Fermentation of Peptides and Amino Acids by a Monensin-Sensitive Ruminal Peptostreptococcus

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A monensin-sensitive ruminal peptostreptococcus was able to grow rapidly (growth rate of 0.5/h) on an enzymatic hydrolysate of casein, but less than 23% of the amino acid nitrogen was ever utilized. When an acid hydrolysate was substituted for the enzymatic digest, more than 31% of the nitrogen was converted to ammonia and cell protein. Coculture experiments and synergisms with peptide-degrading strains of Bacteroides ruminicola and Streptococcus bovis indicated that the peptostreptococcus was unable to transport certain peptides or hydrolyze them extracellularly. Leucine, serine, phenylalanine, threonine, and glutamine were deaminated at rates of 349, 258, 102, 95, and 91 nmol/mg of protein per min, respectively. Deamination rates for some other amino acids were increased when the amino acids were provided as pairs of oxidized and reduced amino acids (Stickland reactions), but these rates were still less than 80 nmol/mg of protein per min. In continuous culture (dilution rate of 0.1/h), bacterial dry matter and ammonia production decreased dramatically at a pH of <6.0. When dilution rates were increased from 0.08 to 0.32/h (pH 7.0), ammonia production increased while production of bacterial dry matter and protein decreased. These rather peculiar kinetics resulted in a slightly negative estimate of maintenance energy and could not be explained by a change in fermentation products. Approximately 80% of the cell dry matter was protein. When corrections were made for cell composition, the yield of ATP was higher than the theoretical maximum value. It is possible that mechanisms other than substrate-level phosphorylation contributed to the energetics of growth.

Much of the amino acid nitrogen that enters the rumen is deaminated, and rates of ammonia production often exceed the needs of ammonia-utilizing microorganisms (2). Because excess ammonia is absorbed across the rumen wall and subsequently converted to urinary urea, deamination represents a loss of dietary protein to the animal. The ionophore monensin is commonly fed to beef cattle as a methane inhibitor, but this feed additive causes a decrease in amino acid deamination in vitro (35, 44) and in vivo (13). In spite of its widespread use, the protein-sparing effect of monensin has never been satisfactorily explained.

Monensin is primarily effective against gram-positive ruminal bacteria (33), but all of the previously isolated and most active ammonia-producing ruminal bacteria have been gram negative (4). However, a gram-positive, monensin-sensitive peptostreptococcus was recently isolated from the rumen (37). This bacterium was unable to utilize carbohydrates as an energy source, and it grew rapidly on peptides and on amino acids. Since ammonia production by this species was far greater than that of other ruminal bacteria, it appeared that the new isolate could be an important contributor to ruminal ammonia production. The peptostreptococcus is not proteolytic (37), but proteolysis is usually not a rate-limiting step in the degradation of soluble proteins.

The aim of this work was to examine the ability of the ruminal peptostreptococcus to utilize various amino acid sources and identify factors affecting deamination and growth. The results indicated that the ruminal peptostreptococcus (i) was only able to deaminate a few amino acids rapidly, (ii) used Stickland reactions to deaminate some amino acids, (iii) sometimes fermented amino acids in the absence of growth, (iv) was unable to transport certain peptides or hydrolyze them extracellularly, (v) was sensitive to growth at a pH of less than 6.0, and (vi) exhibited peculiar growth kinetics in continuous culture.

MATERIALS AND METHODS

Cell growth. The peptostreptococcus (37) was grown anaerobically in basal medium containing (per liter) 292 mg of K2HPO4, 292 mg of KH2PO4, 480 mg of Na2SO4, 480 mg of NaCl, 100 mg of MgSO4·7H2O, 64 mg of CaCl2·2H2O, 500 mg of Na2S·9H2O, 4 g of Na2CO3, vitamins, and microminerals (11). Trypsinase (BBL Microbiology Systems, Cockeysville, Md.), Casamino Acids (Difco Laboratories, Detroit, Mich.), gelatin hydrolysate (U.S. Biochemicals, Cleveland, Ohio), or purified L-amino acids (15 g/liter on an ash-free basis) were provided as the energy and nitrogen source (in amounts specified in the text, tables, and figures). The method for preparation of hydrophilic and hydrophobic peptides was described previously (9); these preparations were also added at 15 g/liter.

