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Inhibition of Methanogenesis in Rumen Fluid Cultures

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Summary

We identified 32 compounds that inhibit 13 to 100% of the methane produced by in vitro cultures of rumen fluid and have the potential to inhibit enteric methanogenesis in ruminant animals. The compounds are analogous to a substrate in the methane biosynthesis pathway, and may inhibit methane production yet not affect other organisms in the rumen.

Introduction

Ruminal methanogens consume CO₂ and H₂, thereby depleting substrates used by bacteria to make volatile fatty acids. Methanogenesis accounts for a 3 to 12% loss of feed gross energy. Retention of lost feed gross energy would be a direct addition to the amount of energy available for gain, which is typically 30% of the feed gross energy. Methane is also a greenhouse gas and cattle account for approximately 15% of methane emissions to the atmosphere. Therefore, a strategy to inhibit ruminal methanogens could improve feed efficiency by up to a third and also be environmentally advantageous.

The enzyme 4-(β-D-ribofuranosyl)aminobenzene-5'-phosphate (RFAP) synthase, is a key to methane synthesis. Blocking this enzyme could inhibit methanogens. Because RFAP synthase is a methanogen specific enzyme, we expect that its inhibition would be selective for methanogens. The objective of this work was to determine if ruminal methane synthesis could be inhibited by analogs to a substrate of RFAP synthase.

Procedure

Analogues of para-amino benzoate were synthesized in the laboratory.

Table 1. Inhibition of methane production by selected compounds at multiple concentrations.

Compound and concentration ^c	% Inhibition	Compound and concentration	% Inhibition
A24		C33	
5.0 mM	65.5 ^a	5.0 mM	99.2 ^a
2.5 mM	61.1 ^a	2.5 mM	98.8 ^a
1.0 mM	39.1 ^a	1.0 mM	98.5 ^a
0.1 mM	0	0.1 mM	19.2 ^a
A61		C34	
5.0 mM	36.9 ^a	5.0 mM	100.0 ^a
2.5 mM	21.2 ^a	1.0 mM	88.8 ^a
1.0 mM	16.9 ^b	0.5 mM	65.0 ^a
0.1 mM	0.9	0.1 mM	13.0 ^a
B11		C42	
5.0 mM	37.7 ^a	5.0 mM	100.0 ^a
2.5 mM	11.8	2.5 mM	92.5 ^a
1.0 mM	6.8	1.0 mM	85.4 ^a
0.1 mM	6.6	0.1 mM	0

^aComparison to untreated vials ($P < 0.05$).

^bComparison to untreated vials ($P < 0.10$).

^c1 mM = 6.02×10^{17} molecules/mL.

Para-amino benzoate is one substrate of the enzyme targeted for inhibition. The analogs are identified by sequential numbers and collectively referred to as candidate inhibitors. The candidates were evaluated for ability to inhibit ruminal methane synthesis by use of an in vitro culture system.

McDougall's buffer (100 mL), distilled H₂O (100 mL), cellobiose (0.5 g), trypticase (0.5 g), Resazurin (0.25 mg), a micro mineral solution (25 μL), and ruminal fluid (53 mL) were gassed with CO₂ to create oxygen-free media. Candidates dissolved in dimethyl sulfoxide (DMSO) were added to individual 9.4 mL glass vials, in quadruplicate. Oxygen-free gas (H₂/CO₂, 80:20) was projected into the vials as the fermentation medium (4 mL) was added. The vials were pressurized to 100 kPa (1 atmosphere), and allowed to incubate in a water bath (37°C) for 22 hours.

Following incubation, pressure in the headspace of the vials was measured. Methane concentration was determined by gas chromatography using a silica packed column and thermal conductivity detector.

Results

Initially, 118 candidate RFA-P synthase inhibitors were tested at a concentration of 5 mM. The results of

these tests are presented in Figure 1. Compounds such as A36, A83, C23, and C39 inhibited methane production from 14 to 20%, which was indicated by a tendency ($P < 0.10$) for treated vials to contain less methane than control vials following incubation. Methane production was decreased by 32 of the 118 compounds tested ($P < 0.05$). Inhibition ranged from 13 (A41) to 100% (C34 and C42).

Several compounds that inhibited methane production by greater than 30% were tested again at lower concentrations (Table 1). Some of these were effective at concentrations of 1 mM or less (A24, A61, C33, C34, and C42).

