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Evidence for a Specific Glutamate/H^+ Cotransport in Isolated Mesophyll Cells[1]

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ABSTRACT

Mechanically isolated *Asparagus sprengeri* Regel mesophyll cells were suspended in 1 millimolar $CaSO_4$. Immediate alkalinization of the medium occured on the addition of 1 millimolar concentrations of L-glutamate (Glu) and its analog L-methionine-D,L-sulfoximine (L-MSO). D-Glu and the L isomers of the protein amino acids did not elicit alkalinization. L-Glu dependent alkalinization was transient and acidification resumed after approximately 30 to 45 minutes. At pH 6.0, 5 millimolar L-Glu stimulated initial rates of alkalinization that varied between 1.3 to 4.1 nmol $H^+/10^6$ cells·minute. L-Glu dependent alkalinization was saturable, increased with decreasing pH, was inhibited by carbonyl cyanide-*p*-trichloromethoxyphenyl hydrazone (CCCP), and was not stimulated by light. Uptake of L-[U-^{14}C]glutamate increased as the pH decreased from 6.5 to 5.5, and was inhibited by L-MSO. L-Glu had no influence on K^+ efflux. Although evidence for multiple amino acid/proton cotransport systems has been found in other tissues, the present report indicates that a highly specific L-Glu/proton uptake process is present in *Asparagus* mesophyll cells.

Recent data suggest that the immediate source of energy driving amino acid uptake is a proton electrochemical gradient across the plasma membrane (13). A plasma membrane located ATPase pumps protons out of the cell establishing both electrical and pH gradients which favor the reentry of protons (16, 17). Influx of the amino acid is believed to be coupled to and driven by the downhill influx of protons. Models of amino acid/proton cotransport suggest that a plasma membrane carrier binds exogenous amino acids and protons prior to entry into the cell (13).

The model predicts that amino acid addition would result in alkalinization of the bathing medium as proton cotransport occurs. Alkalinization has been observed on the addition of L-Gln and various other L-amino acids to detached *Ricinus* cotyledons. D-Gln did not result in alkalinization (14). Similarly, alkalinization was observed on the addition of L-Thr and α-amino isobutyrate to *Vicia faba* leaf tissue (6). Inhibitor studies demonstrated a parallel inhibition of proton and amino acid uptake (6, 14). Work with oat coleoptiles indicated that neutral amino acids and the acidic amino acids L-Asp and L-Glu caused alkalinization of the medium and a rapid but transient depolarization of the membrane potential. Depolarization was attributed to the entry of positively charged protons. Since repolarization occurred, while alkalinization continued, it was proposed that a passive K^+ efflux represented the repolarizing current (10, 13). Evidence for a repolarizing flux of K^+ was obtained when cultured sugarcane cells were used to obtain rates of amino acid uptake and simultaneous measurements of amino acid dependent proton influx and K^+ efflux. The presence of three distinct proton co-transport systems for neutral, basic, and acidic amino acids was indicated and a role of K^+ efflux in charge compensation was suggested in each case (23).

Although the leaf cells of many plants receive their nitrogen via an apoplastic route in amino acid form (12) there is little published information on amino acid/proton uptake processes in photosynthetic leaf cells. Mechanically isolated photosynthetically competent mesophyll cells from *Asparagus sprengeri* (5), can be maintained in a stirred, aerated, and defined suspension medium and have been used to study energy dependent proton efflux (2, 3). This system does not suffer from diffusion problems inherent in studies conducted with fragile nonaerated leaf protoplasts or bulky leaf slices. The objective of the present study was to determine whether amino acid proton cotransport processes could be detected and characterized in these cells.

MATERIALS AND METHODS

All amino acids and CCCP,[3] L-MSO, and DES were purchased from the Sigma Chemical Co. L-[U-^{14}C]glutamate was from New England Nuclear Corp. PCS scintillation fluid and ^{14}C labeled *n*-hexadecane were from Amersham Illinois.

Asparagus sprengeri Regel was grown in vermiculite under greenhouse conditions. Gentle mechanical tissue disruption was used to isolate approximately 50×10^6 cells daily (5). Cells were counted using a hemocytometer and approximately 85% of the cells had intact protoplasts as determined by their ability to exclude Evans Blue (8).

Between 10 to 15×10^6 aerated (500 ml/min) and stirred cells in 10 ml of 1 mM $CaSO_4$ were maintained at 30°C in a water jacketed glass vessel. Net rates of acidification or alkalinization were calculated after using a Radiometer (Copenhagen) recording pH meter to determine the rate of pH change in pH units/min and the buffering capacity of the medium in nmol H^+/pH unit. These two values together with knowledge of cell numbers allow calculation of changes in H^+ concentration in units of nmol $H^+/10^6$ cells·min (2). In addition, rates of alkalinization were measured using a Radiometer recording pH-stat apparatus (3). The rate of addition of 1 mM H_2SO_4 required to maintain a constant preselected pH value was also used to calculate the rate of alkalinization. In both cases a combination pH electrode (Radiometer GK 2321C) was employed. Illumination, when required, was supplied with a 300 W reflector lamp (Sylvania) which gave an irradiance at the surface of the vessel of 4.6×10^{-4} mol·m^{-2}.

