Glutamine Synthetase Activity in the Ruminal Bacterium Succinivibrio dextrinosolvens

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Succinivibrio dextrinosolvens C18 was found to possess glutamine synthetase (GS), urease, glutamate dehydrogenase, and several other nitrogen assimilation enzymes. When grown in continuous culture under ammonia limitation, both GS and urease activities were high and glutamate dehydrogenase activity was low, but the opposite activity pattern was observed for growth in the presence of ample ammonia. The addition of high-level (15 mM) ammonium chloride to ammonia-limited cultures resulted in a rapid loss of GS activity as measured by either the γ-glutamyl transferase or forward assay method with cells or extracts. No similar activity losses occurred for urease, glutamate dehydrogenase, or pyruvate kinase. The GS activity loss was not prevented by the addition of chloramphenicol and rifampin. The GS activity could be recovered by washing or incubating cells in buffer or by the addition of snake venom phosphodiesterase to cell extracts. Manganese inhibited the GS activity (forward assay) of untreated cells but stimulated the GS activity in ammonia-treated cells. Alanine, glycine, and possibly serine were inhibitory to GS activity. Optimal pH values for GS activity were 7.3 and 7.4 for the forward and γ-glutamyl transferase assays, respectively. The glutamate dehydrogenase activity was NADPH linked and optimal in the presence of KCl. The data are consistent with an adenylylation-deadenylylation control mechanism for GS activity in S. dextrinosolvens, and the GS pathway is a major route for ammonia assimilation under low environmental ammonia levels. The rapid regulation of the ATP-requiring GS activity may be of ecological importance to this strictly anaerobic ruminal bacterium.

Ammonia is the main nitrogen source used for growth by the numerically important bacterial species of the rumen (7, 8) and by many other anaerobic species inhabiting the mammalian gastrointestinal tract (5, 37). Ammonia incorporation by enteric bacteria is mediated by two major pathways: the low-affinity glutamate dehydrogenase (GDH) pathway is used when environmental ammonia levels are high, and the high-affinity glutamine synthetase (GS) pathway is used when the levels are low (40, 43). The GS activity is subject to a complex regulation system including enzyme synthesis, covalent modification by adenylylation-deadenylylation, and feedback inhibition. Little knowledge is available on the ammonia assimilation pathways (and their regulatory aspects) for the anaerobic bacteria of the rumen and mammalian gastrointestinal tracts. Both GS and GDH have been detected in whole rumen contents (11), continuous cultures of mixed ruminal bacteria (14), and pure cultures of several ruminal bacteria (18, 23, 33). However, the properties and regulation of these enzymes have been studied in detail with only one ruminal anaerobe, Selenomonas ruminantium (38, 39). The GS in this gram-negative organism differs substantially from the GS in enteric bacteria, particularly in that an adenylylation-deadenylylation regulatory mechanism is absent.

Succinivibrio dextrinosolvens is found in the rumen of cattle and sheep fed diets containing high levels of rapidly fermented carbohydrates such as starch. Under some conditions, Succinivibrio dextrinosolvens may be one of the major rumen bacteria fermenting dextrans and grass levs

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MATERIALS AND METHODS

Organism and culture conditions. Succinivibrio dextrinosolvens C18 and 24 were obtained from the culture collection of the Department of Dairy Science, University of Illinois, Urbana. Stock cultures were maintained on carbohydrate maintenance agar slants (6). The anaerobic culture techniques of Hungate (21) was modified by Bryant (4) and used throughout this study. Continuous cultures were grown in an anaerobic chemostat maintained at 39 ± 0.5°C (26, 31). Steady-state conditions were verified by constant absorbance measurements (660 nm) and constant cell RNA levels measured as described below. Verification of ammonia-limited growth was made by measurement of both glucose and ammonia levels in spent culture medium by the glucose oxidase (bulletin 510; Sigma Chemical Co., St. Louis, Mo.) and indophenol (12) methods, respectively.

