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Generation of a Proton Motive Force by Histidine Decarboxylation and Electrogenic Histidine/Histamine Antiport in *Lactobacillus buchneri*

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Lactobacillus buchneri **ST2A vigorously decarboxylates histidine to the biogenic amine histamine, which is excreted into the medium. Cells grown in the presence of histidine generate both a transmembrane pH gradient, inside alkaline, and an electrical potential ($\Delta\psi$), inside negative, upon addition of histidine. Studies of the mechanism of histidine uptake and histamine excretion in membrane vesicles and proteoliposomes devoid of cytosolic histidine decarboxylase activity demonstrate that histidine uptake, histamine efflux, and histidine/histamine exchange are electrogenic processes. Histidine/histamine exchange is much faster than the unidirectional fluxes of these substrates, is inhibited by an inside-negative $\Delta\psi$ and is stimulated by an inside positive $\Delta\psi$. These data suggest that the generation of metabolic energy from histidine decarboxylation results from an electrogenic histidine/histamine exchange and indirect proton extrusion due to the combined action of the decarboxylase and carrier-mediated exchange. The abundance of amino acid decarboxylation reactions among bacteria suggests that this mechanism of metabolic energy generation and/or pH regulation is widespread.**

Several precursor/product antiport mechanisms have been shown or were anticipated to be involved in generation of metabolic energy (16). Metabolic energy can be obtained from these mechanisms by substrate level phosphorylation (5, 17) or by the formation of transmembrane ion gradients (ion and proton motive force). Characteristic for all of these precursor/product antiport mechanisms is that the product is structurally similar to the precursor. Transport of precursor and product can be catalyzed by one membrane transport protein which binds both precursor and product with high affinity. The usual mode in which these carrier proteins function is that of exchange: i.e., reorientation of the substrate binding site to either side of the membrane takes place while a substrate is bound. When the product is more positively charged than the precursor, this antiport generates an inside-negative electrical potential gradient across the membrane. Two examples of such carriers which have been studied in detail are the oxalate/formate antiporter of *Oxalobacter formigenes* (22) and the malate/lactate carrier of *Lactococcus lactis* (18). Because the metabolic conversion from oxalate to formate or from malate to lactate is a decarboxylation whereby the carboxylic group leaves the cytoplasm as neutral carbon dioxide or dihydrogen carbonate, the net effect of metabolism and electrogenic precursor/product antiport is the extrusion of one proton. Consequently, a proton motive force is generated from the free energy of decarboxylation.

A different mechanism by which metabolic energy is generated from a decarboxylation reaction is found in the Na^+-translocating oxaloacetate decarboxylase (3). In this system, decarboxylation is catalyzed by a membrane-bound protein which uses the free energy directly to translocate sodium ions across the membrane.

In this study, it will be shown that *Lactobacillus buchneri* generates metabolic energy from the decarboxylation of histidine and the electrogenic antiport of histidine and histamine. This mechanism resembles the decarboxylation of oxalate to formate by *O. formigenes* and of malate to lactate by *L. lactis*. The histamine-producing *L. buchneri* ST2A was isolated from Swiss cheese implicated in an outbreak of food poisoning (24). The occurrence of histamine and other biogenic amines like cadaverine, putrescine, tyramine, and tryptamine in food products can result in food poisoning (19, 24, 25). These biogenic amines are formed by many different bacteria by decarboxylation of the corresponding amino acids (6, 20). Therefore, it is anticipated that generation of metabolic energy by precursor/product antiport and decarboxylation is more widespread among bacteria than was realized until now.

MATERIALS AND METHODS

Strain and growth conditions. A histamine-producing strain of *L. buchneri*, ST2A, originally isolated from Emmenthaler cheese, implicated in food poisoning (24), was kindly provided by H. M. L. J. Joosten (NIZO, Ede, The Netherlands). Cells were grown overnight to an optical density at 660 nm of approximately 1.6 in MRS broth (Merck, Darmstadt, Germany) supplemented with 10 mM histidine at 37°C under semi-anaerobic conditions, i.e., in completely filled bottles with screw caps and rubber seals.

Measurement of electrical and pH gradients across the membrane. The intracellular pH was calculated from the fluorescence of BCECF [2′,7′-bis-(2-carboxyethyl)-5(and -6)-carboxyfluorescein; Molecular Probes, Eugene, Oreg.] as described previously (14). The $\Delta\psi$ (electrical potential difference across the membrane [potential inside minus potential outside]) was calculated from the distribution of the tetraphenylphosphonium ion (TPP^+), assuming proportional binding to cellular components (10). The distribution of

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TPP^+ was deduced from changes of the concentration in the buffer, which was measured with a TPP^+-selective electrode (23).