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[3] Abbreviations: CCCP, carbonyl cyanide-*p*-trichloromethoxyphenyl hydrazone; L-MSO, L-methionine-D,L-sulfoximine; DES, diethylstilbestrol.

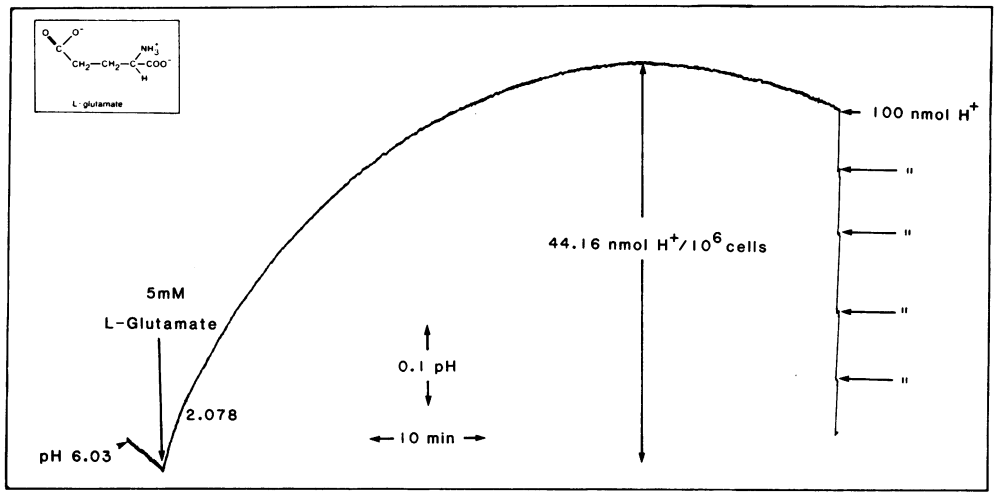

FIG. 1. L-Glu dependent alkalinization. Cells were incubated in 10 ml of illuminated, aerated, and stirred 1 mM $CaSO_4$. L-Glu was added as indicated. The initial rate of alkalinization is expressed in nmol $H^+/10^6$ cells·min. The extent of alkalinization prior to resumption of acidification was calculated from the maximum change in pH, the buffering capacity of the medium, and the number of cells present.

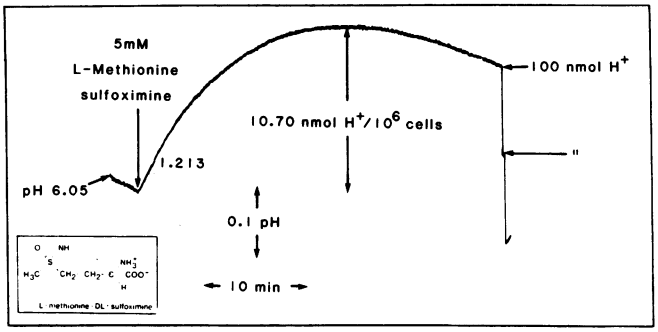

FIG. 2. L-MSO dependent alkalinization. Cells were incubated in 10 ml of illuminated, aerated, and stirred 1 mM $CaSO_4$. L-MSO was added as indicated. The initial rate of alkalinization is expressed in nmol $H^+/10^6$ cells·min. The extent of alkalinization prior to resumption of acidification was calculated from the maximum change in pH, the buffering capacity of the medium, and the number of cells present.

s^{-1}.

When amino acids were surveyed for their ability to cause medium alkalinization or when the uptake of labeled L-[U-^{14}C] glutamate was studied with or without simultaneous alkalinization measurements, a 2 ml cell suspension was employed. The 30°C suspension medium containing approximately 4×10^6 cells was stirred, and subjected to normal laboratory lighting. Medium alkalinization was recorded using a Fisher flat surface polymer combination electrode (13-639-83) designed for work with small volumes. Rates of L-Glu uptake were determined after addition to the 2 ml cell suspension of 20 or 40 μl volumes of 50.17 mM L-[U-^{14}C]glutamate (2946 dpm/nmol) to give 0.5 or 1 mM final concentrations. At zero time and 1 min intervals, 0.2 ml aliquots were removed. The cells were immediately collected on Millipore filters (HA type 0.45 μm) and washed with 5 ml then 2×10 ml of ice-cold suspension medium. The filters were placed in a scintillation vial with 1 ml of 100% methanol and 14 ml of a scintillation cocktail (ACS, Amersham). The efficiency of the counting system was determined using the internal standard [^{14}C] *n*-hexadecane. Rates of uptake were calculated as nmol L-Glu/10^6 cells·min.

