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Catabolism of Amino Acids by *Megasphaera elsdenii* LC1

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The amino acids in an acid hydrolysate of casein were catabolized more extensively by *Megasphaera elsdenii* than those in an enzymic hydrolysate. Threonine and serine were most actively degraded, but no resultant increase in growth yield occurred. Branched-chain volatile fatty acid production, which increased as the dilution rate of a glucose-limited chemostat decreased, seemed to be associated with maintenance rather than with growth.

Amino acid catabolism by rumen microorganisms generally results in an inefficient utilization of amino acid-nitrogen by the ruminant (19). This detrimental effect on ruminant nutrition is sometimes offset by an increased rate of fiber digestion, resulting from an increased availability of branched-chain fatty acids for the growth of cellulolytic bacteria (21).

Megasphaera elsdenii is considered to be one of the main species of bacteria involved in amino acid catabolism and branched-chain fatty acid production in the rumen (1, 10). The present experiments were undertaken to investigate the influence of bacterial growth rate on amino acid catabolism by *M. elsdenii* and to determine whether *M. elsdenii* obtained useful energy from this metabolism while growing on glucose as principal energy source.

M. (formerly *Peptostreptococcus*) *elsdenii* LC1 (NCIB 8927) was maintained on medium 1 of Kurihara et al. (11). The continuous culture apparatus was as described by Hobson (9) with some modifications (22). Batch cultures were grown in Hungate tubes, incubated at 39°C for 24 h. The gas phase was oxygen-free CO_2 in all experiments, and the inoculum was at a concentration of 0.4%.

The composition of basal continuous culture medium was similar to the defined medium described previously (22), which contained minerals, vitamins and glucose as the sole carbon source, except that ammonium salts were omitted, the glucose concentration was 0.8 g liter^{-1}, and L-amino acids or casein acid hydrolysate (10 g liter^{-1}; Oxoid Ltd., London, England) were added as required. The pH of continuous cultures was maintained between 6.8 and 7.1 by the automatic addition of sterile NaOH. Batch culture medium was similar, except that dithiothreitol replaced sodium hydrosulfite as the reducing agent and the glucose concentration was 4 g liter^{-1}. Casitone (Difco Laboratories, Detroit, Mich.) was added (10 g liter^{-1}) to some batch cultures, to provide an enzymic hydrolysate of casein. The initial pH of the batch cultures was 6.3.

Continuous cultures were sampled after 5 or more culture volumes had passed. Samples (12 ml) were immediately chilled in an ice water slurry, and then a portion (10 ml) was centrifuged (27,200 × *g*, 15 min, 4°C) to obtain cell-free supernatant fluid which was stored at −70°C until it was analyzed.

Cell density was calculated from the optical density of diluted suspensions at 640 nm by using calibration factors determined for *M. elsdenii* grown in both batch and continuous cultures over the range of dilution rate (*D*) used in these experiments. This factor increased as *D* increased, from 0.24 to 0.27 g (dry weight) liter^{-1} optical density unit^{-1} between 0.03 and 0.30 h^{-1}.

Glucose in the cell-free supernatant fluid was measured by an automated glucose oxidase method (13) or by a phenol-sulfuric acid method (14). Volatile fatty acids (VFA) were estimated by gas-liquid chromatography (6), succinate was measured enzymically (2), and lactate was determined by a microdiffusion method (3). Pyruvate and 2-oxobutyrate were estimated enzymically and were distinguished by the different rates by which they were reduced by lactate dehydrogenases from pig heart (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and rabbit muscle (Sigma Chemical Co., St. Louis, Mo.) (8).

The amino acid content of the medium was determined by using a Chromaspek amino acid analyser (Hilger Analytical, Kent, England) with a column (35 by 0.3 cm) packed with 8% cross-linked sulfonated polystyrene divinylbenzene cation exchange resin with a bead size of 6 to 7 μm (Locarte Co., London, England). The amino acid content of harvested (27,200 × *g*, 15 min, 4°C), freeze-dried bacteria was determined by using cells from batch cultures. The dried bacteria were hydrolyzed by incubation in 6 M HCl at 110°C for 18 h and dried before analysis. Portions of the cell-free supernatant fluid of cultures containing Casitone were hydrolyzed in the same way before amino acid analysis. The amino acid cysteine and a product of the fermentation by *M. elsdenii* eluted together in this analyzer. The unknown product was tentatively identified as 2-aminobutyric acid by using high-pressure liquid chromatography (20).