Isolation of membrane vesicles. Membrane vesicles were isolated according to a modified protocol by Otto et al. (15). Cells were grown at 30°C on MRS medium supplemented with 1% (wt/vol) glucose, 10 mM histidine, and 0.75 g of glycine and D,L-threonine per liter to an optical density at 660 nm of 0.8 to 1.0. Cells from 1 liter of culture were collected by centrifugation, washed once with 100 mM potassium phosphate (pH 7), and resuspended in approximately 15 ml of 100 mM potassium phosphate–10 mM $MgSO_2$–10% (vol/vol) glycerol (pH 7). An equal volume of the same buffer, containing 2,000 U of mutanolysin (Sigma, St. Louis, Mo.) and 500 mg of lysozyme (Boehringer GmbH, Mannheim, Germany), was added. The suspension was incubated under gentle stirring at 30°C for approximately 1 h. Samples were taken to monitor the protoplasting process with a microscope. Subsequently, 10 ml of 0.75 M K_2SO_4 was added, after which the suspension was stirred for 5 min. Then, 2.6 ml of 1 M potassium-EDTA (neutralized with potassium hydroxide) was added, and again the suspension was incubated for 5 min. After addition of 1.6 ml of 1 M $MgSO_4$, the suspension was centrifuged for 30 min at 48,200 × *g* and 4°C. The pellet was resuspended thoroughly in 50 mM potassium phosphate–10 mM $MgSO_4$ (pH 7), and the suspension was centrifuged for 1 h at 750 × *g* and 4°C. The supernatant, containing membrane vesicles, was carefully collected and centrifuged for 30 min at 48,200 × *g* and 4°C. The pellet was resuspended in 50 mM potassium phosphate–10 mM $MgSO_4$ (pH 7) to a protein concentration of approximately 5 mg/ml and stored in aliquots of 200 μl in liquid nitrogen.

Solubilization of membrane vesicles and reconstitution of membrane proteins with purified lipids into proteoliposomes. Solubilization of membrane vesicles and reconstitution of membrane protein was essentially performed as described previously (27). To remove proteins loosely associated with the membranes, membrane vesicles (10 mg of protein) were incubated under continuous stirring with 2 ml of freshly prepared 50 mM potassium phosphate–5 M urea (pH 7) for 15 min on ice. After centrifugation for 75 min at 185,000 × *g* and 4°C, the pellet was solubilized with 1.25% (wt/vol) octylglucoside (octyl-β-D-glucopyranoside) in the presence of 0.5% (wt/vol) acetone-ether-washed phospholipids from *Escherichia coli* (Sigma) and 20% (vol/vol) glycerol in 50 mM potassium phosphate (pH 7) to a final concentration of 1 mg of protein per ml (1). After 30 min of incubation on ice, the suspension was centrifuged for 1 h at 200,000 × *g* and 4°C. The supernatant was collected, and 0.5% (wt/vol) acetone-ether-washed phospholipids from *E. coli* and 4% (wt/vol) octylglucoside were added. The final octylglucoside concentration was brought to 1.5% (wt/vol) with 50 mM potassium phosphate (pH 7). The resulting suspension was incubated on ice for 10 min, carefully homogenized, and centrifuged for at least 2.5 h at 142,000 × *g* and 4°C. The pellet of proteoliposomes was resuspended in 50 mM potassium phosphate (pH 7) to approximately 2 mg of protein per ml.

Transport experiments. Efflux of histamine in membrane vesicles was measured. To a suspension of membrane vesicles, 0.5 mM histamine was added and the suspension was centrifuged in an Eppendorf centrifuge at full speed for 10 min. Membrane vesicles were resuspended in a small volume of supernatant to a protein concentration of 15 to 30 mg/ml. [^{3}H]histamine was added (49 kCi/mol; Amersham, Buckinghamshire, United Kingdom) to 1.5 to 6 μCi/mg of protein. This suspension was frozen in liquid nitrogen and slowly thawed. Samples (3 μl) were diluted in 1 ml of 50 mM potassium phosphate (pH 5, 37°C), with or without 10 mM histidine, and in the presence of 1 μM valinomycin. At different times after dilution, the reaction was stopped with 2 ml of ice-cold 0.1 M LiCl, and the mixture was immediately filtered over a cellulose nitrate filter ([pore size, 0.45 μm in diameter] BA85; Schleicher and Schuell, Dassel, Germany) and washed once with 2 ml of 0.1 M LiCl. Zero-time points were taken by carefully positioning the membrane vesicle suspension on the wall of a filled tube and subsequently washing the tube with ice-cold LiCl and then filtering as described above.

Uptake of histidine in proteoliposomes was measured. Proteoliposomes were washed once in 50 mM potassium phosphate (pH 7), centrifuged for 45 min at 130,000 × *g* and 4°C, and resuspended to 15 mg of protein per ml in the same buffer. They were loaded with 0.5 mM histamine by sonication five times for 15 to 20 s with a probe sonicator. Nonloaded proteoliposomes were used for control experiments. Samples (10 μl) were diluted into 1 ml of 50 mM potassium phosphate (pH 5) at 37°C with 1 μM valinomycin and 25 to 50 nCi of [^{14}C]histidine (uniformly labeled, 336 Ci/mol; Amersham). The stopping procedure was as described above for membrane vesicles.