The net flux of H^+ and K^+ was measured simultaneously using a Radiometer G202C glass pH electrode, an F2312 K^+ ion selectrode containing 10 mM KCl, and a common K701 reference electrode. The reference electrode had a secondary salt bridge filled with 0.1 M NaCl so that K^+ did not leak into the cell suspension medium. The H^+ and K^+ electrodes were connected via a Fisher Accumet 310 pH meter and a Radiometer PHM84 pH meter, respectively, to a Cole-Parmer model 8371-20 two pen recorder. When ionic strength was maintained constant with NaCl the mV output of the K^+ electrode was linear with the log of the KCl concentration between 10^{-5} and 0.1 M. Experiments were conducted with 10 to 12×10^6 aerated and stirred cells in 10 ml of a solution containing 1 mM $CaSO_4$, 10 mM NaCl, and 0.1 mM KCl.

Whenever necessary, suspensions of cells in nonbuffered media

Table I. *Specificity of Amino Acid Dependent Alkalinization*

Cells were stirred in 2 ml 1 mM $CaSO_4$ at a pH between 5.9 and 6.1 under normal laboratory lighting. Rates of acidification were calculated prior to the addition of 1 mM amino acids by determination of the buffering capacity of the medium. Subsequent to addition, the buffering capacity of the medium was again determined and used to calculate rates of acidification or alkalinization. Results were not obtained with histidine due to its strong buffering capacity at pH 6.0. Each value is the mean of three determinations.

Amino Acid	Before Addition	After Addition	
	Acidification rate	Acidification rate	Alkalinization rate
	nmol $H^+/10^6$ cells·min		
L-GLU	0.301	0	0.373
L-MSO	0.326	0	0.146
L-PHE	0.379	0.113	0
L-HIS	0.264		
L-TRY	0.362	0.142	0
L-PRO	0.298	0.078	0
L-ILU	0.336	0.122	0
L-LEU	0.333	0.121	0
L-SER	0.329	0.118	0
L-THR	0.319	0.110	0
L-ALA	0.359	0.171	0
L-GLN	0.317	0.130	0
L-GLY	0.307	0.122	0
L-MET	0.326	0.146	0
L-VAL	0.294	0.118	0
L-ASN	0.323	0.150	0
L-LYS	0.340	0.175	0
L-TYR	0.326	0.205	0
L-ARG	0.300	0.202	0
L-ASP	0.304	0.209	0
L-CYS	0.253	0.199	0
D-GLU	0.217	0.170	0

Table II. *L-Glu and L-MSO Dependent Transient Alkalinization*

Cells were aerated and stirred in 10 ml 1 mM $CaSO_4$ at pH 5.9 to 6.0. After the addition of 5 mM L-Glu or L-MSO the alkalinization responses were followed until acidification resumed. The initial rates and extent of alkalinization were calculated after determining the buffering capacity of the medium. The maximum pH value and the time to reach this value were recorded. Each value is the mean of five determinations (with SD in parentheses).

	L-Glu		L-MSO	
	Light	Dark	Light	Dark
Rate of alkalinization ($nmol/10^6$ cells·min)	1.76 (0.66)	1.67 (0.38)	1.35 (0.33)	1.20 (0.25)
Extent of alkalinization ($nmol/10^6$ cells)	23.74 (16.58)	29.62 (9.80)	8.07 (1.98)	11.64 (3.02)
Maximum pH reached	6.52 (0.08)	6.57 (0.07)	6.29 (0.06)	6.35 (0.05)
Time to reach maximum pH (min)	30 (10)	44 (10)	16 (3)	26 (11)

were adjusted to the desired experimental pH value with either 1 mM H_2SO_4 or 1 mM NaOH. Stock solutions of L-Glu and other amino acids were adjusted to this pH prior to addition. All concentrations quoted are final values obtained after dilution with the cell suspension. Separate cell preparations were used to obtain mean values for replicated experiments.

RESULTS

Asparagus mesophyll cells when incubated in a nonbuffered medium above pH 5.5 will normally acidify the suspension medium (Figs. 1 and 2). This process results from energy dependent H^+ efflux. To rapidly survey the possible stimulation of medium alkalinization by amino acids, cells were suspended in 2 ml of medium. On the addition of 1 mM concentrations of amino acids, only L-Glu and L-MSO resulted in a reversal of acidification and medium alkalinization. Inhibition of the acidification rate on the addition of other amino acids may represent a very low rate of alkalinization or a reduction in the rate of acidification as the pH of the medium declines (Table I). Experiments with additions of 5 mM concentrations of L-Glu analogs indicate that L-Gln may give rise to a very low rate of alkalinization. No evidence for alkalinization in response to L-Asp was obtained (Table I).