The organism was routinely grown in tubes (18 by 150 mm) that were capped with butyl rubber stoppers and aluminum seals. When hydrogen and carbon monoxide (180 kPa) gas were added, the tubes were placed in an orbital shaker (70 rpm). All incubations were at 39°C, and the pH was 6.7 to 7.0. Growth was monitored by measuring the optical density of the tubes (Stasar II spectrophotometer [Gilford Instrument Laboratories, Inc., Oberlin, Ohio], 600 nm). The peptostreptococcus was also grown in continuous culture (34) with 15 g of Trypsinase per liter; in these cases, microminerals, vitamins, and sulfide were replaced by yeast extract (0.5 g/liter) and cysteine hydrochloride (0.6 g/liter). At least a 98% turnover of medium passed through the

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culture vessel (350 ml) before samples were obtained. In the pH experiments, the medium contained 43.8 mM volatile fatty acids (11), and the pH was lowered by adding concentrated HCl to the medium reservoir.

**Bacteroides ruminicola** B4 and **Selenomonas ruminantium HD4** were obtained from M. P. Bryant, University of Illinois, Urbana. **Streptococcus bovis** JB1 has been described previously (36). These bacteria were grown in basal medium containing 2 g of glucose, 0.5 g of yeast extract, 1 g of Trypticase, 0.6 of cysteine hydrochloride, and volatile fatty acids (11). After 24 h of incubation, the tubes were centrifuged (3,000 × g for 15 min) and the supernatant was discarded. Basal medium containing 15 g of Trypticase per liter and the peptostreptococcus were then added to the tubes. Samples for ammonia determinations were removed after 0, 24, 48, and 72 h of incubation.

**Fermentation products.** Cells were separated from medium by centrifugation (10,000 × g for 15 min at 0°C), and the pellets were washed in 0.9% NaCl. Supernatant fractions and cell pellets were stored separately at −15°C. Volatile fatty acids from *meta*-phosphoric acid-treated (6% [wt/vol], final concentration) supernatant samples were measured with a Gow Mac model 580 flame ionization gas chromatograph equipped with a Supelco 1220 column (1% H2PO4, 100/120 mesh). Nonvolatile organic acids were detected by high-pressure liquid chromatography, using a liquid chromatograph (model 334; Beckman Instruments, Inc., Fullerton, Calif.) equipped with a Beckman model 156 refractive index detector and an HPX-87H organic acid column (Bio-Rad Laboratories, Richmond, Calif.; 0.013 N H2SO4 at 0.5 ml/min, 50°C, 20-μl samples). Hydrogen was measured with a Gow Mac model 550 thermal conductivity gas chromatograph and a Supelco S-8100 column. Ammonia was assayed by the colorimetric assay of Chaney and Marbach (8). Supernatant samples for peptide analyses were adjusted to a pH of >10 with K2CO3 and brought to a boil to remove the ammonia. Peptides were then hydrolyzed with 6 N HCl (110°C for 24 h under nitrogen), neutralized with NaOH, and assayed by a ninhydrin method, using glycine as a standard (31). Peptide size was estimated from the ratio of ninhydrin reaction after HCl hydrolysis to ninhydrin reaction without HCl hydrolysis. Aminopeptidase was assayed as described elsewhere (36), using L-alanine-p-nitroanilide as a substrate.

**Cell composition.** Bacterial protein was measured by the method of Lowry et al. (23) after the cells were treated with 0.2 N NaOH for 15 min (100°C). Results obtained by this method were compared with those obtained with a dye-binding assay (5). Cell nucleic acids were extracted with hot 0.5 N perchloric acid. DNA and RNA were determined by the diphenylamine and orcinol procedures of Burton (6) and Schneider (38), respectively. Cell carbohydrate was assayed by the anthrone method, with glucose as a standard (5). Cells washed in 0.9% NaCl were dried at 105°C for 2 h and weighed on aluminum pans. The weight of NaCl was subtracted.