The effects of the $\Delta\psi$ on transport in membrane vesicles and proteoliposomes were studied. An electrical potential gradient was created by washing membrane vesicles or proteoliposomes twice with 20 mM potassium phosphate–100 mM potassium acetate (pH 5) or 20 mM sodium phosphate–100 mM sodium acetate (pH 5) in the presence of 1 μM valinomycin. Samples were diluted in the same buffers, and transport was measured as described above.

Stoichiometry of histamine/histidine exchange was measured in proteoliposomes. Proteoliposomes were, after washing, suspended in 50 mM potassium phosphate (pH 5) to 1.8 mg of protein per ml and loaded with 500 μM histamine containing 28 μCi of [^{3}H]histamine per mg of protein. Samples (5 μl) were diluted in 1 ml of 50 mM potassium phosphate (pH 5) with 0.2 μCi of [^{14}C]histidine and 1 μM valinomycin. Transport was measured in duplicate at different time intervals as described above.

Radioactivity on filters was measured by liquid scintillation counting. In the stoichiometry experiment, the amount of each isotope was calculated from double-label counts in two channels (0 to 12 and 12 to 156 keV) and the distributions of the counts in the same two channels for single-label counts.

RESULTS

Generation of a $\Delta\psi$ and a pH difference across the membrane. After growth in complex medium supplemented with 10 mM histidine, *L. buchneri* ST2A cells had an approximately fivefold higher histidine decarboxylase activity than cells grown in the absence of additional histidine. High-performance liquid chromatography (HPLC) analysis demonstrated that histidine was nearly stoichiometrically converted to histamine by these cells (data not shown). The decarboxylation activity of a washed cell suspension could be monitored in a medium with low buffer capacity as the histidine-dependent increase in pH resulting from the proton consumption in the decarboxylation reaction (18). Rates thus measured agreed well with changes in histidine and histamine concentrations as analyzed by HPLC. At pH 5 and 5 mM histidine, decarboxylation activities of up to 300 nmol/