The growth medium contained (amount per 100 ml): NaCl, 60 mg; KCl, 60 mg; MgSO₄, 26 mg; CaCl₂, 2 H₂O, 20 mg; salts and trace elements, 0.1 ml; resazurin, 100 µg; NH₄Cl, 22 mg; l-leucine, 200 mg; l-serine, 300 mg; dt-methionine, 200 mg; FeSO₄·7H₂O, 2.8 mg; PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid), 150 mg; K₂HCO₃, 400 mg; NaCO₃, 400 mg; vitamin solution, 1.0 ml; glucose, 900 mg; KH₂PO₄, 96 mg; and l-cysteine hydrochloride, 50 mg. Salts and trace elements, glucose, potassium phosphate, vitamins, and sodium carbonate were prepared separately and added after autoclaving as previously described (31). The vitamin
solution (17) contained (milligrams per 100 ml of ethanol): 1.4-naphthoquinone, 20; and para-aminobenzoic acid, 0.5.

**Cell harvesting and extract preparation.** All cell manipulations were carried out with an anaerobic chamber (22 to 24°C; 95% Ar, 5% H2; Coy Laboratory Products, Ann Arbor, Mich.). Culture samples were collected anaerobically, transported into the chamber, and centrifuged to obtain the cells with wide-mouthed polycarbonate centrifuge bottles with airtight sealing cap assemblies (Ivan Sorvall, Inc., Norwalk, Conn). For ammonia-treated cells, ammonium chloride was added (final concentration, 15 mM) to culture samples prior to centrifugation. Cell suspensions to be used for urea and GS assays were centrifuged (8,800 χ g, 10 min, 4°C), and the cells were washed twice with TKD buffer (50 mM Tris hydrochloride, 1% [wt/vol] KCl, 1 mM dithiothreitol, 10−2% [wt/vol] hexadecyltrimethylammonium bromide [CTAB], 2 mM MnCl2 [pH 7.4]). The washed cells were suspended in TKD buffer under argon to 1% (wt/vol) of the original culture volume, and the cell suspension was stored under argon on ice until used (within 12 h). For cell extracts, cultures were centrifuged, the cells were washed twice with TDK buffer (50 mM Tris hydrochloride, 1% [wt/vol] KCl, 1 mM DTT [pH 7.4]), and the cell pellet was frozen (−20°C) until used (within 3 days). The thawed cells were suspended in TDK buffer under argon to 1% (wt/vol) of the original culture volume, and cell extracts were prepared by passing cell suspensions once through a French pressure cell (20,000 lb/in2) at 4°C under argon. After centrifugation (15,000 χ g, 20 min, 4°C), the cell extract supernatant was stored under argon on ice until used (1 to 2 h). Protein in the cell extracts was measured by the method of Lowry et al. (28).

**Enzyme activities.** Urease (EC 3.5.1.5) activity was measured by using Warburg-type incubation vessels with a center well containing 0.05 ml of 2.0 M KOH (CO2 free). The reaction chamber contained 0.5 ml of 25 mM KH2PO4 (pH 7.2), 0.1 ml of 250 mM tetrasodium EDTA (pH 7.2), 0.2 ml of water, and 0.1 ml of [14C]urea (1.0 mM, 150,000 dpm/μmol) (29, 39). The side arm contained 0.05 to 0.1 ml of cell suspension. The assay was begun by mixing the cells in the side arm with the contents of the reaction chamber. After 20 min at 30°C, 0.1 ml of 1 M H2SO4 was added to the side arm and mixed with the contents of the reaction chamber to stop the assay. The KOH in the center well was assayed for 14CO2 with a Beckman scintillation counter with Aquasol-2 (New England Nuclear Corp., Boston, Mass.) as the scintillation fluid. Urease specific activity was defined as nanomoles of urea hydrolyzed per minute per milligram of protein.