**RESULTS**

**Trypticase versus Casamino Acids.** Trypticase is an enzymatic digest of casein containing peptides and relatively few free amino acids, whereas Casamino Acids is an acid hydrolysate of casein which contains mostly free amino acids. When the ruminal peptostreptococcus was incubated with less than 15 g of Trypticase or Casamino Acids per liter as the energy and carbon source, cell growth was proportional to the substrate concentration (Fig. 1). Casamino Acids allowed approximately 10% more growth than did Trypticase when each was provided at 15 g/liter, but at higher concentrations there was little further increase in optical density or difference between the two amino acid sources. On the basis of initial rates of growth, the apparent \( K_m \) and \( V_{max} \) values for Trypticase or Casamino Acids were 4 g/liter and 0.5/h or 2.4 g/liter and 0.6/h, respectively. Growth on hydrophilic peptides (insoluble in 90% isopropyl alcohol) was 32% faster (0.42 versus 0.32/h) than growth on hydrophobic peptides (soluble in 90% isopropyl alcohol).

When cultures were incubated with 15 g of Trypticase per liter for 24 h, the fermentation products were 25.0 mM ammonia, 7.0 mM acetate, 0.7 mM propionate, 3.6 mM isobutyrate, 0.7 mM butyrate, 2.9 mM isovalerate, and 6.1 mM isocaproate. Casamino Acids (15 g/liter) was more completely fermented, and in this case the products were 35.5 mM ammonia, 11.8 mM acetate, 0.7 mM propionate, 7.0 mM isobutyrate, 2.3 mM butyrate, 4.9 mM isovalerate, 0.2 mM valerate, and 6.6 mM isocaproate. Hydrogen gas was also detected in amounts of 1.0 and 0.7 mmol of gas per liter of culture for Trypticase and Casamino Acids, respectively.

When the bacterium was incubated with 15 g of Trypticase or Casamino Acids per liter, there was little increase in cell protein after 12 h (Fig. 2). Less than 7% of the nitrogen was retained as cell protein in either case, and once again Casamino Acids allowed more growth than did Trypticase. Even though growth had ceased, fermentation and ammonia production continued, particularly if Casamino Acids was the substrate. By 60 h, cultures containing Casamino Acids had produced 40% more ammonia than had those containing Trypticase. In each case, however, less than one-third of the amino acid source was actually utilized.

**Individual amino acids.** When the bacterium was grown in a medium containing purified amino acids (15 g/liter) in the same proportions as those in Casamino Acids, the optical density was lower (1.0 versus 1.4 optical density units) than that observed after growth with Casamino Acids. Deletion of individual amino acids from the purified amino acid medium showed that leucine, phenylalanine, and tyrosine were essential for growth (optical density decreased more than 65%). Deletion of serine caused a 30% decrease in growth, but stepwise (one at a time) removal of the other 16 amino acids
acids had little, if any, effect on final optical density. Addition of leucine, serine, or phenylalanine to cultures that had stopped growing after 24 h in Casamino Acids medium caused a significant increase in bacterial protein (Fig. 3a). Addition of tyrosine did not cause further growth. With Trypticase-grown cultures, the response due to added serine or leucine was twice as great, but addition of phenylalanine did not produce further growth (Fig. 3b). Growth was observed in medium containing only phenylalanine, leucine, and tyrosine (5 g/liter each) but at a very slow rate (0.016/h). The growth rate increased to 0.03/h if serine was also present.

When the peptostreptococcus was incubated with single amino acids, leucine, serine, phenylalanine, glutamine, and threonine were deaminated rapidly (Table 1), and gas-liquid and high-pressure liquid chromatography indicated the following products (carbon recoveries [C] and electron recoveries [E] are given in parentheses):