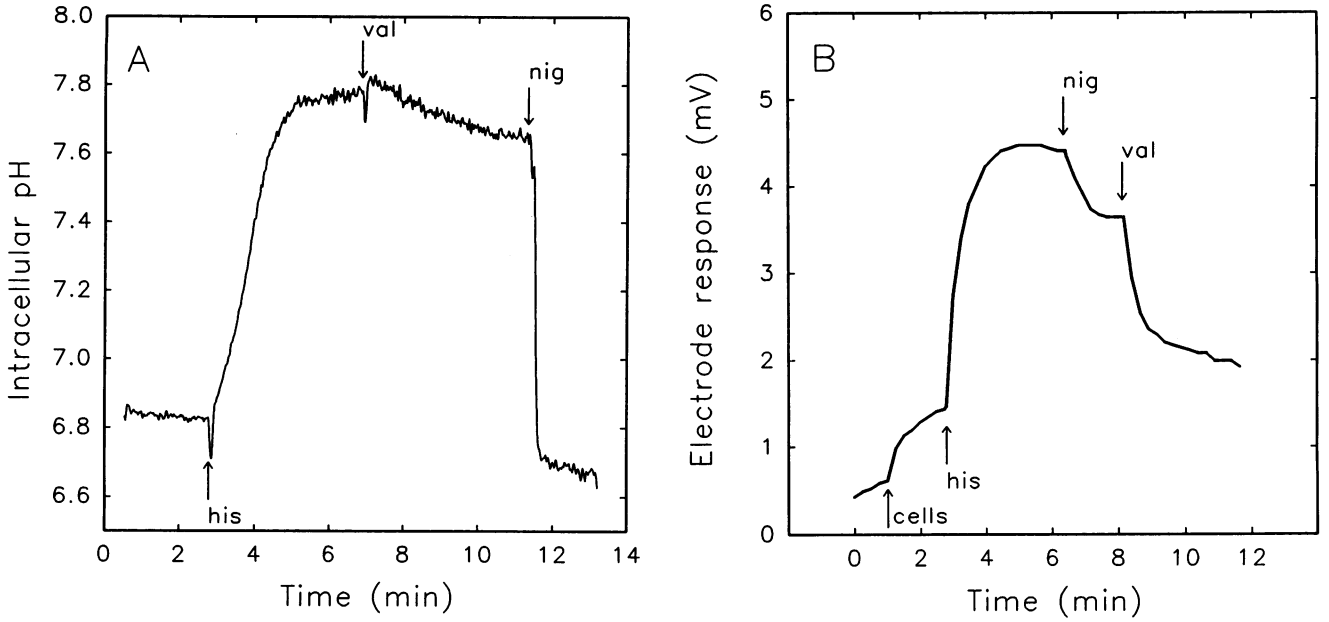

FIG. 1. Generation of both a pH difference across the membrane (A) and $\Delta\psi$ (B) by histidine decarboxylation. The intracellular pH was calculated from the BCECF fluorescence. Resting cells, loaded with BCECF, were resuspended in 50 mM potassium phosphate (pH 6.8). The following were added: 2 mM histidine (his), 1 μM valinomycin (val), and 0.5 μM nigericin (nig). (B) Generation of a $\Delta\psi$. The recording of the TPP^+-specific electrode is shown. Resting cells were suspended to 0.15 mg of protein per ml in 50 mM potassium phosphate (pH 6.5) with 4 μM TPP^+. Additions were the same as in panel A. An increasing electrode potential indicates a decrease of the TPP^+ concentration in the buffer or, consequently, TPP^+ uptake by the cells. With the assumption that the combination of nigericin and valinomycin reduces the $\Delta\psi$ to virtually zero, the maximal $\Delta\psi$'s after addition of histidine or nigericin were estimated to be −85 and −75 mV, respectively.

min/mg of protein were recorded. The decarboxylation activity showed saturation kinetics with respect to histidine concentration and was half-maximal at approximately 1 mM. The activity decreased with increasing pH. After cell lysis by French pressure treatment or sonication and after high-speed centrifugation, most of the histidine decarboxylase activity was recovered with the cytosolic fraction (not shown). In the following experiments, cells which had histidine decarboxylase activity induced by growth on complex medium supplemented with 10 mM histidine were used.

To establish whether histidine decarboxylation is coupled to proton motive force generation, cells were loaded with the fluorescent pH indicator BCECF, which allows instant monitoring of changes in intracellular pH (14). Addition of histidine to BCECF-loaded cells at pH 6.8 resulted in a rapid increase in intracellular BCECF fluorescence to a level equivalent to an internal pH of about 7.8 (Fig. 1A). Valinomycin, an ionophore that mediates electrogenic transport of potassium ions, caused a slow decrease of the intracellular pH. Subsequent addition of nigericin resulted in an immediate collapse of the transmembrane pH gradient. Generation of an electrical potential ($\Delta\psi$), inside negative, upon the addition of histidine was monitored by the use of a TPP^+-selective electrode (10, 23). The endogenous $\Delta\psi$ of resting *L. buchneri* ST2A cells is low. Addition of histidine led to rapid uptake of TPP^+ by the cells, indicating that a $\Delta\psi$ was generated. The maximal $\Delta\psi$ was estimated to be about −85 mV (Fig. 1B). TPP^+ was slowly released upon the addition of nigericin, and further release required the addition of valinomycin. These data strongly suggest that *L. buchneri* ST2A is able to generate a proton motive force across the membrane by using the free energy of the histidine decarboxylation reaction.

Histidine uptake and histamine efflux are mediated through electrogenic antiport. During histidine decarboxylation, histidine is taken up from the medium, whereas the end product histamine is excreted by the cells into the growth medium. Because both processes are metabolically coupled, uptake of histidine and efflux of histamine may be coupled processes and may be mediated by an antiporter. Histamine bears one or two positive charges at physiological pH values. The relevant pK_a is that of the imidazole ring, which has a value of 5.8. In vivo, histamine excretion thus occurs against an existing $\Delta\psi$ and is *trans*-positive. On the other hand, histidine may bear one positive charge at physiological pH. In this case, the relevant pK_a is also that of the imidazole ring, with a value of 6.0. To determine the $\Delta\psi$ dependency of histidine uptake and histamine efflux, the effect of an imposed valinomycin-mediated potassium diffusion gradient on these transport processes in membrane vesicles was investigated. Membrane vesicles loaded with histamine displayed a rapid efflux of histamine (Fig. 2). Histamine efflux is electrogenic, because it is accelerated by an inside-positive $\Delta\psi$ (4- to 5-fold) and inhibited by an inside-negative $\Delta\psi$ (1.3- to 1.4-fold). These data indicate that histamine efflux is an electrogenic process in which one or more positive charges are transported to the outside. Because membrane vesicles still contained histidine decarboxylase activity, experiments in which histidine was used were performed with proteoliposomes reconstituted from a membrane-detergent extract. This reconstitution procedure resulted in a marked decrease in histidine decarboxylase activity to levels below detectability. Proteoliposomes mediated rapid histidine uptake in the presence of an imposed inside-negative $\Delta\psi$ (Fig. 3). When no $\Delta\psi$ was imposed, uptake of histidine was negligible. These data demonstrate that histidine uptake, analogous to hista-