The GS (EC 6.3.1.2) activity was measured by two methods: the forward assay of Bender et al. (1) as modified by Smith et al. (38) and the γ-glutamyl transferase (γ-GT) assay as described by Bender et al. (1). The forward assay reaction mixture (0.4 ml) contained (in micromoles): imidazole hydrochloride, 50; hydroxylamine hydrochloride, 45; monosodium glutamate, 81; MgCl2, 27; and when whole cells were used, CTAB (final concentration, 45 μg/ml). The forward reaction mixture was prepared daily, and the pH was adjusted with 10 N KOH to pH 7.3. The reaction mixture plus cells was equilibrated at 37°C for 5 min, and the reaction was initiated by adding ATP (12 μmol [pH 7.3]). The reaction was terminated after 10 to 40 min by the addition of 1.0 ml of stop mix (55 g of FeCl3·6H2O, 20 g of trichloroacetic acid, 31 ml of 6 M HCl, and distilled water to 1 liter), followed by immediate mixing. After centrifugation (1,000 χ g, 5 min, 4°C) to remove protein, the absorbance (340 nm) of the fluid was measured. Controls without ATP were included for all assays, but these never had significant color development. The γ-GT reaction mixture (0.4 ml) contained (in micromoles): imidazole hydrochloride, 80; hydroxylamine hydrochloride, 18; MnCl2, 0.4;KH2ASO4, 15; ADP, 0.22; and L-glutamine, 10. When GS activity was measured in whole cells, the reaction mixture was modified by the addition of CTAB (final concentration, 45 μg/ml) as described by Bender et al. (1). The reaction mixture was prepared daily from stock solutions and adjusted to pH 7.4 with KOH. The reaction was initiated at 37°C for 5 min, and the reaction was initiated by adding cells or extracts. Controls (minus L-glutamine or minus KH2ASO4 and ADP) were run, but these never had significant color development. The reaction was stopped, and color development was measured as described for the forward assay procedure. The amount of product formed was determined by comparison to standard curves prepared with genuine γ-glutamyl hydroxymate. GS specific activity was defined as nanomoles of γ-glutamyl hydroxymate formed per minute per milligram of protein.

The ODH (EC 1.4.1.2) activity was measured by determining NADPH oxidation at 340 nm by the procedure of Meers et al. (30) as modified by Smith et al. (38). The reaction mixture (3 ml) contained (in micromoles): Tris hydrochloride (pH 7.7), 75; KCl, 960; NH4Cl, 300; and 2-ketoglutarate, 15. The reaction was run at room temperature. After extracts were allowed to equilibrate with the reaction mixture for 5 min, the reaction was initiated by adding 0.6 μmol of NADPH. The NADH-linked GDH activity was assayed with the same reaction mixture minus KCl and was initiated with 0.4 μmol of NADH. Controls without NH4Cl or 2-ketoglutarate were also run. GDH specific activity was defined as nanomoles of NADPH or NADH oxidized per minute per milligram of protein.

Pyruvate kinase (EC 2.7.1.40) activity was measured at room temperature by determining the oxidation of NADH (19). The reaction mixture contained (in micromoles) PIPES (pH 7.0), 220; ADP, 2; MnCl2, 0.4; and MgCl2·6H2O, 2; NADH, 0.4; and L-lactic dehydrogenase, 9.6 U. The reaction mixture and extract were equilibrated for 5 min, and the reaction was initiated by the addition of phosphoenolpyruvate (5). Pyruvate kinase specific activity was defined as nanomoles of NADH oxidized per minute per milligram of protein.

Snake venom phosphodiesterase (SVD) (EC 3.1.4.1) was suspended (10 mg/ml) in Tris hydrochloride buffer (50 mM [pH 7.4], 2 mM MnCl2). The SVD had a specific activity of 3 μmol of p-nitrophenol produced per min per mg of protein at 37°C with bis(p-nitrophenyl phosphate) as the substrate. When measuring GS activity in extracts of *Sucinivibrio dextrinosolvens*, 0.1 ml of SVD solution was added to 1.0 ml of extract and the mixture was incubated at 37°C. At the times indicated, samples were removed and placed directly into the GS assay buffer (for both the forward and γ-GT assays) to start the assay. GS was measured as described previously.

**RESULTS**

**Enzyme activities.** Both strain C18 and strain 24 of *Sucinivibrio dextrinosolvens* possessed a number of enzyme activities associated with ammonia and nitrogen metabolism (Table 1). Only strain C18 had urease activity, as expected from previous studies (17). Substantial GS activities were detected in cells and extracts, and the levels with the γ-GT assay were much greater than those measured with the forward assay. High levels of GDH activity were also found.

Vol. 50, 1985 GS ACTIVITY IN *SUCCINIVIBRIO DEXTRINOSOLVENS* 1015
This activity was NADPH linked and required KCl in the assay mixture. In both strains, the GS activity increased and the GDH activity decreased as cultures entered stationary-growth phase with depletion of ammonia, the sole nitrogen source of the medium other than the amino acids required for growth (leucine, serine, methionine). Neither alanine dehydrogenase nor aspartase activities could be detected, but aspartate synthetase and glutamate-oxaloacetate transaminase activities were present (Table 1) at levels equal to or greater than the activities of catabolic glycolytic enzymes.

