Arginine catabolism by *Thermanaerovibrio acidaminovorans*

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Abstract

The arginine catabolism of *Thermanaerovibrio acidaminovorans* was investigated. *T. acidaminovorans* was able to produce approximately 0.4–0.5 mol citrulline and 0.5–0.6 mol ornithine from 1 mol of arginine. However, in a methanogenic coculture with *Methanobacterium thermoautotrophicum* Z245 1 mol arginine was converted to approximately 1 mol of propionate, 0.5 mol acetate, 4 mol ammonia and 4 mol hydrogen; citrulline and ornithine were not formed. Enzyme measurements indicated the presence of the arginine deiminase pathway (ADI) in cells of *T. acidaminovorans* growing on arginine. ß 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Arginine catabolism; ADI (arginine deiminase) pathway; Interspecies hydrogen transfer

1. Introduction

Arginine can be used by a variety of anaerobic microorganisms as an energy source for growth [1]. Energy rich ureido (R-NH-CO-NH$_2$) compounds can be formed as intermediates in the energy metabolism of bacteria that can grow on arginine by substrate level phosphorylation. The ureido compounds are degraded by phosphorolytic cleavage to carbamyl phosphate. The carbamyl phosphate is then further metabolized to form ATP via carbamate kinase. The intermediate fermentation product from arginine is citrulline. Furthermore, ornithine, bicarbonate and 2 mol of ammonia are formed during arginine degradation.

\[
\Delta G^0 = -37.7 \text{ kJ/mol}
\]

\[
L - \text{Arginine} + H_2O + H^+ \rightarrow \text{citrulline} + NH_4^+
\]

\[
\Delta G^0 = +28.5 \text{ kJ/mol}
\]

Carbamyl phosphate + ADP → ATP + carbamate

\[
\Delta G^0 = -24.2 \text{ kJ/mol}
\]

\[
L - \text{Arginine} + H_2O \rightarrow L - \text{ornithine} + \text{urea}
\]

(Gibbs’ free energy changes were obtained from [2].) The generation of carbamyl phosphate from citrulline is thermodynamically unfavorable. However, the overall process is exergonic and allows the formation of 1 ATP. Degradation of arginine as shown above occurs via the arginine deiminase (ADI) pathway, involving the combined activity of arginine deiminase (ADI), ornithine transcarbamylase (OTC) and carbamate kinase (CK). The ADI pathway has been demonstrated in many bacteria, but there are wine lactic acid bacteria that convert arginine to ornithine and ammonia via another pathway. This is the arginase pathway, involving arginase and urease, catalyzing the following reactions [3]:

\[
\Delta G^0 = -7.5 \text{ kJ/mol}
\]

Carbamate + H$_2$O + H$^+$ → HCO$_3^-$ + NH$_4^+$

\[
\Delta G^0 = -3.3 \text{ kJ/mol}
\]

Overall:

\[
L - \text{Arginine} + 2H_2O + H^+ + ADP + P_i \rightarrow
\]

\[
L - \text{ornithine} + 2NH_4^+ + \text{HCO}_3^- + \text{ATP}
\]

\[
\Delta G^0 = -20.0 \text{ kJ/mol}
\]
Urea + H2O + H+ → CO2 + 2NH4+

$\Delta G^0^r = -27.6$ kJ/mol

(Gibbs’ free energy changes were obtained from [2].)

Both pathways yield the same products: ornithine, ammonia and CO2. However, only the ADI pathway may yield citrulline, either as an intermediate or as end product.

The function of the ADI pathway in microorganisms is diverse. The ADI pathway can serve as a protectant against acid damage. The ammonia production from arginine by *Carnobacterium* spp. and oral Streptococci protects the organisms against the acid produced from sugar fermentation [4,5]. Carbamyl phosphate may serve as a source of phosphate for glycogen synthesis in oral Streptococci [6]. Furthermore, the ADI pathway provides a source of ATP in the conversion of carbamyl phosphate to carbamate in many Lactobacilli.

*Thermanaerovibrio acidaminovorans* is a versatile thermophilic anaerobe able to grow on a variety of amino acids [7]. The organism is able to ferment arginine, yielding ornithine as a major fermentation product. Up to 60% of the arginine ends up as ornithine. In this study we investigated the pathway used for arginine conversion by *T. acidaminovorans* and the possibility of ATP generation from carbamyl phosphate.

### 2. Materials and methods

#### 2.1. Cultivation of the organisms

A bicarbonate buffered mineral salts medium with 0.05% yeast extract as described previously [8] was used in all experiments. Cultures of *T. acidaminovorans* (DSM 6589) and *Methanobacterium thermoautotrophicum* Z245 (DSM 3720) were incubated at 55°C in the dark.

#### 2.2. Preparation of cell free extracts

Cells were harvested anaerobically by centrifugation and washed twice with 50 mM Tris–HCl (pH 7.2), containing 0.1 mM dithioerytritol (DTE). Cell extracts were prepared anaerobically by ultrasonic disintegration. Cell debris was removed by centrifugation at 13 000 rpm for 20 min. Supernatants were stored anaerobically in the dark at 4°C.

