Exchange of Aspartate and Alanine

MECHANISM FOR DEVELOPMENT OF A PROTON-MOTIVE FORCE IN BACTERIA*

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We examined the idea that aspartate metabolism by Lactobacillus subsp. M3 is organized as a proton-motive metabolic cycle by using reconstitution to monitor the activity of the carrier, termed AspT, expected to carry out the electrogenic exchange of precursor (aspartate) and product (alanine). Membranes of Lactobacillus subsp. M3 were extracted with 1.25% octyl glucoside in the presence of 0.4% Escherichia coli phospholipid and 20% glycerol. The extracts were then used to prepare proteoliposomes loaded with either aspartate or alanine. Aspartate-loaded proteoliposomes accumulated [3H]aspartate in a heterologous antiport reaction that was stimulated or inhibited by an inside-positive or inside-negative membrane potential, respectively. Several lines of evidence suggest that these homologous and heterologous exchange reactions were catalyzed by the same functional unit. Thus, [3H]aspartate taken up by AspT during self-exchange was released by a delayed addition of alanine. In addition, the spontaneous loss of AspT activity that occurs when a detergent extract is held at 37°C prior to reconstitution was prevented by the presence of either aspartate (Kₐ = 0.4 mM) or alanine (Kₐ(alanine) 10 mM), indicating that both substrates interact directly with AspT. These findings are consistent with operation of a proton-motive metabolic cycle during aspartate metabolism by Lactobacillus subsp. M3.

Nutrient transport by bacteria is usually thought of as consuming metabolic energy, since this step is typically driven by an ion-motive gradient (e.g., ΔΨm, or ΔρNa⁺) or by hydrolysis of a phosphoester bond (e.g., ATP or PEP) (1, 2). Recently, however, a new class of nutrient transport reactions has been identified, one in which substrate transport is actually used to generate rather than consume energy. The first and best understood of these reactions is found in Oxalobacter formigenes (3, 4), a Gram-negative, obligate anaerobe that exploits the decarboxylation of oxalate to support transmembrane ion-motive gradients (5). This cell mediates the exchange of divalent oxalate with the product of its intracellular decarboxylation, monovalent formate (6), using a membrane transporter named OxIT (4). The one-for-one exchange of oxalate²⁻ and formate¹⁻ polarizes the membrane (electrically negative, inside), while the decarboxylation reaction serves to generate an internal alkalinity, since a single cytosolic proton is consumed during production of formate. As a result, the metabolic sequence, oxalate entry, oxalate decarboxylation, formate exit, acts as a proton pump (3) or “proton-motive metabolic cycle” (Refs. 3 and 4; reviewed in Ref. 7). In the same way and in other bacteria, the transport (vectorial) and decarboxylation (scalar) reactions associated with conversion of malate to lactate (8–10) or histidine to histamine (11) have been shown to act as proton-motive metabolic cycles.

Such precedents suggest a new way of interpreting the relationship between anion transport and decarboxylation reactions in microorganisms. For example, some strains of the Lactobacilli catalyze the decarboxylation of either L-aspartate³⁻ or L-glutamate⁴⁻, with a near-stoichiometric release of the products, L-alanine or γ-aminobutyrate (and CO₂), respectively¹⁻². These decarboxylations support ATP synthesis in a manner consistent with the idea that processing of these anions involves a proton-motive metabolic cycle¹⁻² (and see below).

As their central element, proton-motive metabolic cycles have a vectorial component(s) that mediates the electrogenic exchange of precursor and product. Accordingly, the specific goal of work reported here was to determine whether this transport reaction is found in Lactobacilli subsp. M3, a cell which readily converts aspartate to alanine by intracellular decarboxylation. To approach this issue, we used reconstitution of membrane protein as an analytical tool to probe for the expected exchange of aspartate and alanine in proteoliposomes. Our experiments document that membranes of Lactobacilli subsp. M3 display an electrogenic aspartate:alanine exchange of the sort required by a proton-motive metabolic cycle. This precursor:product antiport is catalyzed by a single element, termed “AspT” (for aspartate transporter), which catalyzes both the homologous self-exchange of aspartate and the heterologous antiport of aspartate and alanine.