These observations indicate it is possible to block synthesis of methane by ruminal organisms by using chemicals that inhibit RFAP synthase. The development of this approach into a commercially feasible application we will require the identification of compounds capable of inhibiting the enzyme at lower dosages. It would not be practical to manufacture an amount of the current inhibitors that would be required to achieve a 1 mM concentration in the rumen.

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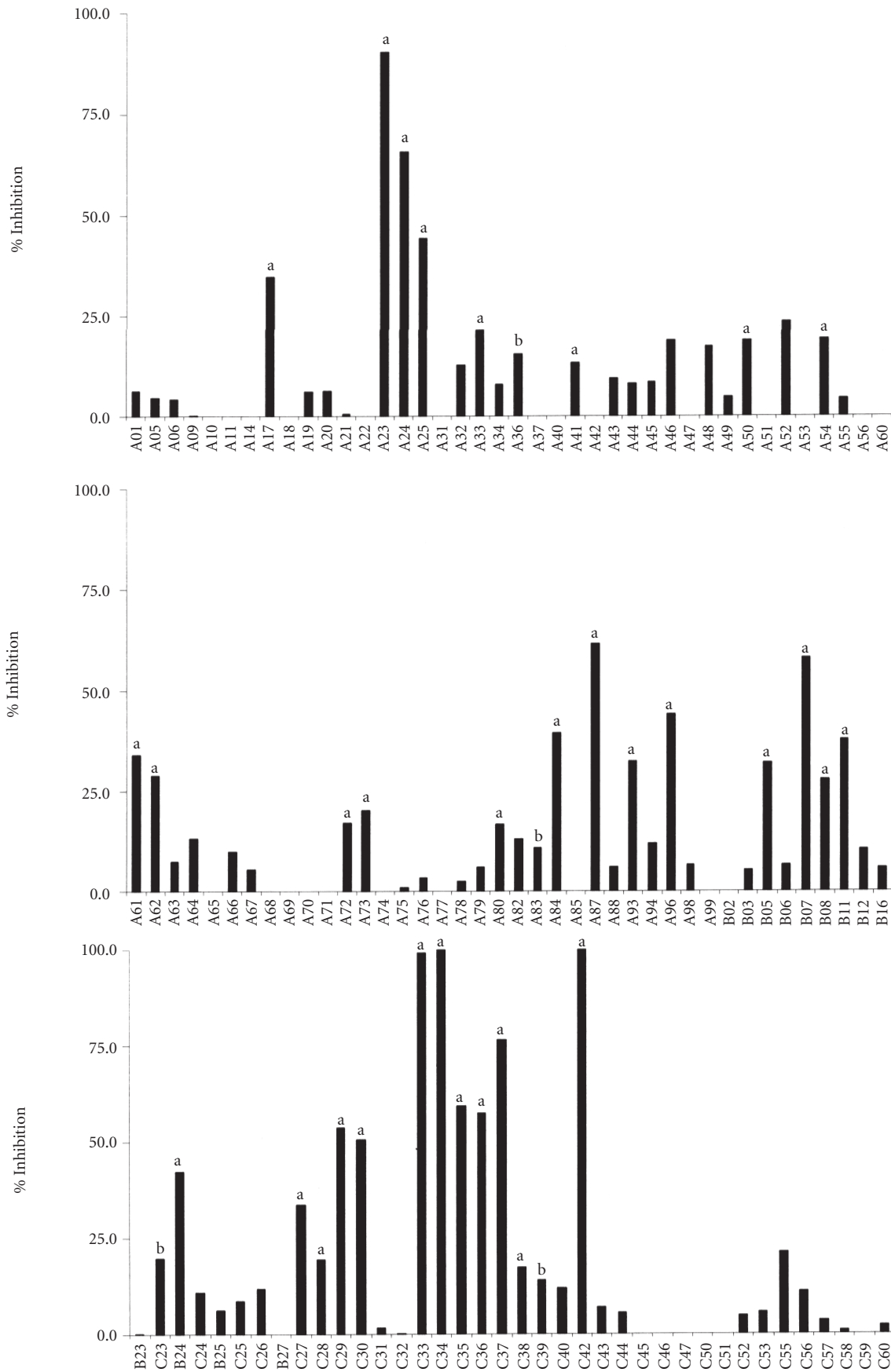


Figure 1. Percent inhibition of methane production (y-axis) exhibited by compounds (x-axis) tested at a concentration of 5 mM. ^a indicates a difference ($P < 0.05$) and ^b indicates a tendency ($P < 0.10$) for treated vials to produce less methane than untreated vials.