The alkalinization resulting from L-Glu or L-MSO addition was characterized in the absence or presence of illumination. In both cases, addition of 5 mM concentrations gave rise to an immediate rate of alkalinization which appeared independent of illumination. Whereas rates for L-Glu dependent alkalinization were around 1.70 nmol $H^+/10^6$ cells·min those for L-MSO varied around 1.25 nmol $H^+/10^6$ cells·min. Alkalinization was transient and net acidification resumed after the medium pH reached a maximum value of approximately 6.2 to 6.6. Compared to L-Glu, L-MSO dependent alkalinization was of shorter duration and peaked at a lower pH value (Table II; Figs. 1 and 2). Alkalinization rates varied between cell preparations. Rates of 1.3 to 4.1 nmol $H^+/10^6$ cells·min were obtained with 5 mM L-Glu at pH 6.0. However, consecutive measurements of alkalinization by measurement of medium pH changes or the pH-stat method gave almost identical rates.

The relationship between the rate of alkalinization and the concentration of L-Glu was investigated with the pH-stat apparatus. L-Glu was varied either by sequential additions to give stepwise increments in concentration (Fig. 3) or by a single addition of L-Glu and determination of the intial rate of alkalinization (Fig. 4). Both methods indicated that the alkalinization response to L-Glu was saturable and no reproducible influence of illumination was obtained. At pH 6.5 the first method indicated a V_{max} of approximately 6 nmol $H^+/10^6$ cells·min and an $S_{0.5}$ value for the concentration of L-Glu giving half V_{max} of approximately 3 mM. Initial rates of alkalinization at pH 5.5 indicated V_{max} values for illuminated and nonilluminated cells of approximately 18 nmol $H^+/10^6$ cells·min and $S_{0.5}$ values between 2 and 3 mM L-Glu (Fig. 4).

The pH-stat method was also used to determine the influence of pH on the rate of alkalinization after addition of 10 mM L-Glu. With both illuminated and nonilluminated cells, rates of alkalinization decreased by approximately 30 to 40% as the pH increased from 5.5 to 6.5 (Table III). The addition of 10 μM CCCP, which renders membranes permeable to H^+, inhibited the rate of alkalinization by 60% (Table IV).

Amino acid dependent alkalinization and depolarization are often associated with a stimulation of K^+ efflux. Consequently, L-Glu dependent alkalinization and K^+ efflux were measured simultaneously. On the addition of cells to a medium containing 0.1 mM K^+, 10 mM Na^+ and 1 mM Ca^{2+} initial rates of H^+ efflux

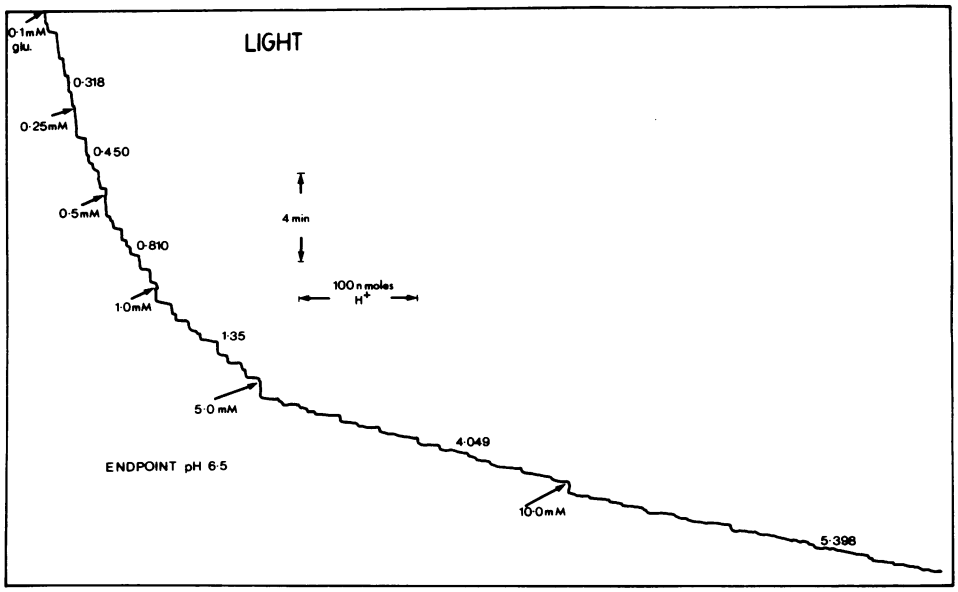

FIG. 3. pH-stat determination of the alkalinization rate as a function of stepwise increases in L-Glu concentration. Cells were incubated in 10 ml of illuminated, aerated, and stirred 1 mM $CaSO_4$. L-Glu was added to give the increasing concentrations indicated. Rates of alkalinization are indicated and are expressed in nmol $H^+/10^6$ cells·min and were calculated using the rate of H_2SO_4 addition required to maintain the medium pH at 6.5.