**EXPERIMENTAL PROCEDURES**

Organism, Growth Conditions, and Preparation of Membrane Vesicles—Lactobacillus subsp. M3 was grown under anaerobic conditions at 30°C in MRS broth (Difco) supplemented with 30 mM L-aspartate and 5% sodium chloride. After growth to stationary phase (~3 days), cells were harvested by centrifugation, washed with 100 mM potassium phosphate (pH 7), and membrane vesicles were prepared by high pressure lysis in the presence of 100 mM potassium phosphate (pH 7), as described (12); vesicles were stored at −70°C as a concentrated stock (10–20 mg/ml protein).

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Solubilization and Reconstitution of AspT—Membrane vesicles (1–2 mg of protein) were solubilized (12), using 1.25% (w/v) octylglucoside in the presence of 0.4% (w/v) acetonitrile washed E. coli phospholipid, 100 mM potassium phosphate (pH 7), 4 mM MgSO4, 1 mM dithiothreitol, 0.75 mM phenylmethylsulfonyl fluoride, and 20% glycerol. Control extracts were prepared in the same way, but without added protein.

Reconstitution was in a final volume of 1 mL, using 400 µL of detergent extract (or control lipid extract), 130 µL of bath-sonicated liposomes (5.9 mg of E. coli phospholipid), 18 µL of 15% octylglucoside, with the balance comprised of 100 mM phosphate (pH 7) as the potassium or NMG3 salt, and 1 mM dithiothreitol. After 20 min on ice, proteoliposomes (or control liposomes) were formed at 23 °C by rapid injection into 20 mL of a loading buffer containing 100 mM potassium phosphate (pH 7) and 1 mM dithiothreitol, along with 25–150 mM aspartate (potassium, or NMG salts, as specified). After a further 20 min, the substrate-loaded proteoliposomes (or liposomes) were recovered by centrifugations and washings (12), with resuspension in 100 mM potassium or NMG sulfate plus 100 mM potassium or NMG-phosphate (pH 7) and 1 mM dithiothreitol. The final resuspension volume was usually 300 µL, giving protein and lipid at ~50–250 µg/ml and 13 mg/ml, respectively (12). When proteoliposomes (liposomes) were loaded with 100 mM alanine, the same procedure was followed except that the buffer for washing and resuspension was the same as the loading buffer, and the resuspension volume was reduced to 80 µL.

Assays of Transport—For assays of 3H-aspartate transport by substrate-loaded particles, proteoliposomes were diluted 20-fold from their concentrated stock into an appropriate volume of assay buffer (resuspension buffer lacking dithiothreitol) along with other required materials. After 1–3-min preincubation at 23 °C, labeled substrate was added to a nominal concentration of 100 µM, and at the required times aliquots of 100 µL, corresponding to about 0.065 µL internal volume (12), were removed for membrane filtration (0.22-µm pore sized GSTF Millipore filters), followed by two washes with 5 mL of assay buffer (12). For transport assays of alanine-loaded proteoliposomes, or those prepared at pH 8, proteoliposomes were diluted 133-fold into assay buffer containing labeled substrate.

A simplified assay (13, 14) was used to monitor AspT activity during tests of its stabilization, in vitro, by substrate. In these experiments, a detergent extract (250–450 µg of protein/ml) was placed at 37 °C, along with desired additives. To quench the reaction, a 100-µL aliquot was removed and placed in a chilled tube containing 100 µL of 20 mM potassium aspartate and other components required for reconstitution (above), using amounts scaled to a final volume of 250 mM. Bath-sonicated liposomes (1.36 mg) were added after 5 min, and the mixture was allowed to remain on ice for 20 min before adding 5 mL of a solution of 100 mM potassium phosphate (pH 7) plus 100 mM potassium aspartate to form substrate-loaded proteoliposomes. To assess transport, duplicate 0.2-mL aliquots were then applied, under vacuum, to the center of GSTF Millipore filters (0.22-µm pore size). External aspartate was removed by two 5-mL rinses with assay buffer, and after release of the vacuum, the reaction was initiated by overlaying proteoliposomes, on the filter, with 0.3 mL of this same buffer containing 100 µM 3H-aspartate. The reaction was terminated by vacuum filtration after the exchange reaction had reached its steady state (10 min); this was followed by the quick rinses with buffer to remove residual external radioactivity.