1.0 leucine + 0.4H₂O → 0.4 isovalerate + 0.6 isocaproate + 1.0NH₃ + 0.1H₂ (C = 100%, E = 101%)
1.0 serine → 0.2 lactate + 0.3 malate + 0.5 acetate + 0.2CO₂ + 1.0NH₃ (C = 96%, E = 107%)
1.0 phenylalanine + 0.2H₂O → 0.4 phenylpropionate + 0.5 phenyllactate + 0.1 phenylacetate + 0.2CO₂ + 1.0NH₃ (C = 100%, E = 107%)
1.0 threonine + 0.6H₂O → 0.5 propionate + 0.1 ketobutyrate + 0.2 butyrate + 0.1 acetate + 0.1 lactate + 1.0NH₃ + 0.6CO₂ (C = 95%, E = 91%)
1.0 glutamine → 0.9 pyroglutamate + 0.1 acetate + 1.0NH₃ + 0.1CO₂ (C = 94%, E = 91%)

The remaining 15 amino acids yielded much less ammonia.
after 24 h, and the specific activities of ammonia production were also much lower (Table 1).

**Stickland reactions.** The remaining 15 amino acids were deaminated slowly, but the rate of ammonia production was increased somewhat when they were provided as pairs of reduced and oxidized amino acids (Table 2). The combination of alanine and proline produced more than 2.6 times as much ammonia as did the combination of proline and alanine. Synergisms between proline and valine, glycine and alanine, proline and isoleucine, and glycine and valine were 1.9-, 1.5-, 1.3-, and 1.2-fold, respectively (Table 2). No positive synergisms were observed with glycine or proline and phenylalanine, glutamate, tyrosine, threonine, or histidine, and addition of selenium (1.5 μM) had no effect. When hydrogen gas was added to cocultures containing leucine, isoleucine, and valine, there was no effect on ammonia production; the hydrogenase inhibitor carbon monoxide (43) was also without effect (data not shown).

**Cocultures.** When the ruminal bacterium *S. bovis* JB1 was incubated with Trypticase for as long as 72 h, there was little production of ammonia (Fig. 4a). However, if *S. bovis* was cocultured with the ruminal peptostreptococcus, there was a large increase in ammonia production (1.6-fold at 72 h). *B. ruminicola* B4 alone was able to produce some ammonia, but a coculture of the peptostreptococcus and *B. ruminicola* was able to produce more ammonia than either bacterium alone (Fig. 4b). *S. bovis* JB1 or *B. ruminicola* B4, *S. bovis* and *B. ruminicola* were initially present at 370 mg of protein per liter.

**TABLE 2.** Stickland reactions by the ruminal peptostreptococcus

<table>
<thead>
<tr>
<th>Oxidized amino acid</th>
<th>Reduced amino acid</th>
<th>Combination</th>
<th>Fold stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proline (4.1)</td>
<td>Alanine (3.0)</td>
<td>Proline + alanine (18.4)</td>
<td>2.6</td>
</tr>
<tr>
<td>Proline (4.1)</td>
<td>Valine (1.0)</td>
<td>Proline + valine (9.8)</td>
<td>1.9</td>
</tr>
<tr>
<td>Glycine (1.6)</td>
<td>Alanine (3.0)</td>
<td>Glycine + alanine (7.5)</td>
<td>1.5</td>
</tr>
<tr>
<td>Glycine (1.6)</td>
<td>Valine (1.0)</td>
<td>Glycine + valine (3.4)</td>
<td>1.3</td>
</tr>
<tr>
<td>Proline (4.1)</td>
<td>Isoleucine (2.3)</td>
<td>Proline + isoleucine (7.6)</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* The bacterial protein concentration was 134 mg/liter, and each amino acid was present at 5 g/liter. Ammonia production values (millimolar after 24 h of incubation) are given in parentheses.

* Synergism (more than can be explained by an additive response).
was able to produce 1.5 times more ammonia than the total produced by the pure cultures (Fig. 4b). No synergism was noted between *Selenomonas ruminantium* HD₄ and the ruminal peptostreptococcus. The peptostreptococcus was unable to grow on gelatin hydrolysate (15 g/liter); if Trypticase-grown cells were incubated with gelatin hydrolysate, ammonia production was very low (1.8 mM). When the peptostreptococcus was provided with gelatin hydrolysate and cocultured with *S. bovis* and *B. ruminicola*, the synergisms in ammonia production were 1.8- and 2.7-fold, respectively.