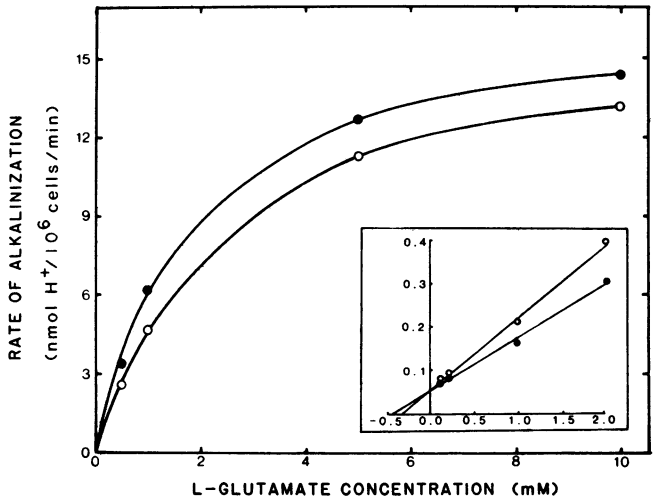

FIG. 4. pH-stat determination of the initial rates of alkalinization as a function of L-Glu concentration. Cells were incubated in 10 ml of aerated and stirred 1 mM $CaSO_4$. The L-Glu concentration was varied by addition to give the concentrations indicated. The resulting initial rates of alkalinization were calculated using the rate of H_2SO_4 addition required to maintain the pH at 5.5. Each data point is the mean of four separate determinations. The inset represents the same data plotted as the reciprocal of the alkalinization rate against the reciprocal of the L-Glu concentration. Without illumination (●); with illumination (○).

Table III. *pH-stat Determination of the Rate of L-Glu Dependent Alkalinization as a Function of pH*

Cells were incubated in the light and dark in 10 ml aerated and stirred 1 mM $CaSO_4$. Immediately prior to the addition of 10 mM L-Glu the pH was adjusted to the pH values indicated. The rates of alkalinization were calculated using the rate of acid addition required to maintain these pH values. Each value is the mean of five determinations (with SD in parentheses).

pH	Rate of Alkalinization	
	Light	Dark
	nmol $H^+/10^6$ cells·min	
5.5	9.7 (2.9)	11.7 (3.2)
6.0	7.6 (2.0)	9.5 (2.0)
6.5	6.2 (2.5)	6.8 (2.6)

Table IV. *Inhibition of L-Glu Dependent Alkalinization by CCCP*

Cells were aerated and stirred in 10 ml illuminated 1 mM $CaSO_4$ at a pH between 5.9 and 6.0. Addition of 10 μM CCCP resulted in an increase in pH. Once this increase ceased, the pH was adjusted with HCl to the original pH. Five mM L-Glu was then added. The initial rates and extents of alkalinization were calculated after determination of the buffering capacity of the medium. Control experiments at the same pH were performed without CCCP addition. Each value was obtained from a minimum of three trials (with SD in parentheses).

	−CCCP	+CCCP	Inhibition
			%
Rate of alkalinization (nmol $H^+/10^6$ cells·min)	2.24 (0.23)	0.92 (0.13)	60
Extent of alkalinization (nmol $H^+/10^6$ cells)	57.3 (11.8)	35.3 (13.4)	38

and K^+ efflux were detected. On the addition of L-Glu alkalinization was observed but, despite repeated attempts, no evidence for L-Glu stimulated K^+ efflux was obtained. The sensitivity of the K^+ electrode ensured that a stimulation would have been observed if the rate of K^+ efflux had been stimulated by an amount equal to the rate of L-Glu dependent alkalinization (Fig. 5). DES was applied to determine whether agents known to cause membrane depolarization would stimulate K^+ efflux from *Asparagus* cells. The results demonstrate that 100 μM DES resulted in an immediate alkalinization of the cell suspension medium and an equally rapid 15-fold increase in the rate of K^+ efflux to 15.7 nmol $K^+/10^6$ cells·min (Fig. 5).

L-[U-^{14}C]glutamate was employed to probe the relationship, if any, between L-Glu uptake and L-Glu dependent alkalinization. Labeled L-Glu was added to give a 1 mM concentration in a 2 ml cell suspension and uptake and alkalinization were monitored simultaneously over a 75 min period. The typical transient alkalinization response to added L-Glu was observed with a maximal pH being reached after 30 to 45 min. Thereafter, acidification resumed and the pH of the medium declined. An uptake of label into the cells was observed which appeared approximately linear for 30 min and then appeared to decline. However, the results indicate that the uptake of labeled L-Glu continued when net alkalinization had been superceded by net acidification (Fig. 6). The influence of pH on L-Glu uptake was investigated by incubating cells in 2 ml phosphate buffer at pH 5.5, 6.0, and 6.5 prior to the addition of 1 mM labeled L-Glu. Rates of uptake were linear for at least 25 min and increased by 130% as the pH decreased from 6.5 to 5.5 (Fig. 7). At pH 5.5, 10 μM CCCP inhibited the rate of uptake by 53% (data not shown).

Unlabeled L-Glu and several of its analogs were tested for their ability to inhibit the uptake and stimulate the alkalinization observed on the addition of 0.5 mM labeled L-Glu to cell suspensions. As expected, 5 mM L-Glu stimulated alkalinization by over 360% and inhibited the uptake of ^{14}C-labeled L-Glu by 67%. L-MSO at the same concentration stimulated alkalinization by 111% and inhibited uptake by 40% indicating that L-MSO and the labeled L-Glu were competing for the same uptake system. Results with 5 mM concentrations of D-Glu, L-Asp, and L-Gln suggest that only the latter may weakly stimulate alkalinization and inhibit the uptake of labeled L-Glu (Fig. 8).