**Assay conditions for GS activity.** When CTAB was added to cultures prior to harvesting, there was a substantial increase in the level of GS activity detected by the forward or γ-GT assay method (Table 2). In contrast, the addition of ammonia to the culture resulted in a large decrease in GS activity as measured by both assay methods, and the concomitant addition of CTAB only partially prevented these losses. Furthermore, when manganese chloride was added to cultures (final concentration, 2 mM) prior to harvesting, there was about 1.5-fold increase in GS activity observed with ammonia-treated cells but not with untreated cells (data not shown). Thus, both CTAB and manganese chloride were routinely added to cultures prior to harvesting.

When the γ-GT assay method was used, the limiting substrate was always glutamine, the assay was linear from 10 to 100 μg of cell protein per assay. Controls run without the addition of ADP or potassium arsenate showed little color development, suggesting the absence of glutaminase activity. The pH profile for GS activity measured with extracts from untreated and ammonia-treated cells was determined (Fig. 1). The pH was very broad for both types of extracts, and an overlapping, isoactivity point was not observed when the γ-GT assay was used. A similar, broad pH profile was also observed for GS activity in extracts as determined by the forward assay. Based on these data, the γ-GT and forward assays were run at their optimal pH values of 7.4 and 7.3, respectively.

**Loss of GS activity with ammonia treatment.** As noted previously (Table 2), the addition of ammonium chloride to ammonia-limited cultures of *Succinivibrio dextrinosolvens* resulted in a rapid loss of GS activity. This loss appeared to be specific for GS as decreases in other enzyme activities were not observed (Table 3). The activity levels of urease, pyruvate kinase, and GDH in ammonia-treated cells or extracts were similar to those in untreated cells or extracts. In contrast, the GS activities of treated cells were only about 18 and 14% of those found with untreated cells as determined by the γ-GT and forward assays, respectively. When untreated and ammonia-treated cells or extracts were mixed, the resultant GS activities were appropriately additive. Thus, the loss of GS activity did not appear to be caused by the presence of a soluble inhibitory substance. The addition of chloramphenicol and rifampcin (final concentration, 50 and 25 μg/ml, respectively) to cultures of *Succinivibrio dextrinosolvens* resulted in a rapid cessation of growth as determined by optical density measurements. When both of these antibiotics were added to samples from ammonia-limited chemostat cultures (D = 0.2 h⁻¹), followed by the addition of ammonium chloride, the GS activity of the cells decreased to the same level as that observed with cells that were not exposed to the antibiotics (Table 4). In addition,
TABLE 3. Effects of ammonia addition on GS and other enzyme activities with cells or extracts of _Succinivibrio dextrinosolvens_ C18

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Enzyme</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells, untreated</td>
<td>GS (forward)</td>
<td>580</td>
</tr>
<tr>
<td></td>
<td>GS (γ-GT)</td>
<td>16,800</td>
</tr>
<tr>
<td></td>
<td>Urease</td>
<td>580</td>
</tr>
<tr>
<td>Cells, treated</td>
<td>GS (forward)</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>GS (γ-GT)</td>
<td>3,000</td>
</tr>
<tr>
<td></td>
<td>Urease</td>
<td>660</td>
</tr>
<tr>
<td>Cell extract, untreated</td>
<td>GS (forward)</td>
<td>580</td>
</tr>
<tr>
<td></td>
<td>GS (γ-GT)</td>
<td>14,300</td>
</tr>
<tr>
<td></td>
<td>GDH</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Pyruvate kinase</td>
<td>16</td>
</tr>
<tr>
<td>Cell extract, treated</td>
<td>GS (forward)</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>GS (γ-GT)</td>
<td>2,250</td>
</tr>
<tr>
<td></td>
<td>GDH</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Pyruvate kinase</td>
<td>13</td>
</tr>
</tbody>
</table>

These antibiotics had little or no effect on the GS activity of cells not exposed to ammonium chloride.

