Exchange of Glutamate and γ-Aminobutyrate in a Lactobacillus Strain

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Lactobacillus sp. strain E1 catalyzed the decarboxylation of glutamate (Glu), resulting in a nearly stoichiometric release of the products γ-aminobutyrate (GABA) and CO₂. This decarboxylation was associated with the net synthesis of ATP. ATP synthesis was inhibited almost completely by nigericin and about 70% by N,N′-dicyclohexylcarbodiimide (DCCD), without inhibition of the decarboxylation. These findings are consistent with the possibility that a proton motive force arises from the cytoplasmic proton consumption that accompanies glutamate decarboxylation and the electrogenic Glu/GABA antiporter and the possibility that this proton motive force is coupled with ATP synthesis by DCCD-sensitive ATPase.

Recently, a new class of nutrient transport reactions, one in which substrate transport is actually used to generate rather than consume energy, has been identified. The first and best understood of these reactions is found in Oxalobacter formigenes (3, 13), an organism that exploits oxalate decarboxylation to sustain transmembrane ion motive gradients generated as a result of the one-for-one exchange of oxalate2⁻ and formate⁻ (2, 4). In the same way and in other bacteria, the transport and decarboxylation reactions of several carboxylic acids, including amino acids, have been shown to act as proton motive metabolic cycles (8, 9, 11, 12, 14).

Some strains of lactobacilli catalyze the decarboxylation of glutamate, resulting in the stoichiometric release of the end products γ-aminobutyrate (GABA) and CO₂ (7). In this report we show that this decarboxylation process can be coupled with energy production, consistent with the possibility that the processing of glutamate involves a proton motive metabolic cycle resembling those described above.

Lactobacillus sp. strain E1 was isolated as a contaminant from a low-salt soy sauce carbonated by glutamate decarboxylation. Cells were grown at 30°C in a broth (pH 5) containing the following ingredients: 10% soy sauce (Kikkoman, San Francisco, Calif.), 0.3% yeast extract (Difco, Detroit, Mich.), 1% glucose, 3.3% NaCl (to give a final NaCl concentration of 5%), 50 mM sodium glutamate, and 0.001% pyridoxine hydrochloride. After growth to stationary phase (72 h), cells were harvested by centrifugation, washed twice and resuspended in MM buffer (100 mM MES [morpholineethanesulfonic acid]-KOH buffer with 2 mM MgSO₄ [pH 5.0]).

ATP production associated with glutamate decarboxylation was examined as described previously (1), with a modification. For starvation, 0.2 ml of a washed-cell suspension (24 mg/ml) was added to 1.6 ml of MM buffer and incubated for 15 min at 30°C. After starvation, 0.2 ml of 100 mM potassium glutamate or KCl in MM buffer was added and incubation at 30°C was continued. When ionophores were used, they were added to starved cells 1 min prior to the addition of glutamate. For extraction of intracellular ATP, 0.2 ml of the reaction mixture was removed at various times and extracted in 1.8 ml of 40 mM HEPES buffer (pH 7.75) at 100°C for 5 min. ATP content was then determined by a modification of the method of Fromme and Graber (6), with a firefly ATP assay kit (Kikkoman, Noda, Japan).

Glutamate decarboxylation was monitored by appearance of the product GABA as follows. After incubation of cells or cell extracts with glutamate (above), the reaction was terminated by boiling for 5 min. The solution was then clarified by centrifugation, and the concentration of GABA in the supernatant was determined by a modification of the method of Okada and Shimada (10), without NADP oxidation and NADPH cycling reagent. The assay mixture contained 0.3 M Tris buffer (pH 8.9), 10 mM α-ketoglutarate, 2 mM 2-mercaptoethanol, 0.5 mM NADP, and 250 mU of GABase (a mixture of 4-aminobutyrate:2-oxoglutarate aminotransferase and succinate-semialdehyde:NAD[P] oxidoreductase; Boehringer GmbH, Mannheim, Germany) per ml. The appearance and concentration of NADPH were monitored at 340 nm (37°C).

To test whether glutamate decarboxylation was associated with net ATP synthesis, intracellular ATP levels were measured in the presence or absence of glutamate. The addition of potassium glutamate but not KCl gave an increase of intracellular ATP (Fig. 1), suggesting that ATP synthesis was associated with glutamate decarboxylation.