DISCUSSION

The alkalinization response can be characterized as specific (Table I), transient (Table II; Figs. 1 and 2), saturable (Figs. 3 and 4), and inhibited by CCCP (Table IV) and higher pH values (Table III). These characteristics meet several of the criteria proposed for the identification of an amino acid/proton co-transport system driven by the proton electrochemical gradient and the amino acid electrochemical gradient (13). The possibility that alkalinization results from the release of a basic product of L-Glu metabolism was investigated by addition of the glutamine synthetase inhibitor L-MSO, a nonphysiological L-Glu analog. Since *Asparagus* is a C3 plant (5) the conversion of L-Glu to L-Gln by cytosolic glutamine synthetase (22) represents its most probable first step in metabolism. The unexpected finding that L-MSO also stimulates alkalinization (Figs. 2 and 8) indicates that alkalinization results from an influx of H^+ and not L-Glu metabolism. Fusicoccin stimulation of H^+ efflux and labeled L-Glu uptake (data not reported) represents further evidence for the L-Glu/proton co-transport origin of alkalinization (6, 13).

Transient alkalinization and the resumption of acidification is predicted by an amino acid/proton cotransport mechanism of uptake (13), and has been observed when amino acids are added to various plant tissues (6, 14, 23). Proton co-transport requires that ATP driven proton efflux continues to maintain the proton electrochemical gradient required for uptake. The initial alkalinization represents a net process in which energy dependent proton efflux is masked by a larger proton influx. Thus, continued L-Glu uptake during the resumption of net acidification (Fig.

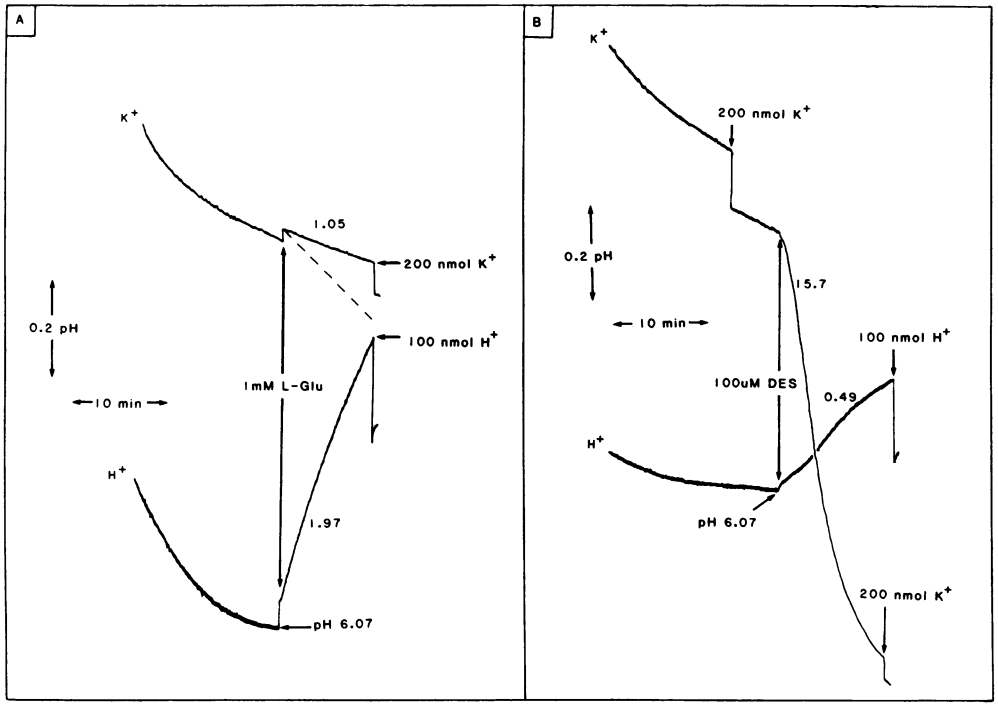

FIG. 5. The influence of L-Glu and DES on rates of H^+ influx and K^+ efflux. Cells were incubated in 10 ml of a nonilluminated stirred medium containing 1 mM $CaSO_4$, 10 mM NaCl, and 0.1 mM KCl. L-Glu (A) and DES (B) were added as indicated to give 1 mM and 100 μM concentrations, respectively. H^+ and K^+ concentrations were recorded simultaneously on a two-pen recorder and rates of H^+ influx and K^+ efflux were calculated in nmol/10^6 cells·min after addition of standard amounts of H^+ and K^+ to calibrate the system. The dotted line represents the rate of K^+ efflux that would result if L-Glu dependent H^+ influx resulted in a stoichiometric efflux of K^+.