**Recovery of GS activity after ammonia treatment.** The GS activity with ammonium chloride-treated cells could be increased in several ways. When cells were simply washed three times by centrifugation with TKDCM buffer, the GS activity increased from 20 to 70% of the initial level observed with untreated cells. This washing procedure had essentially no effect on the GS activity of untreated cells (Table 4). If ammonia-treated cells were incubated in buffer with glucose, the cells regained 60% of the initial GS activity of untreated cells after 120 min, whether or not the treated cells had been extensively washed prior to incubation. The addition of both chloramphenicol and rifampin to the culture prior to cell harvesting and to the buffers used for cell washings and incubations had no substantial effects on the changes in GS activity resulting from these cell manipulations (Table 4). Furthermore, similar results were obtained when these antibiotics were added to the cell suspensions after harvesting instead of being added to the culture.

Extracts were prepared from untreated and ammonia-treated cells and incubated at 37°C. Periodically, samples were removed and assayed for enzyme activities. In both extracts, the pyruvate kinase and GDH activities were stable, and, in fact, gradually increased about 15 to 20% over 60 min of incubation. Similar results were found for the GS activity when measured by the γ-GT assay (Fig. 2). In _Escherichia coli_ (20, 40) and a variety of other gram-negative bacteria examined (1, 22, 24), the deadenylylation of the GS enzyme can be catalyzed by the addition of SVD to the enzyme preparation. The addition of SVD to _Succinivibrio dextrinosolvens_ extracts from untreated cells resulted in little change of GS activity even after 60 min of incubation. In contrast, the GS activity in extracts from ammonia-treated cells increased more than sixfold within 10 min after the addition of SVD (Fig. 2). After 60 min, the GS activity was about eightfold greater and approximately 40% higher than that observed with the untreated cell extract supplemented with SVD.

In enteric bacteria, the adenylylated GS enzyme is inhibited by magnesium when activity is measured by the γ-GT assay (1, 36). The addition of magnesium chloride (final concentration, 60 mM) to the assay mixture had no or slightly stimulating effects on the GS activity of untreated cells when measured by the γ-GT assay, but with ammonia-treated cells magnesium inhibited GS activity by 40 to 70%. In contrast, when GS activity was measured by the forward assay with untreated cells, magnesium was necessary for maximal activity, whereas manganese was very inhibitory (Table 5). However, manganese was quite stimulatory to the GS activity of ammonia-treated cells when the forward assay method was used.

**Glutamine metabolism products.** Inhibition of GS activity by products of glutamine metabolism has been demonstrated with a number of diverse organisms (1, 20, 42). Several of

![FIG. 2. Effects of SVD on GS activity of _Succinivibrio dextrinosolvens_ C18 cell extracts. Activities were measured (γ-GT assay) with extracts from untreated (circles) or ammonia-treated (triangles) cells without (filled symbols) or with (open symbols) the addition of SVD (1 mg/ml) to the assay mixture. Units are nanomoles per minute per milligram of protein.](image-url)
these compounds were tested for their effects on GS activity of *Succinivibrio dextrinosolvens* (Table 6). Alanine, serine, and glycine inhibited GS activity in cell extracts, particularly when measured by the γ-GT assay method. The other amino acids tested and AMP were slightly stimulatory or without effect.

### DISCUSSION

The results of this study establish that *Succinivibrio dextrinosolvens* possesses a GS and that the GS activity is regulated by an adenylylation-deadenylylation mechanism that might be similar to the one found in *E. coli*. These conclusions are supported by several lines of evidence: (i) the presence of γ-GT and forward assay activities in cells and extracts; (ii) the rapid loss of GS activity when cells grown under ammonia limitation are exposed to high levels of ammonium chloride; (iii) the recovery of GS activity in extracts from ammonia-treated cells by SVD treatment; (iv) the loss and recovery of GS activity in the presence of antibiotics affecting protein synthesis; and (v) the effects of magnesium and manganese on GS activity. The GS in *Succinivibrio dextrinosolvens* is also similar to that in *E. coli* in having both γ-GT and forward assay activities which are partially inhibited by alanine or glycine. With *E. coli*, adenylylation of GS decreases both forward and γ-GT activities, but conditions (pH, divalent cations) can be found in which both activities are the same regardless of adenylylation state. However, an important difference with *Succinivibrio dextrinosolvens* is that both γ-GT and forward activities are lost upon ammonia treatment of cells or, presumably, adenylylation. In addition, no pH inactivity point for GS activities with untreated and treated cells or extracts could be established with either γ-GT or forward assay methods. Thus, these findings preclude measurement of the adenylylation level by magnesium inhibition. Attempts to measure adenylylation state differential affinity for ADP in the γ-GT assay (16, 41) were also unsuccessful (data not shown).