From the amino acid composition of *M. elsdenii* and the amino acids metabolized in continuous culture (Table 1), it was clear that direct incorporation into cell material used only a small proportion of the amino acids which were removed from the medium. Thus catabolism was the main fate of the amino acids lost from the medium. With casein acid hydrolysate as a source of N, substantial quantities of only five amino acids were removed from the medium (Table 1). Serine and threonine concentrations were decreased by 70% in all samples, with up to 90% being removed at the lowest values of *D*, whereas increasing amounts of the branched-chain amino acids, isoleucine, valine, and leucine, were utilized as *D* decreased. Corresponding increases were seen in the concentrations of branched-chain VFAs produced (Table 2). Some of the other amino acids tended to increase in concentration, including glutamate, alanine, methionine, and proline. Cysteine and 2-aminobutyric acid were not differentiated in these samples.

A similar pattern of metabolism was found in batch

TABLE 1. Amino acid metabolism by *M. elsdenii* in glucose-limited chemostat culture

Amino acid	*M. elsdenii* cells[a]		Extracellular medium concn (mM)[b] at D of:						Reservoir (mM)
	μmol g (dry wt)$^{-1}$	mmol liter of culture^{-1}[c]	0.058	0.106	0.155	0.211	0.249	0.281	
Asp	420	0.08	2.56	2.50	2.38	2.26	2.22	2.27	2.33
Thr	217	0.04	0.15	0.18	0.17	0.20	0.31	0.21	1.02
Ser	201	0.03	0.16	0.25	0.23	0.30	0.49	0.33	1.68
Glx	470	0.10	5.82	5.34	4.94	4.70	5.58	4.58	5.09
Gly	380	0.07	1.26	1.19	1.11	1.16	1.03	1.05	1.06
Ala	488	0.09	1.47	1.66	1.65	1.60	1.59	1.64	1.36
Met	109	0.02	0.47	0.51	0.29	0.60	0.43	0.56	0.23
Val	245	0.05	0.20	0.24	0.37	0.27	0.70	0.51	0.83
Ile	171	0.03	0.09	0.11	0.16	0.27	0.33	0.23	0.43
Leu	277	0.06	0.38	0.48	0.67	0.98	1.24	0.90	1.41
Tyr	114	0.02	0.52	0.72	0.45	0.47	0.42	0.42	0.37
Phe	141	0.03	0.80	0.77	0.80	0.82	0.64	0.73	0.83
His	86	0.02	0.38	0.34	0.37	0.36	0.32	0.33	0.32
Lys	254	0.04	1.42	1.45	1.46	1.46	1.33	1.34	1.30
Arg	158	0.03	0.59	0.51	0.43	0.54	0.50	0.49	0.49
Pro	188	0.03	3.74	3.28	3.33	3.31	3.59	3.04	2.54

[a] Means of triplicate determinations.
[b] Means of duplicate determinations.
[c] Calculated from cell density at D = 0.058 h^{-1} and the amino acid composition of *M. elsdenii* grown in batch culture.

cultures, except that 0.7 mM glutamate and 1.2 mM aspartate were utilized and the catabolism of valine and isoleucine was greatly decreased. This pattern was altered when a pancreatic hydrolysate of casein replaced the acid hydrolysate. Aspartate, glutamate, threonine, and serine utilization decreased by 86, 57, 57, and 67%, respectively, whereas 0.6 mM methionine and 0.2 mM glycine were degraded. Similar differences can be observed between the results of Scheifinger et al. (18) with free amino acids and those of Cotta and Russell (4) with Trypticase (BBL Microbiology Systems, Cockeysville, Md.). This altered pattern of utilization presumably reflects a difference in the ability of *M. elsdenii* to take up free amino acids compared with peptides.

