maximum in about 1 min. This was followed by a slower repolarization. The membrane potential did not repolarize to its initial level after 5 min of AIB exposure, although the trend was toward more negative values at this time. When the AIB-containing solution was replaced with the original AIB-free solution, repolarization of the membrane potential was accelerated, often resulting in membrane potentials which were hyperpolarized when compared with initial levels. The magnitudes of the repolarizations in the presence of AIB or the hyperpolarizations when AIB-free solutions replaced AIB-containing solutions were not evaluated in detail because it was felt that these values could not be measured reliably.

Figure 4 also shows the effect of pH on AIB-induced membrane potential changes. In general, the maximum depolarization was increased by more acid conditions. The curves also illustrate the considerable variability in the results, but the trend is clear.

Figure 5b shows the averages of AIB-induced depolarizations of membrane potentials at pH 6.5, 5, and 4. The lower pH solutions promoted larger AIB-induced depolarizations. Membrane potentials in AIB-free solutions were also more positive

Table I. Effect of K^+ on acid-stimulated uptake of AIB

Salt Added	pH 6.5	pH 4.0	Stimulation by acid
	nmoles·section^{-1}·hr^{-1}		%
None	0.28	0.30	7
10 mM KCl	0.21	0.27	29
100 mM KCl	0.10	0.21	110
None	0.37	----	---
50 mM KCl	0.15	0.28	87
50 mM KNO_3	0.14	0.25	79
25 mM K_2SO_4	0.14	0.22	57

After a 3 hr preincubation on nutrient solution with or without added K^+ salts, the coleoptile sections were transferred to the same solution containing 40 μM ^{3}H-AIB for 15 min.

Table II. Effect of Na^+ concentration, IAA and FC on AIB uptake.

Addition	pH	Na^+ Concentration 0	25 mM $-NaN_3$	25 mM $+NaN_3$ (2mM)
		% of Control		
None	6.5	100	100	13
+IAA (30 μM)	6.5	105	132	12*
+FC (10 μM)	6.5	103	124	--
None	4.0	---	126	08

Coleoptile sections were preincubated in the usual nutrient solution (pH 6.5) or in nutrient solution supplemented with 25 mM NaCl for 3 hr. IAA was present 45 min before and during the 15 min uptake period; FC and NaN3 were added only during the uptake period. The final concentration of AIB was 40 μM. Data are expressed as a percent of the pH 6.5 control at 0 or 25 mM added Na^+. In a typical experiment, 25 mM Na^+ decreased uptake at pH 6.5 from 0.39 to 0.27 nmoles·section^{-1}·hr^{-1}.

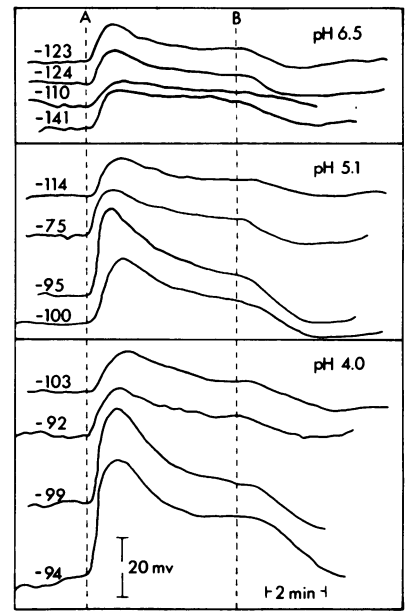

FIG. 4. Tracings of chart records showing the time course of AIB-induced depolarizations of membrane potentials at different pH values. AIB (4 mM) replaced the control (S1) solution at A which was in turn replaced by the S1 solution at B. The curves shown were selected at random from a large population of curves. Numbers on the curves are membrane potentials of cells prior to the addition of AIB.