Other Assays—Protein was measured using a modification of method of Schaffner and Weissman (15). ATP in boiled cell extracts was determined with the firefly assay, as described by Mason et al. (16).

Chemicals—[2,3-3H]Aspartate acid (26.3 Ci/mmol) was purchased from DuPont NEN Corp. Phospholipid was purified from the crude E. coli lipid provided by Avanti Polar Lipids, Inc. (12). Octylglucoside was from Boehringer Mannheim.

RESULTS

Aspartate-dependent ATP Production by Intact Cells—In Gram-positive anaerobes such as the Lactobacilli, the proton-coupled F0F1-ATPase hydrolyzes ATP made during fermentation of sugars or amino acids to generate a proton-motive force. As a consequence, ATP levels do not change when such cells are treated with an ATPase inhibitor (e.g. DCCD) or with proton ionophores (e.g. CCCP, or the combination of nigericin and valinomycin) (17–20). It was unexpected, therefore, that these inhibitors strongly reduced ATP synthesis associated with the decarboxylation of aspartate and production of alanine plus carbon dioxide in Lactobacillus subspecies M3 (Table I). By contrast, this inhibitor sensitivity is understandable if aspartate decarboxylation is organized as a proton-motive metabolic cycle (3, 4, 7) involving the antiport of precursor (aspartate) and product (alanine), since in that case ATP synthesis would arise from reversal of the ATPase. With this possibility in mind, we used reconstitution of membrane protein to probe for the presence of a carrier which could mediate the required antiport reaction.

Identification and Characterization of the Aspartate Self-Exchange Reaction—To identify the putative aspartate:alanine exchange carrier, AspT, our initial focus was a study of substrate-loaded proteoliposomes, with the expectation that AspT would display an aspartate self-exchange. For this reason, proteoliposomes were loaded with 100 mM aspartate and suspended in a sulfate-based medium to which 100 µM external [3H]aspartate was added. For these general conditions, we found substantial transport of the labeled substrate. In the experiment shown (Fig. 1), the steady state incorporation of [3H]aspartate was approximately 500 nmol/mg of protein. Assuming [3H]aspartate had been taken up uniformly by all proteoliposomes (but see below), this corresponded to a 30-fold accumulation of substrate over its concentration in the me-
Moreover, the incorporated material was readily chased by a later addition of excess unlabeled aspartate, as expected if [3H]aspartate had been taken up by an exchange reaction. And since accumulated [3H]aspartate was released by either aspartate or alanine (Fig. 1), it seemed feasible that both compounds served as substrates. No accumulation of [3H]aspartate was observed when liposomes (no protein) were tested (Fig. 1), nor did aspartate accumulate in proteoliposomes in which internal sulfate replaced aspartate (not shown in Fig. 1; see Fig. 5). In other experiments of this sort we found transport of [3H]aspartate to be unaffected by the presence of the potassium ionophore, valinomycin (1 μM), or by addition of the protonophore, carbonyl cyanide carboxymethoxyphenylhydrazone (1 μM) (data not shown). Nor was [3H]aspartate transport influenced by substitution of sodium for potassium or by the presence of Mg2+ or EDTA (each at 10 mM; data not given). Based on these observations, we concluded that membranes of Lactobacillus subsp. M3 contain a carrier capable of mediating an aspartate self-exchange, as expected of AspT, and that this reaction is an electroneutral event operating independently of coupling cations such as Na+ or Mg2+.

