Competition for hydrogen by human faecal bacteria: evidence for the predominance of methane producing bacteria

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Abstract

Studies of sludge have shown that some species of sulphate reducing bacteria outcompete methane producing bacteria for the common substrate H₂. A similar competition may exist in human faeces where the methane (CH₄) producing status of an individual depends on the faecal concentration of sulphate reducing bacteria. To determine if non-methanogenic faeces outcompete CH₄ producing faeces for H₂, aliquots of each type of faeces were incubated alone or mixed together, with or without addition of 10% H₂ and/or 20 mmol/l sulphate. Methane producing faeces consumed H₂ significantly more rapidly and reduced faecal H₂ tension to a lower value compared with non-methanogenic faeces. The mixture of the two types of faeces yielded significantly more CH₄ than CH₄ producing faeces alone (mean (SD) 8.5 (1.3) v 2.9 (0.45) mmol/l of homogenate per 24 hours, p<0.01). Faecal sulphide concentrations were similar in CH₄ producing and non-producing homogenates both before and after 24 hours of incubation. The addition of sulphate to the homogenates did not significantly influence CH₄ production or sulphide formation. Our results suggest that in human faeces methane producing bacteria outcompete other H₂ consuming bacteria for H₂.

Methane (CH₄), a metabolic product of a group of anaerobic bacteria, is excreted consistently in appreciable quantity by some subjects but not others. In various population groups the prevalence of CH₄ excretors has been found to range from 24% to 95%. Since CH₄ is not metabolised in man, the ability of subjects to excrete this gas reflects the number or activity, or both, of the methanogenic flora present in the colon. Why only select subjects harbour a CH₄ producing flora has piqued the interest of numerous investigators.

The sole source of energy of most species of methane producing bacteria is via the oxidation of H₂ produced by other organisms and the activity of methanogens is limited by low H₂ availability. Methanogenesis consumes 4 moles of H₂ to reduce 1 mole of CO₂ to CH₄, a process that greatly reduces the volume of gas that would otherwise be present in the colon. Thus, understanding the factors that regulate the activity of the CH₄ producing flora could provide both clinically relevant information with regard to flatulence, as well as basic knowledge concerning the factors that regulate the proliferation and/or activity of colonic bacteria.

Sulphate reducing bacteria (SRB) also use H₂ to reduce sulphate to sulphide, and studies of sludge and sediments have shown the SRB outcompete methane producing bacteria for H₂ when adequate sulphate is available. As a result, the presence of a high concentration of SRB limits methanogenesis. The mystery of why some subjects consistently excrete CH₄, while others do not, was apparently solved by Gibson and co-workers who carried out a number of studies suggesting that a similar competition between methane producing bacteria and SRB exists in the human colon lumen. Thus the inability of a subject to excrete CH₄ apparently reflects the presence of a non-methanogenic colon flora that outcompete methane producing bacteria for H₂.

If this hypothesis were correct, it follows that CH₄ formation by CH₄ producing faeces should be reduced appreciably by admixture with non-methanogenic faeces, and this reduction should be reversed in the presence of a large excess of H₂. This concept was tested in the present study by incubating CH₄ producing and non-methanogenic faeces, individually or mixed together, with and without the addition of H₂ and/or sulphate. Surprisingly, we found that methanogenesis actually was enhanced by the presence of non-methanogenic faeces, even when large quantities of sulphate were provided for SRB metabolism.

Methods

FAECAL HOMOGENATES

We studied faeces from eight healthy adult volunteers who were all on a conventional diet and who had not taken antibiotics during the two months before the study. On the basis of previous results, faeces of four of them were known to produce large quantities of CH₄, while faeces of the other four produced little or no CH₄.

Faecal homogenates were prepared by homogenising faeces (1:5 w/v) in 0.1 M phosphate buffer (pH 7.0). Strict anaerobiosis was maintained during the procedure and all vessels, syringes, and solutions were exhaustively purged with argon before use. The four CH₄ producing faecal samples were paired with the four non-producing samples and a series of four experiments were then carried out in which faeces from the producer and the non-producer were incubated singly or mixed together. Incubations were carried out in 12 50 ml gas-tight syringes sealed with stopcocks. Four syringes contained 5 ml aliquots of the CH₄ producing homogenate plus 5 ml of phosphate buffer, four
Competition