#### 2.3. Enzyme assays

Arginine deiminase (ADI), ornithine transcarbamylase (OTC) and carbamate kinase (CK) were measured according to [9]; arginase according to [10] and ornithine transaminase according to [11]. Pyruvate:ferredoxin oxidoreductase was measured as described by [12]. All assays were conducted under anoxic conditions in the dark at 50-55°C. To calculate the specific activities of *T. acidaminovorans* in cell free extracts of cocultures, a correction was made for the biomass produced by the methanogen, using the molar growth yield (2.2 g dry weight x (mol CH4⁻¹)) of *M. thermoautotrophicum* on H2/CO2.

#### 2.4. Analytical methods

Growth yields of the organisms were quantified by measuring the dry weight content. For yield studies triplicate bottles were used for each arginine concentration tested. Growth yields were corrected for the biomass formed from

<table>
<thead>
<tr>
<th>Enzyme</th>
<th><em>T. acidaminovorans</em> (arginine)</th>
<th>Coculture a (arginine)</th>
<th><em>T. acidaminovorans</em> (glutamate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADI</td>
<td>0.02–0.04</td>
<td>0.005–0.02</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>OTC</td>
<td>1.0–1.5</td>
<td>2.0–2.5</td>
<td>n.d. b</td>
</tr>
<tr>
<td>CK</td>
<td>0.1–0.22</td>
<td>0.1–0.12</td>
<td>n.d.</td>
</tr>
<tr>
<td>Arginase</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.005</td>
</tr>
<tr>
<td>Ornithine transaminase</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Pyruvate:ferredoxin oxidoreductase</td>
<td>0.1–0.15</td>
<td>0.5–1.2</td>
<td>0.5–0.7</td>
</tr>
</tbody>
</table>

*Activity was corrected for the presence of the methanogen.

b N.d.: measured, but not detected.

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Table 1

Conversion of arginine by *T. acidaminovorans* in the absence and presence of *M. thermoautotrophicum* Z245

<table>
<thead>
<tr>
<th>Arginine degraded (mmol l⁻¹)</th>
<th>Products formed (mmol l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetate</td>
</tr>
<tr>
<td>Pure culture</td>
<td>8.6</td>
</tr>
<tr>
<td>Coculture</td>
<td>8.2</td>
</tr>
</tbody>
</table>

*CH4 formation was expressed as hydrogen (4 mol H2 are equivalent to 1 mol CH4). H2 was never detected in cocultures.
and CH₄ were analyzed as described previously [8]. Pro-
yeast extract. Arginine, citrulline, ornithine and glutamate
were analyzed using the HPLC technique as described by
arginase activity in glutamate grown cultures. It is possible
with glutamate as substrate. However, it is not possible
to determine the presence of arginase activity in glutamate
grown cells.

The absence of ornithine and citrulline after growth of
arginine metabolism of T. acidaminovorans, which can only
be overcome by the addition of a methanogen. Only then arginine is completely converted to propionate, acetate, NH₄⁺, CO₂ and H₂.

Table 3
Yield of T. acidaminovorans grown on different arginine concentrations

<table>
<thead>
<tr>
<th>Arginine converted (µmol)</th>
<th>mg dry weight</th>
<th>Yield (g dw mol⁻¹ arginine)</th>
<th>µmol² dry weight</th>
<th>Citrulline formed (µmol)</th>
<th>Ornithine formed (µmol)</th>
<th>Citrulline/ornithine ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>3</td>
<td>21.4</td>
<td>12</td>
<td>55</td>
<td>90</td>
<td>0.61</td>
</tr>
<tr>
<td>590</td>
<td>11</td>
<td>18.6</td>
<td>44</td>
<td>185</td>
<td>320</td>
<td>0.57</td>
</tr>
<tr>
<td>1030</td>
<td>12</td>
<td>11.6</td>
<td>48</td>
<td>260</td>
<td>530</td>
<td>0.49</td>
</tr>
<tr>
<td>1260b</td>
<td>25</td>
<td>19.8</td>
<td>99</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

* Mₐ, organic fraction = 114; 50% dry weight = organic fraction; 90% organic fraction = protein.
* T. acidaminovorans grown in the presence of M. thermoautotrophicum.

3. Results and discussion

T. acidaminovorans was able to convert arginine to cit-
rulline, ornithine and NH₄⁺ (Table 1). The ratio between
citrulline and ornithine varied in different incubations
from 1:1.2 to 1:2. In coculture with the hydrogen scav-
enger M. thermoautotrophicum Z245 arginine was con-
verted to acetate, propionate, NH₄⁺ and H₂, the latter
being used to reduce CO₂ to CH₄. No intermediate citrul-
ine or ornithine could be detected in these cocultures.

Enzyme measurements showed activity of ADI, OTC
and CK in low to moderately high activities (Table 2). In
the coculture activities of ADI were somewhat lower
and OTC activities somewhat higher. This suggested
the presence of the ADI pathway for arginine degradation by
T. acidaminovorans. No activity of arginase, a key enzyme
of the arginase pathway, could be detected. Remarkable is
that cells grown on glutamate showed no measurable ac-
tivity of the enzymes from the ADI pathway, but low
activity of arginase could be detected. It has been found
in experiments with concentrated cell suspensions of T.
adaminovorans that glutamate could be metabolized to
proline and ornithine (data not shown). These reactions
are probably used for anabolic purposes and might ex-
plain the presence of arginase activity in glutamate grown
cells.

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References