In contrast to both *E. coli* and *Succinivibrio dextrinosolvens*, the GS of the ruminal anaerobe *Selenomonas ruminantium* lacks the γ-GT activity, is not inhibited by amino acids arising from glutamine metabolism, and does not have an adenylylation-deadenylylation regulation. Based on inhibition by nucleotides, it has been suggested that GS activity in *Selenomonas ruminantium* may be regulated in part by the energy status of the cell (38).

When grown in batch culture, *Succinivibrio dextrinosolvens* has high levels of GDH but low levels of both GS and urease (Table 1). With ammonia-limited chemostat cultures, the opposite pattern of enzyme levels is seen (Table 2). Thus, these data are consistent with the use of mainly the GS pathway for ammonia assimilation when environmental ammonia is low and of mainly the GDH pathway when ammonia levels are ample, as observed with many other bacterial species (43). For efficient synthesis of glutamate when ammonia is fixed by GS, glutamate synthase activity is required as clearly shown by studies with mutants of *E. coli* (32) and *Klebsiella aerogenes* (2). We were unable to reliably detect glutamate synthase in *Succinivibrio dextrinosolvens* (data not shown), but this may be more related to determining the appropriate electron carrier. Compared with *E. coli* and other species, strictly anaerobic bacteria appear to use low-potential electron carriers as indicated by studies with *Selenomonas ruminantium* and *Bacteroides amylophilus* (37). *Succinivibrio dextrinosolvens* possesses ample glutamate-oxaloacetate transaminase (Table 1) which should be sufficient for ammonia assimilation via GDH.

Urea hydrolysis in the rumen is very important to the overall nitrogen metabolism of the animal (44), and both bacteria associated with rumen contents and the rumen wall are ureolytic (13, 46). Ureolytic activity in ruminal bacteria is a highly variable trait between and within species. For example, relatively few strains of *Selenomonas ruminantium* are not ureolytic (25), but most strains of *Succinivibrio dextrinosolvens* are ureolytic (17, 46). Our data indicate that variations in GS and urease activities parallel one another (Tables 1 and 3), except when cells are treated with ammonia. An inverse relationship between GS and GDH activities was observed. The synthesis of GS and urease are coordinately regulated in *Selenomonas ruminantium* and fluctuate as a function of environmental ammonia levels (39). These patterns of enzyme activities in ruminal bacteria are consistent with the types of regulation observed in detailed studies with nonanaerobic bacteria (2, 15, 27, 32).

### TABLE 5. Effects of magnesium and manganese on the GS activity (forward assay) of *Succinivibrio dextrinosolvens* C18α

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Assay conditions</th>
<th>GS activitya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MgCl₂</td>
<td>MnCl₂</td>
</tr>
<tr>
<td>Untreated cells</td>
<td>54</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Ammonia-treated cells</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

a Samples were from ammonia-limited chemostat (D = 0.2 h⁻¹) and harvested directly (untreated) or after the addition of ammonium chloride to 15 mM to the sample (ammonia treated).

b Final concentration (mM) in assay mixtures.

c Means of two experiments; values are nanomoles per minute per milligram of protein.
Succinivibrio dextrinosolvens often is isolated in large numbers from the rumens of cattle and sheep fed high levels of grain or other rapidly fermentable carbohydrates (10, 34, 46). In these types of feeding regimens as well as many others, the ruminal ammonia levels can vary greatly with time after feeding and with ruminal site (45). Although strain 24 has a poor affinity for ammonia, strains C18 and 22B have affinities (17) similar to the 50 μM level for half-maximal growth rate of many major ruminal bacteria (35). The abilities of Succinivibrio dextrinosolvens to grow easily under low environmental ammonia levels, to rapidly turn off the ATP-consuming GS activity when ammonia levels are ample, and to ferment starch breakdown products (maltose, dextrins) may be important ecological factors in allowing the organism to proliferate in the rumen under high grain feeding conditions. In addition, these conditions also lead to low ruminal fluid pH values and to Succinivibrio species most probably more tolerant to low pH than are other ruminal bacteria, as indicated by the occurrence of large numbers of Succinivibrio strains from animals with low (5.7 to 5.9) rumen pH values (9).

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LITERATURE CITED