PH2

methanogenic

types

show the results

supplemented

represented

10%.
The graph on the right

shows the results obtained

when the homogenates were

supplemented with 20 mM

Na2SO4. Data are represented as mean (SEM).

contained 5 ml aliquots of the non-methanogenic

homogenate plus 5 ml of phosphate buffer, and four

contained 10 ml of a mixture (1:1) of the two

types of faeces. One of the following was then

added to one of the four syringes that comprised

the above sets: (a) argon (30 ml); (b) H2 (3 ml)

and argon (27 ml); (c) 20 mM Na2SO4 and argon

(30 ml); or (d) 20 mM Na2SO4, H2 (3 ml) and

argon (27 ml). A rubber sleeve attached to a

septum was slipped over the male end of the

stopcock. At the time of removal of a gas sample

from the syringe, the stopcock was turned from

the position where the syringe was sealed, to a

position where the syringe was open to the

septum. A 21 gauge needle (attached to a 1 ml

syringe) was then inserted through the septum

and the stopcock into the gas space of the

syringe, and a 1 ml gas sample was obtained.

Incubation was carried out at 37°C on a

rotating wheel. Gas samples were obtained for

analysis at 0, 1, 2, 4, and 24 hours of incubation.

Aliquots of each homogenate were collected

before and after 24 hours of incubation for

sulphide analysis; 12% zinc acetate was ana-

erobically added to each aliquot in a ratio of 1:4

to prevent oxidation of sulphide.

ANALYSES

Gas samples were analysed for H2 and CH4

within six hours of collection using a gas chro-

matograph equipped with a molecular sieve

column, a reduction detector for H2, and a flame

ionisation detector for CH4.

The method of Cline for the measurement of

sulphide in water was modified for faecal sul-

phide measurements. Briefly, the homogenate

was diluted 1:20 with distilled water and three

aliquots of 0.909 ml were used. The first aliquot,

that was treated with 0.72 μl of 50% HCl and

g rigorously stirred for 30 minutes to drive off all

sulphide, served as a blank. The second was

spiked with 18.2 μl of zinc acetate–sodium

sulphide standard (2·6 mM) to evaluate sulphide

recovery. The third aliquot was used for the
determination of sulphide content of the speci-

dmen. The colorimetric reaction was carried out

in 1.5 ml Eppendorf tubes that were immediately

sealed following the addition of 0.80 μl of

diamine–ferric chloride reagent made up in 50%

HCl. At the time of reagent addition, 50% HCl

(0.72 μl) was added to aliquots two and three

and zinc acetate solution (18.2 μl) was added to

aliquots one and three. After 30 minutes of

incubation at room temperature, samples were

centrifuged at 12000 g for three minutes and the

absorbance of the supernatant was spectrophot-

ometrically determined at 670 nm. Percentage

recovery of sulphide from spiked aliquots

averaged 87% (range 73–99%). Sulphide con-

centration of a given sample of homogenate was

calculated from the optical density of the sample

minus that of the HCl treated sample, corrected

for the percentage recovery determined from the

spiked sample.

CALCULATIONS

The volume of H2 or CH4 present at any time

point was calculated from the concentration of

the respective gas and the volume of gas present

in the syringe, plus the volume of H2 or CH4

calculated to have been previously removed for

analysis. The consumption rate of H2, deter-

mined from samples incubated with 10% H2, was

normalised for H2 tension (P(H2)) and expressed as

μmol/hour per litre of homogenate per atmos-

phere of PH2. The P(H2) of a given time period

was considered equal to the arithmetic mean of

the H2 tensions at the beginning and end of the

time period. Data were expressed as mean (SEM).

Statistical analyses for significance were per-

formed using the Student t tests for paired and

for unpaired data.

Results

Figure 1 shows mean H2 consumption (nor-

malised for PH2) by CH4 producing faeces, non-

methanogenic faeces, and the mixture of the two,
in the absence and presence of additional