The presence of an aspartate-linked antiporter was also supported by an analysis of how steady state levels of [3H]aspartate accumulation were influenced by the relative sizes of the internal and external aspartate pools. For example, [3H]aspartate incorporation increased in direct proportion to elevation of internal substrate concentration (Fig. 2A). We also addressed this issue quantitatively by experiments in which [3H]aspartate transport was monitored as the external pool was expanded by known amounts (Fig. 2B). This led to predictably increased ratios of external to internal [3H]aspartate and that relationship was used to calculate an aspartate-accessible internal mass of 5.3 ± 1.3 nmol/mg lipid (Fig. 2, legend). Given an overall internal volume of about 1 μl/mg lipid (12, 21) and the internal aspartate concentration of 100 mM, the total internal aspartate pool should be about 100 nmol/mg lipid (12). Therefore, the observed accessible mass (5.3 nmol/mg lipid) indicates that only a small fraction (~5%) of proteoliposomes carried out the aspartate self-exchange reaction. This level of activity was typical of the work reported here, and for this reason, presuming a random distribution of AspT among proteoliposomes, we concluded that no single proteoliposome contained more that one functional unit of AspT.

We performed two additional experiments to characterize more directly the interaction between AspT and its substrate, aspartate. In one case, we undertook a simple kinetic study, relying on samples filtered after 10 s to estimate initial velocities (Fig. 3). In that experiment, we found that the self-exchange reaction had a Michaelis constant (Km) of 0.36 ± 0.03 mM and a maximal velocity of 0.40 ± 0.03 μmol/min/mg of protein (means ± S.E.).

We also obtained a direct measurement of the dissociation constant, Kd(aspartate), by monitoring the kinetics with which aspartate stabilized solubilized AspT. When a detergent extract was placed at 37 °C, reconstitution at periodic intervals showed that recoverable AspT activity decayed in an exponential fashion, with a half-life of about 10 s (0.15 min) (Fig. 4). Added substrate was clearly protective, and AspT lifetime was more stable than the unliganded carrier, a realistic view given the observed response to excess aspartate (Fig. 4), the stabili-
of [3H]aspartate movement (from 7.9 to 15.1 nmol/15 s). In internally positive potential was present, there was a stimulation achieved by substrate can be analyzed quantitatively to
derive the dissociation constant of the AspT-aspartate complex
with external or internal potassium to generate a membrane
potential that should accelerate or retard such an electrogenic
movement of aspartate at 2.5 mM (K\text{aspartate}) by the following expression,
\[ R = 1 + (S)/(K_d \text{[aspartate]}) \] (Eq. 1)
where (S) represents the aspartate concentration used for sta-
fabilization. For the aspartate levels tested here (2.5–20 mM), this relationship suggests a K_d (aspartate) of 0.3 ± 0.02 mM
(Fig. 4, legend).

Heterologous Exchange Catalyzed by AspT—Intact cells of
Lactobacillus subsp. M3 convert aspartate to alanine plus CO_2, and if this reflects operation of a proton-motive metabolic cycle, it seemed possible that AspT would carry out the heterologous exchange of aspartate and alanine. This was implied by the finding that substrate taken up during aspartate self-exchange was released by added aspartate or alanine (Fig. 1). Since conversion of aspartate to alanine is very nearly stoichiometric in Lactobacillus subsp. M3, one further expects the aspartate:alanine exchange ratio to be a one-for-one antiport, and for that case one anticipates that the reaction will be electrogenic, since near physiological pH (pH 7) the net charge on aspartate is -1
\((pK_1(COOH) = 2.09, pK_2(NH_3^+) = 9.82, pK_3(COOH) = 3.86)\), while that on alanine is 0 \(pK_2(COOH) = 2.34, pK_2(NH_3^+) = 9.69)\). This view was examined in studies of aspartate transport by alanine-loaded proteoliposomes, using valinomycin along with external or internal potassium to generate a membrane potential that should accelerate or retard such an electrogenic reaction. In such experiments (Fig. 5), control proteoliposomes were prepared and assayed using NMG as the internal and external cation. These controls, whose behavior was largely unaffected by valinomycin, took up \(^{[3}H\text{[aspartate]}\) at an initial rate of about 2.2–2.8 nmol/15 s/mg of protein (Fig. 5; Table II). Note, however, that when proteoliposomes were made with internal potassium, so that an internally negative potential was established in the presence of valinomycin, there was profound inhibition of \(^{[3}H\text{[aspartate]}\) transport (from 2.7 to 0.1 nmol/15 s/mg of protein), while in the reciprocal trial, when an internally positive potential was present, there was a stimulation of \(^{[3}H\text{[aspartate]}\) movement (from 7.9 to 15.1 nmol/15 s). In addition, the presence of external, but not internal, potassium