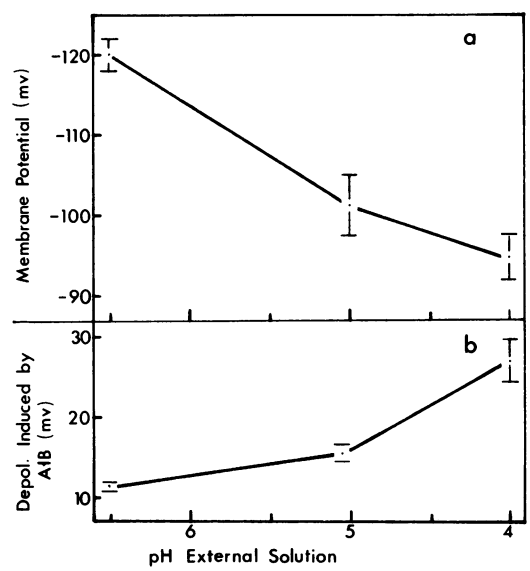

FIG. 5. Effect of pH on the resting membrane potentials (upper curve) and AIB-induced depolarizations (lower curve) of oat coleoptile cells. Data were derived from recordings like those shown in Figure 4. Membrane potentials were measured after tissues were exposed to a treatment pH for at least 5 min. After an additional 3 to 5 min, the AIB-free solution was replaced by one containing AIB at the same pH. Bars above and below each point delimit the standard error of the mean. Number of measurements were: pH 6.5, 22; pH 5.1, 19; pH 4, 16.

when the external solution had a lower pH (Fig. 5a). These results can be compared with data in Tables I and II, which show greater AIB transport rates at lower pH values.

Figure 6 shows that the AIB-induced depolarizations increased with increasing AIB concentrations and that the effect appeared to approach a maximum at higher AIB concentrations. These depolarization data can be compared with the AIB transport data in Figure 3, which show a similar trend.

Table III shows that IAA hyperpolarized the membrane poten-

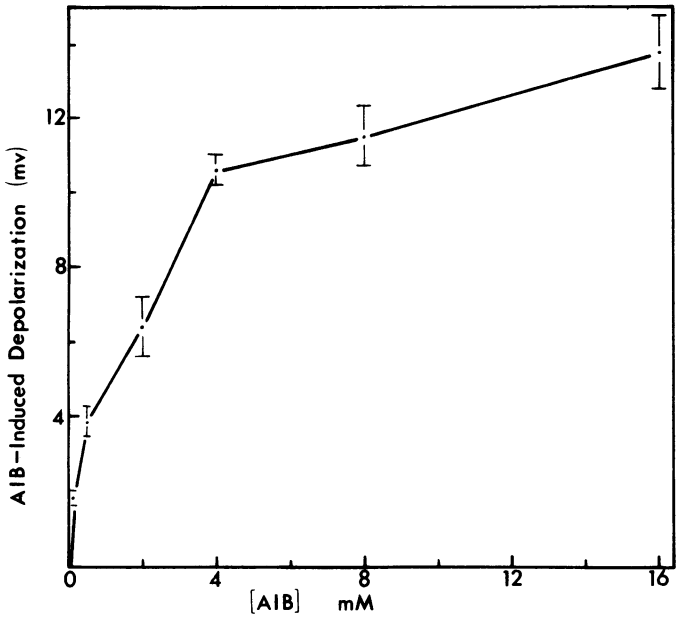

FIG. 6. Effect of AIB concentration on AIB-induced depolarization of membrane potentials at pH 6.5. All solutions had the S1 inorganic ion composition. Bars delimit standard error of the mean. Number of observations for each concentration were: 0.1, 3; 0.5, 8; 1, 5; 4, 33; 8, 18; 16, 11.

Table III. Effect of IAA (30 μM) on membrane potentials and AIB induced depolarizations of membrane potentials of oat coleoptile cells.

	+ IAA	- IAA
Membrane potential (mv)	-102 ± 7 (11)	-88 ± 5 (13)
AIB induced depolarization (mv)	5.3 ± 1.6 (10)	3.5 ± 1 (12)

All solutions were S1 with 25 mM NaCl added for a total NaCl concentration of 29 mM. The pH was 6.5 and the AIB concentration was 4 mM. Data are expressed as mean ± standard deviation (number of observations). The IAA effects on membrane potentials and AIB depolarizations were significant at the .001 and .01 levels respectively.

tial of oat coleoptile cells (as has been shown previously [1, 5, 13]) and also enhanced the depolarization induced by AIB. IAA also stimulated AIB uptake (Table II).

DISCUSSION

In general, our data are consistent with a proton co-transport model (14, 21) for AIB transport into oat coleoptile cells. As predicted by the model (Fig. 1), the presence of a transportable amino acid in the medium caused a depolarization of the membrane potential (Fig. 4) thus supporting an electrogenic nature for the transport process. The model also predicts that both AIB uptake and the AIB-induced depolarization of the membrane potential should be affected similarly by alterations of external pH and by factors that affect the resting potential. For example, the AIB-induced depolarization of the membrane potential (Fig. 6) and AIB transport (Fig. 3) were affected similarly by the AIB concentration of the medium. Furthermore, increases occurred in both AIB transport (Table I) and AIB-induced depolarizations (Fig. 5) after the pH of the medium was lowered; reducing the pH increased the chemical potential difference of protons between the medium and the cell interior. Similar effects of pH on AIB uptake into barley leaf cells were reported by Shtarkshall and Reinhold (20).