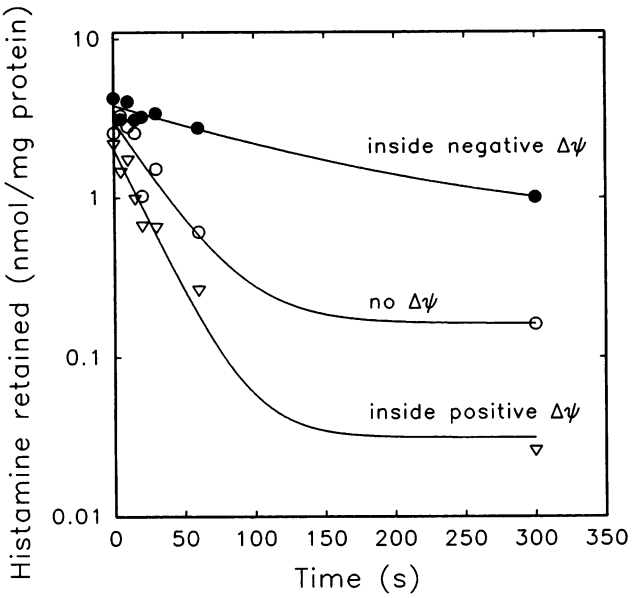

FIG. 2. Electrogenic histamine efflux. Potassium diffusion potentials were imposed on histamine-loaded membrane vesicles. The conditions were as follows: no Δψ, potassium-loaded vesicles diluted into potassium buffer; Δψ, inside negative, potassium-loaded vesicles diluted into sodium buffer; Δψ, inside positive, sodium-loaded vesicles diluted into potassium buffer. The procedures and buffers are described in Materials and Methods. The pH was 5.

mine efflux, is an electrogenic process in which positive charges are translocated across the membrane.

To determine whether histidine uptake and histamine efflux are coupled processes mediated by an antiport system, the effect of external histidine and internal histamine on histamine efflux and histidine uptake, respectively, was studied. Experiments were performed in the presence of the ionophore valinomycin to exclude possible generation of a Δψ by electrogenic histamine efflux, which might cause Δψ-dependent transport. Efflux of histamine from membrane vesicles (Fig. 4) was accelerated very much in the presence of excess external histidine. Assuming an experimental resolution of 5 s or less, the acceleration caused by histidine was at least fivefold. On the other hand, histidine uptake by proteoliposomes was stimulated by loading the liposomes with histamine (Fig. 5). In nonloaded proteoliposomes, no significant uptake of histidine could be detected. These data support a histidine/histamine antiport mechanism of transport.

The effect of Δψ on the exchange reaction was studied by monitoring histidine uptake in histamine-loaded proteoliposomes. In the presence of an inside-negative Δψ (Fig. 6), histidine uptake was lower than in the control experiment, in which no Δψ was imposed. However, an inside-positive Δψ markedly stimulated histidine uptake. Notably, the rate of histidine uptake in the presence of an inside-positive Δψ under conditions of histidine/histamine antiport (Fig. 6) is several orders of magnitude (100 to 200) higher than unidirectional histidine uptake in the presence of an inside-negative Δψ (Fig. 2). These results demonstrate that histidine/histamine antiport is an electrogenic event in which a net positive charge is translocated to the outside. Therefore, histidine/histamine antiport may directly contribute to the generation of an inside-negative Δψ.

Histidine/histamine antiport is stoichiometric. The stoichiometry of the exchange reaction was investigated in double-label experiments with proteoliposomes. The uptake of [^{14}C]histidine and the efflux of [^{3}H]histamine were simultaneously measured (Fig. 7). The histidine/histamine antiport stoichiometry was calculated from the histamine-stimulated histidine uptake and the histidine-stimulated histamine efflux, assuming that the stimulatory effects of the counter-substrate are due to exchange. The experimental histidine/histamine exchange stoichiometry was 0.77 ± 0.44 (mean ± standard deviation, calculated from the datum points of 10 s or later in Fig. 7). These data are in agreement with a mechanistic stoichiometry of 1 for histidine/histamine antiport.

DISCUSSION

Evidence that *L. buchneri* ST2A generates a proton motive force from the decarboxylation of histidine and electrogenic histidine/histamine antiport has been presented. Rapid exchange between external histidine and internal histamine is inhibited by an inside-negative Δψ and accelerated by an inside-positive Δψ. Moreover, in both the absence and presence of a Δψ, histidine uptake and histamine efflux are much faster in the presence than in the absence of the counter-substrate. The results are consistent with a histidine/histamine antiport mechanism in which a net positive charge is transported with histamine. It is not clear whether this transport system mediates only a strictly coupled histidine/histamine antiport reaction or can also catalyze uniport or proton symport of these substrates. The possibility that transport of these compounds in the absence of the counter-substrate is catalyzed by other carriers cannot be excluded. An alternative explanation would be to assume that at least