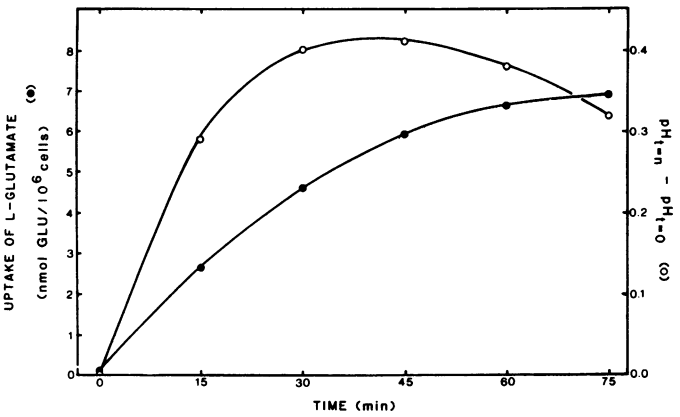

FIG. 6. Simultaneous measurement of L-Glu dependent alkalinization and uptake of L-[U-^{14}C]glutamate. Cells were incubated in 2 ml of stirred 1 mM $CaSO_4$ and exposed to normal laboratory lighting. Labeled L-Glu (2946 dpm/nmol) was added to give a 1 mM concentration. The initial pH was approximately 6.1. L-Glu dependent alkalinization of the medium was followed and samples of the cell suspension were removed at the times indicated to determine uptake. Each value is the mean of three determinations.

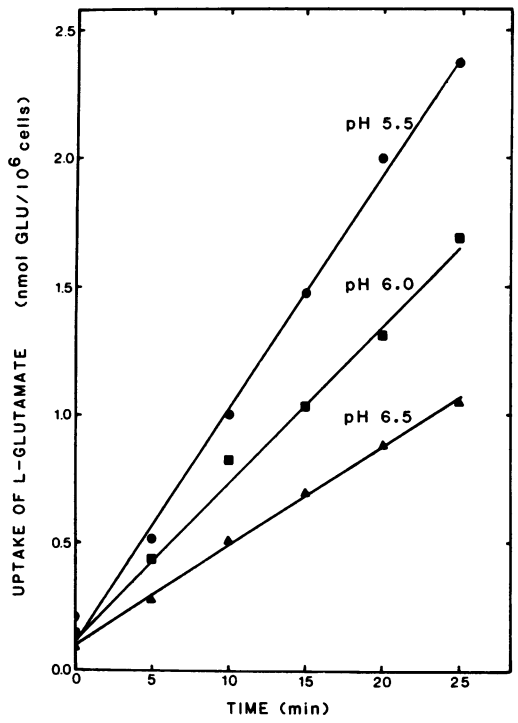

FIG. 7. The influence of pH on the uptake of L-[U-^{14}C]glutamate. Cells were incubated in 2 ml of stirred 20 mM K-phosphate at the pH indicated and exposed to normal laboratory lighting. Labeled L-Glu (2946 dpm/nmol) was added to give a 1 mM concentration and samples of cell suspension removed at the times indicated to determine uptake of label. Each value is the mean of 5 determinations.

6) is not inconsistent with the continuation of L-Glu/H^+ cotransport.

The inhibition of L-Glu dependent alkalinization and L-Glu uptake by CCCP and higher pH values are also predicted from a L-Glu/proton co-transport model (Tables III and IV; Fig. 7). CCCP, which renders membranes permeable to protons, would reduce or eliminate the proton electrochemical gradient and reduce the driving force for proton co-transport (13). Nevertheless, the L-Glu dependent alkalinization which was observed subsequent to CCCP addition (Table IV) is not inconsistent with co-transport since a high L-Glu concentration in the medium will itself constitute a driving force for the coupled influx of protons and L-Glu. Similarly, raising the pH from 5.5 to 6.5 will reduce the proton electrochemical gradient across the membrane and tend to reduce the rate of a co-transport process. The cytoplasmic pH of illuminated *Asparagus* mesophyll cells varies between 6.9 and 7.2 when the external pH varies from 5.5 to 7.5 (7).

Alkalinization on addition of amino acids to plant tissues has been documented by several workers (6, 10, 14, 23). In very few