There were some differences between experimental procedures for measuring membrane potentials and measuring uptake which were due in part to the difficulties inherent in completely coordinating the activities of two separate laboratories. We do not feel that the differences in procedures introduced any serious errors in comparing our results, however, and were probably less serious than the unavoidable uncertainties associated with comparing uptake data on whole tissue sections with membrane potential data from cells exposed at a cut surface.

The IAA- and FC-stimulated increases in AIB transport (Table II) and AIB-induced depolarization (Table III for IAA) were also predictable from the model and the known ability of IAA and FC to hyperpolarize the membrane potential (1, 5, 13). Whether the effect was due exclusively to the IAA- or FC-induced membrane potential hyperpolarization or due also to an increased acidification of the immediate cell surroundings is not known. Wall acidification would have been more of a factor with uptake studies than it would have been in the membrane potential studies where surface pH probably remained more constant; this was because the cells measured were close to the tissue surface and were bathed by the flowing external solution.

The depressions of AIB uptake by increased K^+ concentrations or by the presence of the metabolic inhibitor NaN_3 were expected from studies showing that these substances depolarize the membrane potential (9, 10) and hence decrease this component of the proton chemical potential difference. In addition, the azide may act to reduce the proton gradient, for Spanswick and Miller (23) have shown that in *Nitella*, at least, 1 mM NaN_3 reduces the interior pH as much as 1 to 2 units.

The apparent discrepancy between the observed enhancement of the AIB-induced depolarization by low pH and the enhancement of AIB uptake by low pH only when Na^+ or K^+ concentrations were high could be explained in part by the higher (4 mM) Na^+ concentration in the S1 used for the membrane potential study compared with the 1 mM Na^+ concentration in the 1× solution used for the uptake experiments. Another possibility is that the cuticle and epidermis limited the uptake of AIB into the free space. The rate-limiting effect of this passive step on the uptake of AIB by cells of the tissue was probably less pronounced when the uptake by cells was reduced by K^+ or Na^+. Thus, an effect of H^+ on cellular uptake would be more easily detected when cellular uptake was inhibited. A third possibility is that the movement of AIB and/or protons into the free space of coleoptile sections was somehow promoted by higher ionic strength solutions.

The transient nature of a substrate-induced depolarization, *e.g.* the repolarization in the presence of substrate, has been noted in other systems of suspected co-transport (11, 16, 18, 22), but it is not completely understood. Our data show that the changing depolarizations with time (Fig. 4) is not correlated with the rate of uptake (Fig. 2), if we assume that AIB uptake is linear from time zero as it is from 2.5 min to 30 min. Because of the limited amount of radioactivity taken up by the sections in 2 min or less, it is difficult to determine short term uptake rates, but it is reasonable to assume that some counts always remain associated with tissue free spaces. Thus, a linear extrapolation of the line to zero time may be justified. Slayman (22) has shown that for *Neurospora* the transient change in potential caused by 3-0-methyl glucose is not associated with a change in membrane resistance. In *Neurospora* (22) and in *Samanea pulvini* (16) the transients are associated with transient changes in external pH; *e.g.* an initial rapid net loss of acidity from the medium followed by less of a net loss. This behavior would be consistent with a repolarization caused by an increased rate of electrogenic proton efflux, which would compensate for the amino acid-induced influx. Other possible explanations for the repolarizations could involve changes in fixed charges, carrier positions, or carrier conformations. It is possible that a better understanding of the cause of the transient behavior of the AIB-induced membrane potential change will contribute to a better understanding of how the amino acid carrier functions in the membrane.

For the present, however, our data fit very well with the co-transport theory. This theory is useful in that it provides a mechanism for coupling both proton gradients and membrane potential differences to the uptake of organic compounds; it also helps to

explain why factors which alter the membrane potential or the proton concentration of the medium may have significant effects on the uptake of substances which are uncharged and/or have a pK considerably above or below the pH of the medium.

Acknowledgments—The authors wish to acknowledge the technical assistance of P. Mahar with the AIB flux measurements, and B. Cali with the membrane potential measurements. They also wish to acknowledge the assistance of G. Nuovo who performed many of the preliminary membrane potential measurements and who helped with literature searches and preliminary drafts of this manuscript.

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