Na2SO4. Methane producing faeces consumed
Methane formation* by CH₄ producing faeces, non-methanogenic faeces, and by the mixture of the two during incubation with and without addition of H₂ and/or Na₂SO₄

| Substrate added | Incubation (hours) | None | H₂ (10%) | Na₂SO₄ (20 mM) | H₂ + Na₂SO₄ (10%/20 mM) |
|-----------------|-------------------|------|----------|----------------|-----------------------|
| CH₄ producing   | 1                 | 0-30 (0-09) | 3-5 (0-38) | 0-21 (0-054) | 2-16 (0-0045) |
|                 | 2                 | 0-54 (0-14) | 1-98 (0-50) | 0-43 (0-099) | 1-89 (0-50)     |
|                 | 4                 | 0-99 (0-22) | 2-66 (0-41) | 0-77 (0-14)  | 2-57 (0-45)    |
|                 | 24                | 2-89 (0-45) | 4-37 (0-38) | 2-25 (0-32)  | 4-14 (0-45)    |
| Non-methanogenic| 1                 | 0-0 (0-0)   | 0-0 (0-0)  | 0-0 (0-0)    | ND          |
|                 | 2                 | 0-0 (0-0)   | ND         | ND           | 0-0059 (0-0045) |
|                 | 4                 | ND         | ND         | 0-0086 (0-0068) |
| Mixture         | 1                 | 0-013 (0-012) | ND         | 0-0027 (0-0026) | 0-020 (0-011) |
|                 | 2                 | 0-68 (0-15) | 1-22 (0-36) | 0-72 (0-18)  | 1-35 (0-54)    |
|                 | 2                 | 1-26 (0-18) | 2-16 (0-54) | 1-22 (0-25)  | 2-25 (0-54)    |
|                 | 4                 | 2-30 (0-13) | 3-87 (0-54) | 2-12 (0-36)  | 3-51 (0-72)    |
|                 | 24                | 8-55 (2-26) | 10-4 (1-3)  | 7-20 (1-49)  | 8-55 (1-62)    |

*Data are expressed as average (SEM) in mmol/l/homogenate. ND = <0-0045 mmol CH₄/l of homogenate.

H₂ significantly more rapidly than non-methanogenic faeces during the time periods 0–1 hour (p<0-05), 1–2 hours (p<0-001), and 2–4 hours (p<0-001). The mixture of the homogenates had a H₂ consumption rate comparable to that of CH₄ producing faeces, and significantly (p<0-01) higher than that of non-methanogenic faeces at 1, 2, and 4 hours of incubation. The addition of Na₂SO₄ had no statistically significant effect on H₂ consumption by any of the homogenates at any sampling time. After 24 hours of incubation the P₄₀ of the CH₄ producing homogenates (1950 (325) ppm) was much lower (p<0-0001) than that of the non-methanogenic homogenates (39200 (4600) ppm). The P₄₀ reached in the mixture of homogenates (2900 (450) ppm) was comparable to that of the CH₄ producing homogenates and significantly lower (p<0-0001) than that of non-methanogenic homogenates. Similar results were obtained in the presence of added Na₂SO₄.

The mean CH₄ production by the different incubates is summarised in the Table. Trivial CH₄ production occasionally was observed in faeces considered to be non-methanogenic, but the highest value did not exceed 1% of the values observed with CH₄ producing faeces or with the mixture. This very low production was not significantly enhanced by the addition of 10% H₂ to the gas space, in contrast to the increase found with CH₄ producing homogenates.

In the absence of added H₂, CH₄ formation by CH₄ producing faeces was not inhibited by admixture with non-methanogenic faeces, but rather was enhanced in each of the four pairs of homogenates. This enhancement was statistically significant after 2, 4 and 24 hours of incubation (Fig 2). When H₂ was added, the increase in CH₄ production was statistically significant only after 24 hours. The addition of Na₂SO₄ had no significant effect on CH₄ production by any of the homogenates (Fig 2 and Table).

Before incubation, sulphide concentration averaged 0-18 (0-043) mM for non-methanogenic faeces and 0-15 (0-047) mM for CH₄ producing faeces (NS). Compared to the non-supplemented mixtures, neither the addition of 10% H₂, Na₂SO₄, nor both significantly influenced sulphide concentrations (Fig 3) after 24 hours of incubation. The tendency for faecal sulphide concentration to increase with incubation did not reach statistical significance in either the CH₄ producing or non-methanogenic homogenates. The increase found in CH₄ producing faeces was not significant. This enhancement was found in CH₄ producing faeces supplemented with sulphate. This sulphide production would have consumed only about one twentieth of the H₂ consumed via CH₄ formation.