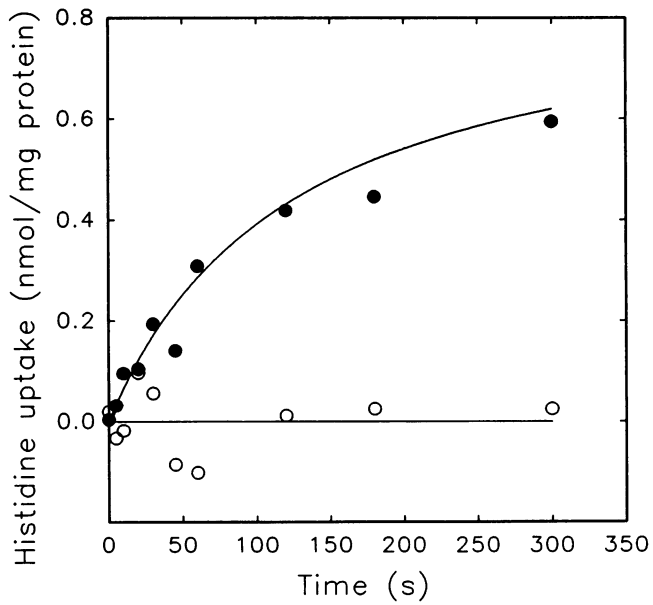

FIG. 3. Electrogenic uptake of histidine. Potassium diffusion potentials were imposed on proteoliposomes in the presence of external histidine. The conditions were as follows: no Δψ (○), potassium-loaded proteoliposomes diluted into potassium buffer; Δψ, inside negative (●), potassium-loaded proteoliposomes diluted into sodium buffer. The procedures and buffers are described in Materials and Methods. The pH was 5.

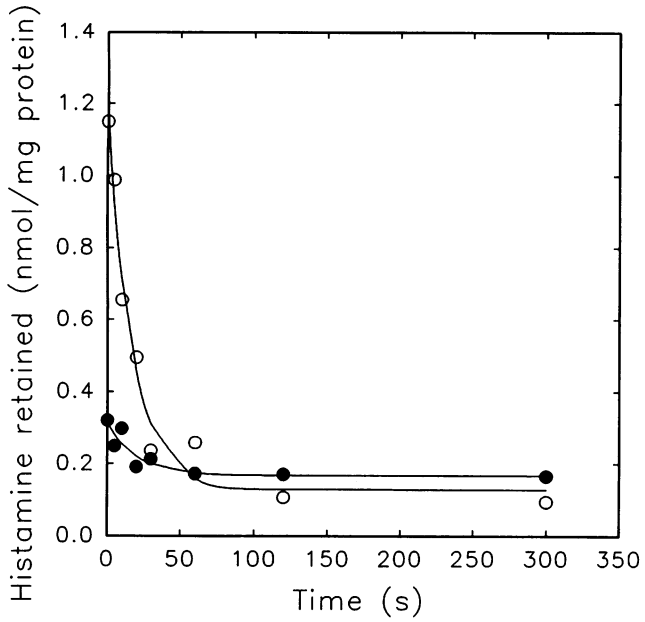

FIG. 4. Stimulation of histamine efflux by exchange with histidine. Efflux of histamine from membrane vesicles was followed in the absence (○) or presence (●) of 10 mM histidine. The pH was 5. The buffer contained 1 μM valinomycin.

part of this transport is catalyzed by the antiporter, functioning in a uni- or symport mode. For the malate/lactate antiporter of *L. lactis*, it has been shown that malate can be transported in a uni- or symport mode (18). The arginine/ornithine antiporter of *Pseudomonas aeruginosa* catalyzes both electroneutral exchange between both substrates as well as their unidirectional fluxes (26). Most secondary transport systems catalyze exchange much faster than uni-

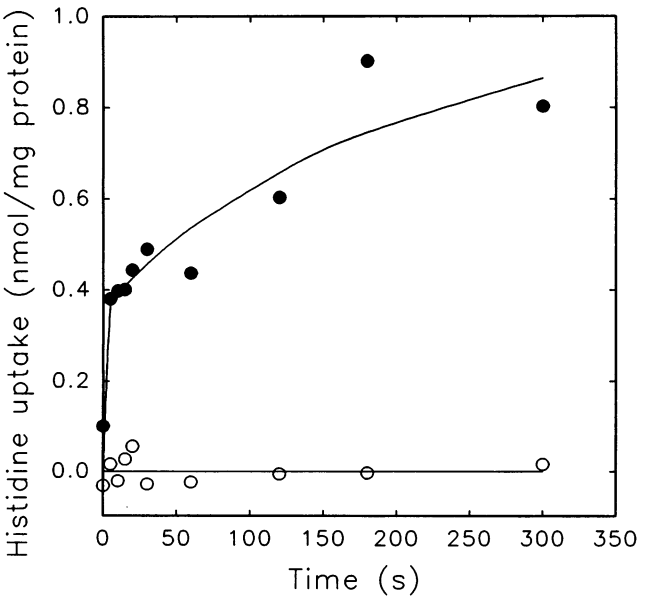

FIG. 5. Histidine uptake by exchange with histamine. Histidine uptake was monitored in control proteoliposomes (○) or in proteoliposomes loaded with 1.5 mM histamine (●). The pH was 5. The buffer contained 1 μM valinomycin.