Addition of threonine or serine to a chemostat culture at D = 0.13 h^{-1} had no effect on the growth yield of *M. elsdenii* (Table 3), although most or all of the added threonine and serine was catabolized. In batch culture, *M. elsdenii* grew poorly in the absence of glucose, even when 5 mM threonine or serine was added to the medium. Again, addition of these acids in the presence of glucose failed to stimulate the growth yield (data not shown).

When 5 mM threonine was added to glucose-containing batch cultures, it was completely metabolized, and additional propionate (1.3 mM), 2-aminobutyrate (1.3 mM), C_5 branched acids (1.0 mM), valerate (0.5 mM), acetate (0.6 mM), 2-oxobutyrate (0.4 mM), and pyruvate (0.2 mM) were produced. The addition of 5 mM serine in the presence of glucose resulted in the metabolism of only an extra 1.4 mM serine, from which the only additional products detected were C_5 branched-chain acids (0.4 mM) and butyrate (0.4 mM). Thus, with the exception of 2-aminobutyrate, which has not been observed previously, these products are consistent with the findings of Lewis and Elsden (12) with washed cell suspensions.

It has been suggested that amino acid catabolism is a major contributor to maintenance energy rather than growth in *M. elsdenii* (17) and *Bacteroides ruminicola* (16). The greatly decreased metabolism of the branched-chain amino acids in batch culture of *M. elsdenii* in the present experiments and the relatively constant rate of branched-chain VFA production in continuous culture (Table 2) are also consistent with the proposal that these particular amino acids contribute to maintenance rather than to growth. Indeed, more branched-chain VFA production occurs in stationary phase than during growth (1). It can be calculated from Table 2 that branched-chain VFA production averaged 1.3 mmol g^{-1} h^{-1} and, by using formulas described by Pirt (15), that the apparent maintenance coefficient in terms of glucose was 0.59 mmol g^{-1} h^{-1}. The former calculation

TABLE 2. Growth yields and branched-chain volatile acid production by *M. elsdenii* in glucose-limited chemostat culture[a]

D (h^{-1})	Glucose molar growth yield[a] (g mol^{-1})	Production of branched-chain volatile acid (mM)		Rate of branched-chain acid production (mmol g^{-1} h^{-1})
		Isobutyrate	2-Methylbutyrate + isovalerate[b]	
0.058	36.0	1.05	2.14	1.16
0.106	42.2	0.84	1.75	1.47
0.155	47.0	0.83	1.43	1.68
0.211	49.2	0.58	1.16	1.68
0.249	47.0	0.31	0.30	0.73
0.281	53.1	0.46	0.53	1.18

[a] Initial glucose concentration = 4.44 mM. Results are the average of two determinations.
[b] These acids were not consistently separated on gas-liquid chromatography.

TABLE 3. Influence of L-threonine and L-serine on the growth of *M. elsdenii* in glucose-limited chemostat culture[a]

Initial concn (mM)		Final concn (mM)		Cell density (mg ml^{-1})
Threonine	Serine	Threonine	Serine	
0.42	0.38	0	0.06	0.227
0.43	1.12	0	0.24	0.233
0.96	1.19	0.10	0.13	0.230
1.98	1.99	0.04	0.12	0.224
1.98	3.73	0.05	0.19	0.234

[a] D = 0.13 h^{-1}. Other amino acids were present at the same reservoir concentration as in Table 1. Residual glucose was not measured.

would be equivalent to an ATP production rate of 1.3 mmol g^{-1} h^{-1} (7), whereas the latter can be converted to ATP by using the extrapolated maximum yield (56.6 g mol^{-1}) and by assuming that the average ATP molar growth yield for rumen bacteria of 15.1 g mol^{-1} (5) applies to *M. elsdenii*. Thus 3.74 mol of ATP would be produced per mol of glucose, and the apparent maintenance energy contributed by glucose becomes 2.2 mmol of ATP g^{-1} h^{-1}. Branched-chain VFA production would therefore contribute 37% of the total maintenance requirement of 3.5 mmol of ATP g^{-1} h^{-1}.

Amino acid catabolism may therefore be concluded to be of minor significance energetically to *M. elsdenii*, except for some provision of maintenance energy. The main importance of amino acid catabolism is probably to other organisms in the rumen ecosystem, in the supply of NH_3 and branched-chain VFA that are essential for the growth of cellulolytic bacteria.

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