![Fig. 4. Substrate protection of solubilized AspT. A detergent extract (342 μg of protein/ml) was placed at 37 °C in the absence (C) and presence of aspartate at 2.5 mM (A), 5 mM (D), 10 mM (I), and 20 mM (A). To follow the decay of AspT, aliquots were removed at the indicated times and placed in chilled quench tubes containing 20 mM aspartate along with the other components required for later reconstitution. After reconstitution, proteoliposomes were tested for residual AspT activity by the abbreviated assay (see "Experimental Procedures"). In the absence of added substrate, recoverable AspT activity disappeared with a half-life of 0.15 min. Aspartate at 2.5 mM, 5 mM, 10 mM, and 20 mM gave half-lives of 1.25, 2.7, 5.5, and 11 min, respectively, and from Equation 1 (see text), these predicted corresponding K_d (aspartate) values of 0.34, 0.23, 0.16, and 0.08 nmol/mg protein).](Image 74x594 to 281x732)

![Fig. 5. The electrogenic nature of aspartate alanine exchange. Proteoliposomes or liposomes were loaded with 100 mM alanine plus 100 mM phosphate (pH 7) as either the NMG (C, D, I) or potassium (C) salts; with one exception (I), 1 μM valinomycin (Val) was also present. Control liposomes and proteoliposomes were assayed using the potassium-based medium, with valinomycin. These controls included liposomes prepared with either NMG or potassium salts of alanine and proteoliposomes containing 75 mM NMG sulfate rather than 100 mM NMG alanine; because these controls gave no significant aspartate transport, they are not individually noted in the figure for reasons of clarity. Aspartate transport was determined for all combinations of potassium or NMG alanine-loaded proteoliposomes using potassium- or NMG-based assay media, with and without valinomycin; data not shown here are included in Table II.](Image 332x593 to 538x732)

**Table II.** Voltage dependence of aspartate transport by alanine-loaded proteoliposomes

| External and internal cation | Aspartate transport |
|-----------------------------|---------------------|
|                             | No valinomycin | Plus valinomycin |
| External NMG                |                 |                 |
| Internal NMG                | 2.8 ± 0.6       | 2.2 ± 0.1       |
| Internal potassium          | 2.7 ± 0.7       | 0.1 ± 0.1       |
| External potassium          |                 |                 |
| Internal NMG                | 7.9 ± 1.3       | 15.1 ± 2.9      |
| Internal potassium          | 8.2 ± 1.5       | 12.0 ± 3.0      |

Data are mean values ± S.E. for the transport of \(^{[3}H\text{[aspartate]}\) by alanine-loaded proteoliposomes, treated with 1 μM valinomycin or ethanol, as described in the legend to Fig. 5B. All parameters were evaluated in three independent experiments.