Because ninhydrin is able to react only with free amino groups (31), the ratio of ninhydrin reaction after HCl hydrolysis to ninhydrin reaction without HCl treatment gives the approximate number of amino acids in a peptide. On the basis of these calculations, Trypticase and gelatin hydrolysate contained peptides with average chain lengths of 3.2 and 12.3 amino acids, respectively. During the 72-h incubation (Fig. 4), the ninhydrin ratio of Trypticase remaining in peptostreptococcus cultures increased to 4.8, while the ratio decreased to 2.6 and 1.4 for the *S. bovis* and *B. ruminicola* cultures, respectively. The ninhydrin ratios of peptostreptococcus-*S. bovis* and peptostreptococcus-*B. ruminicola* cocultures were 3.1 and 2.0, respectively.

**Continuous cultures.** When the dilution rates of continuous cultures (pH 7.0) were increased from 0.078 to 0.316, there was a decrease in total volatile fatty acid production but little change in the ratio of the acids (Fig. 5a). The reduction in volatile fatty acids was proportional to a decrease in ammonia (Fig. 5b). Calculated ATP values also decreased at slow dilution rates (Fig. 5b). ATP values were calculated on the basis of the following assumptions: (i) serine was the major precursor of acetate; (ii) isobutyrate and isocaproate were produced from valine and leucine, respectively; (iii) isovalerate was derived from either leucine or isoleucine; (iv) propionate and valerate were produced from threonine via a Stickland reaction involving either alanine or proline; and (v) ATP production would occur via a kinase reaction.
Bacterial dry matter also decreased at slow dilution rates, but bacterial dry matter declined to a lesser extent than did either ammonia or volatile fatty acids. When reciprocals of yield based on theoretical pathways of ATP formation or ammonia production were plotted against the reciprocal of the dilution rate, the slopes were slightly negative rather than positive (Fig. 6). The peptostreptococcus contained 80% protein, 0.5% DNA, 3% carbohydrate, and 12.5% RNA, and the dilution rate had little effect on this composition. Because the protein value was very high, the method of Lowry et al. (23) for protein determination was compared with the dye-binding method of Bradford (5). Similar amounts of protein were determined with the two assays.

As the pH of Tryptase-limited continuous cultures was decreased by the addition of HCl to the medium reservoir, there was a decline in steady-state bacterial dry matter in the chemostat (Fig. 7). Since the ratio of ammonia to dry weight or protein was reasonably constant, pH must have affected peptide utilization and not just the efficiency of growth. Volatile fatty acids were included in the medium because they can affect the ability of bacteria to grow at low pH (41), but this inclusion confounded the measurement of fermentation products. When ruminants are fed forages, rumen pH is usually near neutral, but pH is often less than 6 if large amounts of concentrates are included in the diet (39).

**DISCUSSION**

Previous mixed-culture studies with ruminal bacteria indicated that monensin caused a large decrease in microbial protein when Tryptase was the fermentation substrate (35). The results of these experiments suggested that monensin was acting directly as an antibiotic. However, monensin had little if any effect on the growth of ruminal bacteria (10) that were also capable of producing ammonia (4). Preliminary experiments confirmed the resistance of *B. ruminicola*, *Megasphaera elsdenii*, and *Selenomonas ruminantium* to monensin when Tryptase was provided as the fermentation substrate (data not shown). Thus, it seemed likely that monensin-sensitive, amino acid-fermenting bacteria had not yet been isolated from the rumen. Subsequent enrichments with high concentrations of peptides as the only energy source yielded a gram-positive peptostreptococcus with a very high specific activity of deamination (37). On the basis of ammonia production rates (>300 nmol of ammonia per mg of protein per min) and most probable numbers in vivo (10^9/ml of ruminal fluid), this species appeared to be an important amino acid-fermenting species, and its sensitivity to monensin was consistent with the protein-sparing effects.