To elucidate whether ATP synthesis during glutamate decarboxylation required a proton motive force, we examined the effects of the ionophores nigericin (2 μM) and valinomycin (2 μM). ATP production was inhibited almost completely by 2 μM nigericin but not inhibited by 2 μM valinomycin (Table 1). Decarboxylation was not inhibited by either ionophore. These data suggest that a pH gradient (ΔpH) across the cytoplasmic membrane was necessary for ATP synthesis.

We also examined the effect of N,N′-dicyclohexylcarbodiimide (DCCD), an inhibitor of F₁F₀ ATPase, on ATP synthesis. When starved cells were incubated with 0.5 mM DCCD at 4°C overnight, 64% of ATP production coupled with glutamate decarboxylation was inhibited, whereas decarboxylase activity was not inhibited.

In separate experiments, we found that the presence of a high concentration of external GABA reduced the rate of ATP synthesis associated with glutamate decarboxylation (Table 1). A similar phenomenon had been observed in Lactobacillus sp. strain M3, in which excess extracellular alanine (>300 mM) inhibited ATP synthesis driven by the aspartate/alanine proton motive metabolic cycle (1). This suggests, then, that GABA...
Lactobacillus sp. strain E1. Resting cells were resuspended in 100 mM MES buffer (pH 5.0) containing 2 mM MgSO₄. To start the experiment, 10 mM tamate decarboxylation was not driven by Na⁺ not shown), we concluded that ATP synthesis coupled to glutamate decarboxylase of E1 was localized in the cytoplasm (data not shown). Since avidin did not inhibit a sodium pump. In such cases, the enzyme is inhibited by a organic acids by a membrane-bound decarboxylase that acts as a sodium pump. In such cases, the enzyme is inhibited by a biotin enzyme inhibitor, avidin (5). Since avidin did not inhibit the electrogenic aspartate/alanine antiporter. The free outward diffusion of CO₂ makes the overall cycle effectively irreversible.

Some bacteria derive energy from the decarboxylation of organic acids by a membrane-bound decarboxylase that acts as a sodium pump. In such cases, the enzyme is inhibited by a biotin enzyme inhibitor, avidin (5). Since avidin did not inhibit glutamate decarboxylase activity of E1, and since the glutamate decarboxylase of E1 was localized in the cytoplasm (data not shown), we concluded that ATP synthesis coupled to glutamate decarboxylation was not driven by Na⁺ motive decarboxylase.

These results support the idea that glutamate decarboxylation generates energy required for ATP synthesis by the organic acids by a membrane-bound decarboxylase that acts as a sodium pump. In such cases, the enzyme is inhibited by a biotin enzyme inhibitor, avidin (5). Since avidin did not inhibit glutamate decarboxylase activity of E1, and since the glutamate decarboxylase of E1 was localized in the cytoplasm (data not shown), we concluded that ATP synthesis coupled to glutamate decarboxylation was not driven by Na⁺ motive decarboxylase.

DCCD-sensitive ATPase by a proton motive metabolic cycle as follows (Fig. 2). (i) The one-for-one exchange of glutamate and GABA polarizes the membrane (resulting in electrically negative conditions inside the membrane). (ii) Intracellular glutamate decarboxylation consumes a cytoplasmic proton, generating a ΔpH across the membrane (resulting in alkaline conditions inside the membrane). (iii) The proton motive force arising in the combination of transport and decarboxylation steps is used for ATP synthesis by DCCD-sensitive ATPase. (iv) The free outward diffusion of CO₂ makes the overall cycle effectively irreversible.

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REFERENCES

TABLE 1. Effects of ionophores or GABA on ATP production in Lactobacillus sp. strain E1 cells by glutamate decarboxylation

<table>
<thead>
<tr>
<th>Addition(s) to mixture</th>
<th>ATP production (nmol of ATP/mg/min)</th>
<th>Glutamate decarboxylation (nmol of GABA/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>127</td>
<td>51.4</td>
</tr>
<tr>
<td>Val.</td>
<td>112</td>
<td>51.4</td>
</tr>
<tr>
<td>Nig.</td>
<td>0.7</td>
<td>50.4</td>
</tr>
<tr>
<td>Val. + Nig.</td>
<td>0.8</td>
<td>61.6</td>
</tr>
<tr>
<td>GABA</td>
<td>22</td>
<td>ND</td>
</tr>
</tbody>
</table>

* The final concentration of each ionophore was 2 μM (Val., valinomycin; Nig., nigericin). The final concentration of GABA was 10 mM. Rates of ATP production and glutamate decarboxylation were measured with respect to the amount of cells (in milligrams [dry weight]) in the mixture.

* ND, not determined.