These results are consistent with a model that aspartate transport is mediated by a heterologous exchange process, where the aspartate:alanine exchange ratio is a one-for-one antiport. However, the presence of external, but not internal, potassium appeared to stimulate the initial phases of aspartate transport (from 2.7 to 7.9 nmol/15 s; Table II), although the reasons for this are unclear. A similar finding was made during reconstitution of the oxalate:formate heterologous exchange from O. formigenes (3), but potassium dependence was not evident for the purified OxlT protein (4). It seems unlikely that aspartate transport is mediated by a heterologous exchange process, where the aspartate:alanine exchange ratio is a one-for-one antiport. However, the presence of external, but not internal, potassium appeared to stimulate the initial phases of aspartate transport (from 2.7 to 7.9 nmol/15 s; Table II), although the reasons for this are unclear. A similar finding was made during reconstitution of the oxalate:formate heterologous exchange from O. formigenes (3), but potassium dependence was not evident for the purified OxlT protein (4). It seems unlikely that aspartate transport is mediated by a heterologous exchange process, where the aspartate:alanine exchange ratio is a one-for-one antiport. However, the presence of external, but not internal, potassium appeared to stimulate the initial phases of aspartate transport (from 2.7 to 7.9 nmol/15 s; Table II), although the reasons for this are unclear. A similar finding was made during reconstitution of the oxalate:formate heterologous exchange from O. formigenes (3), but potassium dependence was not evident for the purified OxlT protein (4).
from 100 to 30 mM. In that case, basal and stimulated rates for proteoliposomes loaded with 100 mM alanine were comparable with those found earlier (12 ± 0.7 and 23 ± 1.3 nmol/15 s), as was the observed 1.9-fold rate stimulation by membrane potential. When internal alanine was reduced to 30 mM, we recorded a lower basal rate of [3H]aspartate transport, but the same stimulated value (5.6 ± 0.7 versus 21 ± 2.1 nmol/15 s), and a correspondingly increased (3.7-fold) stimulation by membrane potential. These supplemental experiments, together with those described earlier (Fig. 5 and Table II), strongly suggest that aspartate:alanine antiport is an electrogenic reaction in which net negative charge moves in parallel with aspartate.

Alternate Substrates of AspT—Having documented that AspT accepts both aspartate and alanine, our concluding work centered on identification of other possible substrates, using two different approaches. In one kind of experiment, we evaluated putative substrates by asking whether [3H]aspartate was taken up by substrate-loaded proteoliposomes (e.g. Fig. 5). Thus, proteoliposomes were loaded with the NMG salts of alanine, β-alanine, glycine, or glutamate (100 mM in each case) and then suspended in the usual assay buffer (i.e. K2SO4 plus potassium phosphate) in the presence of 1 µM valinomycin and 100 µM [3H]aspartate. In each case, proteoliposomes accumulated labeled [3H]aspartate, and in each case a later addition of unlabeled aspartate evoked a rapid discharge of the internalized material (not shown). A comparison of initial velocities of [3H]aspartate transport indicated an order of preference as alanine > glycine > β-alanine > glutamate (rates in the ratios of 1.0, 0.67, 0.37, and 0.04, respectively). By contrast, [3H]aspartate accumulation was not found for proteoliposomes prepared with internal acetate, butyrate, formate, γ-aminobutyric acid, malonate, oxalate, propionate, or pyruvate (each at 100 mM); nor did the addition of these compounds at 30 mM affect the aspartate self-exchange reaction (not shown). From this work, we concluded that along with aspartate and alanine, β-alanine, glycine, and glutamate might serve as substrates for AspT, and that AspT accepts substrates with an amino function in the α or β, but not γ position. In further work we confirmed that these alternate substrates interacted directly with AspT by showing that their presence stabilized the solubilized protein (experiments similar to that of Fig. 4). Thus, when added at 20 mM, alanine, glycine, and β-alanine gave an increase in AspT lifetime comparable with the effect of about 0.65 mM aspartate. This implies that these substrates have a relatively low affinity for AspT (cf. Equation 1) and places their Kd values at ~10 mM. In similar trials, glutamate gave some slight protection, which was not quantitated, while γ-aminobutyric acid did not.