The peptostreptococcus fermented leucine, serine, phenylalanine, threonine, and glutamine at faster rates than it fermented other amino acids (Table 1). Isovalerate and isocaprate were the products of leucine fermentation, while phenylalanine was converted to phenylacetate, phenylpropionate, and phenylacetate. Similar products were detected with clostridia (14, 15). Serine is usually deaminated by a dehydratase (25), and pyruvate can be converted to acetate by many bacteria, including clostridia (7) and the ruminal bacterium *M. elsdenii* (22). Lactate is produced from serine by *Fusobacterium nucleatum* (19). To our knowledge, malate has never before been reported as a product of serine fermentation, but a malic enzyme capable of converting pyruvate to malate is present in *Escherichia coli* (17). Threonine has been found to yield propionate, butyrate, 2-oxobutyrate, acetate, and a small amount of lactate. *Micrococcus aerogenes* produces 2-oxobutyrate via threonine dehydratase (45), and propionate results from the decarboxylation of 2-oxobutyrate (7, 22, 45). *M. elsdenii* produces small amounts of acetate via a direct cleavage of 2-oxobutyrate (22). On the basis of retention times during high-pressure liquid chromatography, pyroglutamate appears to be the product of glutamine fermentation by the peptostreptococcus. Pyroglutamate is found in many plant tissues (16, 30), and it is produced by the enzyme L-glutamine cyclotransferase (24). To our knowledge, this enzyme has not been detected in bacteria, but a similar enzyme, L-glutamate acid cyclotransferase, has been detected in *Pseudomonas cruciata* (1). It is possible that glutamine is deaminated to glutamate and then converted to pyroglutamate. Nearly all of the products of Tryptase or Casamino Acids could be produced from leucine, serine, phenylalanine, threonine, or glutamine. However, only valine yielded isobutyrate, and the rate of valine fermentation was very slow, 17 nmol/mg of protein per min (Table 1); Stickland reactions (27) increased valine deamination somewhat (Table 2), but it is not known whether valine fermentation rates can account for all of the isobutyrate production.

**FIG. 6.** Maintenance energy plot of 1/yield of ATP or 1/yield of ammonia versus 1/dilution rate. The pH was 7.0. DW, Dry weight.

**FIG. 7.** Effect of pH on the production of bacterial dry weight and ammonia in continuous culture at a dilution rate of 0.1/h with 15 g of Tryptase per liter.
When the peptostreptococcus was grown in a medium containing purified amino acids, the deletion of leucine, serine, phenylalanine, or tyrosine caused a significant decrease in final optical density. Because the deletion of other amino acids had little effect on cell growth, it appeared that only four amino acids were essential for growth. However, if the peptostreptococcus was grown in medium containing only serine, leucine, phenylalanine, and tyrosine, the growth rate was less than 10% of that observed with Casamino Acids. The inability of the peptostreptococcus to grow rapidly in the absence of nonessential amino acids suggested that one amino acid could substitute for another (e.g., via transamination).

When serine, leucine, and phenylalanine were added to Casamino Acids cultures that had stopped growing, there was a subsequent increase in cell protein (Fig. 3a). Since these amino acids were the ones showing the highest rates of deamination (Table 1), it is likely that growth was limited by energy rather than carbon. Similar results were obtained for Trypticase-grown cultures, but the stimulation of growth caused by leucine and serine was greater (Fig. 3b). Assuming that the total amino acid compositions of these two preparations are similar, it appeared that the organism was unable to metabolize all of the peptides containing serine and leucine.

The ruminal peptostreptococcus was able to grow rapidly on the peptide source Trypticase, but less than 23% of the nitrogen was converted to ammonia or cell protein (Fig. 2b). When Casamino Acids was substituted for Trypticase, approximately 40% more ammonia was produced (Fig. 2a). These results also suggested that extracellular peptidase activity or transport limited peptide utilization. Gelatin hydrolysate yielded almost no ammonia. Because gelatin is deficient in certain amino acids, lack of growth could have been a result of essential amino acid deficiencies. However, even if the peptostreptococcus was pregrown on Trypticase and then cultured with gelatin hydrolysate, ammonia production was very low. Because gelatin contains nearly half as much leucine, serine, phenylalanine, and threonine as does casein, the absence of ammonia could not be explained solely by amino acid composition.