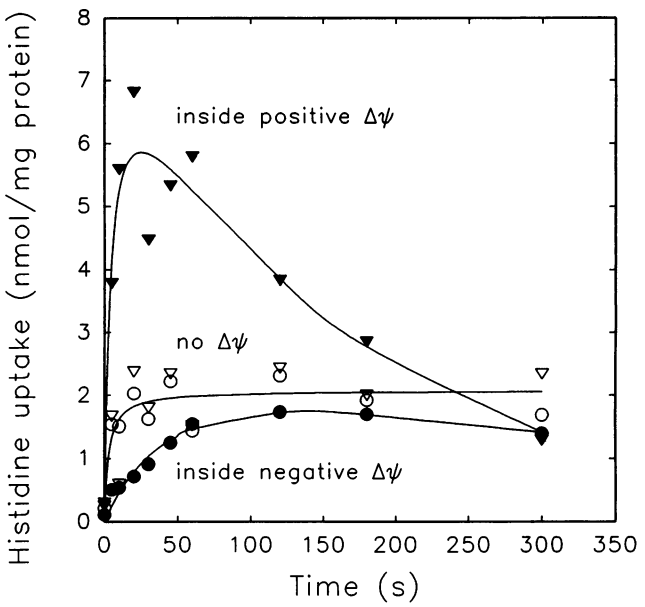

FIG. 6. The effects of the Δψ on histamine/histidine exchange. Histidine uptake in proteoliposomes loaded with 0.5 mM histamine in the presence of different potassium diffusion potentials was monitored. The conditions were as follows: no Δψ, potassium-loaded liposomes diluted into potassium buffer (○) or sodium-loaded liposomes diluted into sodium buffer (▽); Δψ, inside negative (●), potassium-loaded liposomes diluted into sodium buffer; Δψ, inside positive (▼), sodium-loaded liposomes diluted into potassium buffer. The procedures and buffers are described in Materials and Methods. The pH was 5.

or symport systems. Other transport systems, such as the arginine/ornithine antiport in *L. lactis* (4) and sugar-phosphate/phosphate antiport in various bacteria (2), are strictly coupled.

Already by the 1920s, it was suggested that decarboxylation of amino acids by microorganisms might fulfill a function protective against intracellular (and extracellular) acidification (see reference 7 and work cited therein). Many bacteria, both gram positive and negative, have since been shown to possess amino acid decarboxylases and to extrude large quantities of the corresponding amines into the medium (reviewed in reference 6; also see references 9, 20, and 21). These decarboxylases are induced in low-pH media in the late-exponential growth phase. The amino acid has to be present at high concentrations (millimolar range) in the medium. Also, suboptimal growth temperatures are, in many cases, favorable for induction. The lysine decarboxylase system has been studied in more detail with *E. coli* (12, 13). The gene coding for lysine decarboxylase is located in an operon structure together with a gene which seems to code for a transport protein. On the basis of homology with the arginine/ornithine antiporter of *P. aeruginosa* (26), this transport protein was predicted to be a lysine/cadaverine antiporter.

To explain intracellular pH regulation by decarboxylation, the following scheme is proposed (Fig. 8). Charge compensation in the decarboxylation reaction demands the consumption of a proton. The overall result will be a net consumption of protons unless the decarboxylation leads to drastic pK_a changes of the remaining amino and carboxylic groups on the substrate and to subsequent deprotonation of

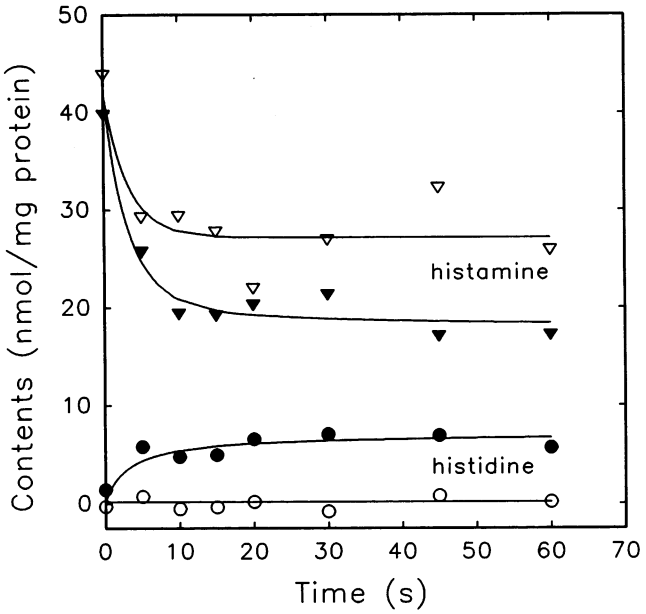

FIG. 7. Stoichiometry of histamine/histidine exchange. Histidine uptake and histamine efflux in proteoliposomes in the absence (open symbols) and presence (solid symbols) of counter-substrate were monitored. The pH was 5. The buffer contained 1 μM valinomycin.