**DISCUSSION**

In Lactobacillus subsp. M3, the inhibitor sensitivity of ATP synthesis associated with aspartate metabolism suggests operation of a proton-motive metabolic cycle (Table I) involving the exchange of the precursor, aspartate, and its decarboxylation product, alanine. To test this idea we used reconstitution to characterize [3H]aspartate transport in this cell and searched for the two reactions likely to characterize the hypothetical antiporter, AspT: the exchange of aspartate with itself in aspartate-loaded proteoliposomes and the heterologous exchange of aspartate and alanine, as tested in alanine-loaded proteoliposomes. Both reactions were demonstrable (e.g. Figs. 1 and 5) for conditions in which only a small fraction (~5%) of the proteoliposomal population contained an exchange carrier. And since [3H]aspartate taken up by aspartate- or alanine-loaded particles was released by adding an excess of ether substrate (e.g. Fig. 1), we conclude a single exchange carrier, AspT, carries out both homologous and heterologous antiport reactions. Equally important, by examining the effect of imposed electrical potential on the heterologous exchange (Fig. 5), it could be shown that negative charge moves in the same direction as aspartate. Given the relevant pK values of the carboxyl and amino groups on these substrates (see above), the heterologous...
exchange catalyzed by AspT likely involves movement of aspartate\(^1\) against alanine\(^2\); by extension, the aspartate self-exchange is probably based on movements of aspartate\(^1\).

The diagram of Fig. 6 summarizes the proposed proton-motive cycle in Lactobacillus subsp. M3 and in other cells that use decarboxylation to convert aspartate into alanine and CO\(_2\). In this model, entry of negatively charged aspartate is followed by its intracellular decarboxylation in a reaction that consumes a single cytosolic proton. The products of this decarboxylation, CO\(_2\) and alanine, are assumed to leave the cell by different routes. Owing to its small size and high lipid solubility, we presume that CO\(_2\) moves outward by passive diffusion through the lipid bilayer. Alanine, however, with its more limited capacity for passive diffusion, requires a specific efflux pathway, and this is provided by AspT itself. In the steady state, then, the result would be a proton-motive cycle in which the vectorial component (AspT) catalyzes import of a single negative charge, while the scalar reaction (decarboxylation) ensures the stoichiometric disappearance of a single internal proton. This association of vectorial and scalar elements resembles that described earlier for O. formigenes (3, 4), but the biochemical nature of the individual proteins differs considerably in the two organisms. Thus, the antiporters, OxlT and AspT, are distinguished by both kinetic behavior and substrate specificity (3, 4), while the decarboxylation reactions differ in their use of cofactor, coenzyme A in O. formigenes (6), but pyridoxal 5′-phosphate in L. subsp. M3.\(^1\)

Our experiments indicate this thermodynamic proton pump (Fig. 6) will characterize the steady state during aspartate metabolism by Lactobacillus subsp. M3. On the other hand, the discrepancy between the affinity of AspT for aspartate and alanine (0.3 mM and \(\geq 10\) mM, respectively) suggests a proton-motive cycle may not come into play until internal alanine rises to an appropriately high value. Therefore, in the pre-steady state, we suggest that AspT mediates the electroneutral exchange of aspartate with hydroxyl ion, or the equivalent, H\(^+\) aspartate symport,\(^4\) whereby ensuring continued influx of aspartate until decarboxylation expands the alanine pool to a suitable size. In this way, AspT could also provide aspartate as a substrate for conventional metabolic pathways or for biosynthetic purposes.

Analysis of anion transport and exchange in O. formigenes provided the first example of a proton-motive metabolic cycle (3, 4), and subsequent work (8–11, 23) has identified additional cycles in both Gram-negative and Gram-positive forms (reviewed in Refs. 7 and 24). For the most part, these examples are linked to decarboxylations (e.g. Fig. 6) (3, 8–11, 23), although it has been clear that more complex metabolic ensembles may be similarly structured. Indeed, among the lactic acid bacteria one now finds useful models of both types. Thus, there is evidence that the processing of malate (8–10), histidine (11), and aspartate (this work) offer cases in which a simple metabolic sequence is arranged so as to generate a proton-motive force by combining physically separated vectorial and scalar events. A more complex, ensemble model is found in Leuconostoc oenos (23), where entry of the anion, citrate\(^1\), is eventually coupled to a steady state proton-motive cycle by a subsequent proton-consuming metabolism. These few precedents suggest we are at the initial stages of understanding such emergent cycles and that it may be useful to consider wider application of this principle in cell biology (3, 4, 7).

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