When either S. bovis or B. ruminicola was cocultured with the ruminal peptostreptococcus, there was a more than additive increase in ammonia production (Fig. 3). S. bovis produces aminopeptidase (36), but we were unable to detect this activity with B. ruminicola (data not shown). However, a decrease in the ninhydrin ratio of residual peptides (with HCl hydrolysis/without hydrolysis) indicated that both of these ruminal bacteria had peptidase activity. The ninhydrin ratio of peptostreptococcus cultures grown on Trypticase increased, and this result suggested that short peptides were used in preference to longer ones. Indeed, gelatin hydrolysate contains much longer peptides than does Trypticase, and it was a very poor fermentation substrate. Cocultures of peptide-degrading ruminal bacteria and the peptostreptococcus also gave a synergistic increase in ammonia production when gelatin hydrolysate was the substrate.

When batch cultures contained 15 g of amino acid source per liter, Casamino Acids gave 10% more optical density than did Trypticase (Fig. 1), but the difference in ammonia production was greater than 40% (Fig. 2). The apparent increase in growth efficiency of Trypticase cultures might be ascribed to an energetic advantage of peptide versus amino acid transport (28), but there was little difference in the ratio of protein to ammonia during periods of rapid growth (Fig. 2). Because much of the difference in ammonia production between Casamino Acids- and Trypticase-grown cultures arose during periods when there was no detectable growth (>24 h), energy spilling may have occurred (26, 41).

When bacteria are grown in continuous culture at slow dilution rates, almost none of the limiting substrate usually is left in the culture vessel (42), and the yield of the bacteria usually declines due to expenditures of maintenance energy (29). With the peptostreptococcus, the reverse was observed. Product formation (Fig. 5) declined at slow dilution rates, and the yield based on ammonia increased (Fig. 6). Little of the increase in yield at slow dilution rates was explained by a change in fermentation pathways (Fig. 5), and even the calculated yield based on ATP increased at low dilution rates (Fig. 6). This aberrant relationship between yield and dilution rate caused a slightly negative estimate of maintenance (slope of 1/yield versus 1/dilution rate). Interpretation of these results is complicated by the fact that Trypticase is not a homogeneous substance like glucose, but previous work has indicated that bacteria can dissipate energy as heat (see comment on energy spilling above) at rapid dilution rates if the energy source accumulates (32). Energy spilling as heat would not, however, explain the decline in ammonia and volatile fatty acids at slow dilution rates.

Calculations by Southamer (40) indicated that the theoretical maximum growth yield of bacteria should be approximately 32 g (dry weight)/mol of ATP, but this value was based on a cell composition of 52.4% protein, 16.6% carbohydrate, 15.7% RNA, 9.4% lipid, and 3.2% DNA. By using the same type of calculation, the cell composition of the peptostreptococcus (80% protein, 0.5% DNA, 3% carbohydrate, and 12.5% RNA), a similar lipid and ash content, and an assumption that carbohydrate was produced via glucoseogenesis, the maximum yield of ATP should have been 23 g (dry weight). With the peptostreptococcus, the estimated yield of ATP was as high as 26. Since the yields produced by most bacteria are significantly lower than the theoretical maximum values (41), it is possible that the amount of ATP produced from amino acid fermentation was underestimated.

Leucine was the amino acid most rapidly fermented, but the isocaproate-isovalerate pathway of leucine fermentation has a stoichiometry of 2 leucines and theoretically produces only 1/3 mol of ATP per mol of leucine via a kinase reaction (14). Such a low level of ATP production would hardly be enough to drive transport, let alone growth. Electron transport via cytochromes can provide additional ATP in anaerobic fermentations (20). Most peptostreptococi do not have cytochromes, but Lanigan (21) reported the presence of cytochromes in Peptococcus (Peptostreptococcus) heliotrin-reducens. It was recently demonstrated that some bacteria (12), including Peptostreptococcus asaccharolyticus (46), have decarboxylases which are capable of generating sodium gradients across cell membranes; that capability together with the presence of sodium gradients could drive nutrient transport or ATP formation (18). Since the rumen is a sodium-rich environment, the involvement of sodium gradients in amino acid metabolism is a particularly interesting possibility.

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