the product. What is usually observed is that massive decarboxylation leads to alkalinization of a medium with relatively low buffer capacity. Second, independent of this, transport (i.e., the net result of substrate uptake and stoichiometric product extrusion) can result in proton consumption primarily in the cytoplasm. Only in the second instance may this lead, because of the activity of proton-coupled transporters and proton leak, to alkalinization of the medium. This is the case if transport is electrogenic, in the sense that a net positive charge is translocated to the outside (or a negative charge to the inside). The difference between the molecule transported outward and the one transported inward is then, apart from the freely diffusible CO_2, exactly one proton. Thus, transport leads to "indirect proton extrusion" (11). This automatically results in generation of a proton motive force driven by the free energy of the decarboxylation reaction. To illustrate the potential of this form of proton motive force generation, the equilibrium intracellular pH may be calculated. As a reasonable value for the standard free energy of amino acid decarboxylation, one may take −25 kJ/mol, which was recalculated from data in reference 8 concerning lysine decarboxylation. (The standard conditions are pH 7 and 1 atm [101.29 kPa] of CO_2 pressure.) The net reaction resulting from electrogenic transport, decarboxylation, and passive electrically neutral CO_2 diffusion will be

$$(\text{R-COO}^-)_o^z + \text{H}_i^+ \rightarrow (\text{R-H})_o^{z+1} + \text{CO}_{2,o}$$
$$(\Delta G_0' = -25 \text{ kJ/mol})$$

where R-COO$^-$ and R-H are the anionic form of the carboxylic acid substrate and the corresponding decarboxylated product, z is the total charge on the molecule, and i and o are the intracellular and extracellular locations, respectively. Assuming a $\Delta\psi$ of −100 mV, a partial CO_2 pressure of 0.05 atm (5.06 kPa), and equal concentrations of the amino acid and amine forms appearing in the reaction equation above, an equilibrium intracellular pH of 11 can be calculated. In reality, the reaction will not proceed to equilibration because of inward proton fluxes or decreasing decarboxylation rates. In fact, kinetic mechanisms may exist that have been selected during evolution to slow down the reaction far from equilibrium, when attaining physiological pH. For example, the decarboxylases studied by Gale (6) had optimal activity at a pH between 4.5 and 6. Also not taken into account is the effect of the extracellular pH on the concentration of the anionic form of the carboxylic acid. The protonation of the anionic form on the outside will reduce its concentration and hence decrease the equilibrium intracellular pH.

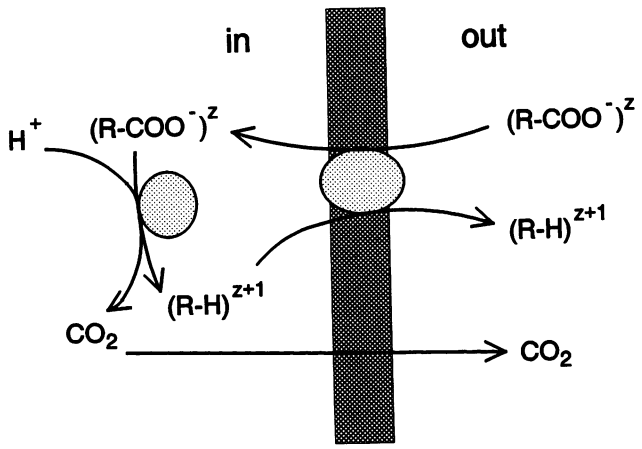

FIG. 8. Generalized scheme of proton motive force generation by decarboxylation and electrogenic antiport. R-COO$^-$ and R-H are the carboxylic acid and the decarboxylated product, respectively. The particles which are recognized and exchanged by the carrier or antiporter are the carboxylic acid with charge z and the decarboxylated product with charge $z + 1$. It is assumed that the carboxylic carbon leaves the cell as neutral CO_2 or H_2CO_3. Consequently, a net charge is transported to the outside, corresponding to the proton consumed in the cell. The particular state of R-COO$^-$ protonation recognized by the decarboxylase is irrelevant to the overall reaction.

Depending on the organism and the conditions, the primary goal of amino acid decarboxylation may be to prolong the period in which a sufficiently high intracellular pH is present or as a source of metabolic energy. In the case of histidine decarboxylation by *L. buchneri* ST2A, this is accomplished by an electrogenic antiport mechanism, whereby amino acid and amine are transported by one carrier in the exchange mode. It is likely that other amino acid decarboxylation reactions described in the literature (6, 9, 12, 20, 21, 24), in which the amine is excreted in large quantities, are involved in generation of metabolic energy or maintenance of pH by using such a transport mechanism as just described.

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