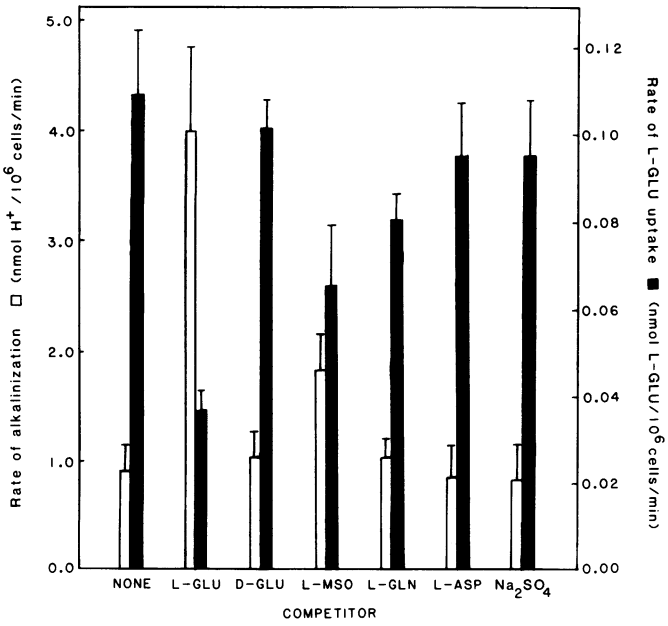

FIG. 8. The influence of L-Glu analogs on the uptake of L-[U-^{14}C] glutamate and alkalinization. Cells were incubated in 2 ml of stirred 1 mM $CaSO_4$ and exposed to normal laboratory lighting. Labeled L-Glu (2946 dpm/nmol) was added to give a 0.5 mM concentration. At the same time, sodium salts of L-Glu analogs or sodium sulfate were added to give 5 mM concentrations. Samples of suspension were removed at the times indicated to determine the uptake of label. Each value is the mean of four determinations. (Error bars indicate 1 SD).

cases, however, has the response been characterized with respect to saturation kinetics. In the present study and a previous report on L-Gln dependent alkalinization mediated by *Ricinus* cotyledons (14) proton uptake became saturated (Figs. 3 and 4). This result contrasts with evidence for a nonsaturable component of uptake which is commonly observed when amino acid uptake is plotted as a function of its concentration (13, 18, 19). Initial results with *Asparagus* mesophyll cells also indicate that a nonsaturable diffusional component facilitates an increasing proportion of uptake at concentrations above 1 mM. Evidence for a saturable amino acid/proton co-transport system and a nonsaturable proton independent system was obtained with studies on valine uptake into *Commelina* mesophyll cells, and α-aminoisobutyric acid uptake into soybean embryos (1, 19).

An influx of protons and an efflux of K^+ has been demonstrated on the addition of neutral, basic and acidic amino acids to sugarcane suspension cells. It has been suggested that efflux of K^+ is an electrophoretic response to membrane depolarization and contributes to the repolarization of the membrane (23). Depolarization in response to amino acids is well documented and thought to result from the amino acid/proton uptake system transporting net positive charge into the cell (9, 10, 13). Despite repeated attempts no evidence for L-Glu stimulation of K^+ efflux could be obtained (Fig. 5). To place this negative result in perspective, DES, an inhibitor of plasma membrane ATPase known to depolarize plasma membranes, was employed. The result was proton influx and a massive K^+ efflux (Fig. 5). Together these results indicate that the L-Glu/proton co-transport system is electroneutral. Electroneutral uptake would result from the influx of one proton with one L-Glu in the anionic form which predominates at physiological pH values. However, a proton to L-Glu ratio of 1.89 was obtained with sugarcane suspension cells (23). In the present study a ratio of approximately 5 to 8 was consistently obtained (Fig. 8). Determination of the stoichiometry of uptake systems driven by a proton electrochemical gradient is notoriously difficult (15). The transport of labeled substrates will be underestimated if decarboxylation or further metabolism results in loss of label from the cell (21). Manometric experiments with *Asparagus* cells indicates a 200 to 300% stimulation of CO_2 release immediately after feeding cold L-Glu (data not reported). Calculation of the stoichiometry of the co-transport process will require knowledge of correction factors for label lost through metabolism, and any uptake not coupled to proton co-transport.

The data in Table I and Figure 8 suggests that the co-transport system is highly specific for L-Glu and L-MSO. Slight stimulation of alkalinization and inhibition of L-Glu uptake by 5 mM L-Gln suggests that this amino acid may bind weakly to the same transport system. A high affinity transport system for L-Glu, L-Gln, and L-MSO has been characterized in *Anabaena variabilis* (4). In contrast, cultured sugarcane cells of *Ricinus* cotyledons and *Avena* coleoptiles exhibit H^+ influx in response to a wide variety of amino acids at concentrations between 0.3 and 5 mM (10, 14, 23). Similarly, uptake studies indicate transport systems that recognize a variety of amino acids. Work with *Avena* coleoptiles and sugarcane cells has resulted in proposals for three independent transport systems specific for neutral, basic, and acidic amino acids. In the latter case, L-Asp and L-Glu compete for the same carrier (9, 23). The specificity for L-Glu/H^+ co-transport reported here may reflect specificity in the pathways by which amino-acids are distributed within the plant. Studies on the distribution of xylem-fed amino acids in the vegetative shoots of *Lupinus albus* demonstrated that only the acidic amino acids, L-Glu and L-Asp, pass directly into leaf mesophyll cells. In contrast, other amino acids are lost from the xylem during passage through the stem tissue (11). Autoradiographic studies also demonstrated that only the acidic xylem-fed amino acids were transported directly into mature mesophyll cells of *Populus deltoides* (20). An investigation into the movement of xylem-fed labeled amino acids through *Asparagus* stems is envisaged.

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