Discussion

The findings of our study sharply contrast with previous reports*⁷⁸ suggesting that the absence of CH₄ production in the colon of certain individuals reflects the presence of high concentrations of organisms, such as SRB, that outcompete methanogens for H₂. These reports have shown that CH₄ producing faeces usually contained less than 10⁶ SRB/g dry weight while non-methanogenic faeces always contained more than 10⁹ SRB/g dry weight.¹² and that the sulphide concentration of CH₄ producing faeces was much lower than that of non-methanogenic faeces. In addition, incubation of CH₄ producing faeces with non-methanogenic faeces was reported to inhibit CH₄ formation.⁷

Our study provided two independent lines of evidence that led us to conclude that competition for H₂ does not explain why some subjects fail to excrete CH₄. First, if a lack of CH₄ production reflects very rapid H₂ consumption by non-methanogenic bacteria, one might expect that faeces that did not produce CH₄ would consume H₂ more efficiently than CH₄ producing faeces. To the contrary, we found that added H₂ was consumed about five times more rapidly by CH₄ producing faeces (see Fig 1). More important, after 24 hours of incubation, CH₄ producing faeces reduced the P₄₀ of the homogenate to one twentieth of that observed in non-methanogenic faeces. Since the two type of faeces have been shown to have similar absolute H₂ production rates,⁷ methanogens apparently are able to consume H₂ at a lower P₄₀ than other H₂ consuming bacteria. These results agree with the in vivo observation that CH₄ producing faeces excrete

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![Figure 2: Methane production (mean (SEM)) by faecal homogenates during 24 hours of incubation without (left) and with 10% H₂ (right). From left to right, the four bars at each time point respectively represent: (a) CH₄ producing faeces without supplement; (b) CH₄ producing faeces supplemented with 20 mmol/l Na₂SO₄; (c) mixture of CH₄ producing faeces with non-methanogenic faeces without supplement; (d) mixture of CH₄ producing faeces with non-methanogenic faeces supplemented with 20 mmol/l Na₂SO₄. Asterisks indicate a significant difference (p<0-02) between the CH₄ producing homogenates and the mixture.](http://gut.bmj.com/firstpublished/10.1136/gut.32.12.1498)
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Pitt P, De Bruijn KM, Beeching MF, Goldberg E, Blendis LM. Studies on breath methane: the effect of ethnic origins and lactulose. Gut 1980; 21:951–9.

Segal I, Walker ARP, Lord S, Cummings JH. Breath methane and large bowel cancer risk in contrasting African populations. Gut 1988; 29:608–13.

Weaver GA, Krause JA, Miller TL, Wolin MJ. Incidence of methanogenic bacteria in a sigmoidoscopy population: an association of methanogenic bacteria and diverticulosis. Gastroenterology 1986; 1:768–704.

Lovley DR, Dwyer DF, Klug MJ. Kinetic analysis of competition between sulphate reducers and methanogens for hydrogen in sediments. Appl Environ Microbiol 1982; 1:1373–9.

Kristjansson JK, Schoenheit P, Thauer RK. Different K, values for hydrogen of methanogenic bacteria and sulphate reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulphate. Arch Microbiol 1982; 1:278–82.

Lupton FS, Zielkus JG. Physiological basis for sulphate-dependent hydrogen competition between sulphidogens and methanogens. Curt Microbiol 1984; 1:7–12.

Gibson GR, Cummings JH, Macfarlane GT. Competition for hydrogen between sulphate-reducing bacteria and methanogenic bacteria from the human large intestine. J Appl Bacteriol 1988; 65:241–7.

Gibson GR, Cummings JH, Macfarlane GT. Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulphate reduction and methanogenesis by mixed populations of human gut bacteria. Appl Environ Microbiol 1988; 54:2750–5.

Gibson GR, Cummings JH, Macfarlane GT, et al. Alternative pathways for hydrogen disposal during fermentation in the human colon. Gut 1990; 31:679–83.

Gline JD. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 1969; 14:454–8.

Gibson GR, Macfarlane GT, Cummings JH. Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. J Appl Bacteriol 1988; 65:103–11.

Strucchi A, Levitt MD. Are varying H2 consumption rates in the colon the major determinant of H2 excretion? Gastroenterology 1990; 98: A205.

Bjorndal A, Jensen E. Relationships between hydrogen (H2) and methane (CH4) production in man. Scand J Gastroenterol 1982; 17:985–92.

Gibson GR, Macfarlane GT, Cummings JH. Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. J Appl Bacteriol 1988; 65:103–11.

Strucchi A, Levitt MD. Are varying H2 consumption rates in the colon the major determinant of H2 excretion? Gastroenterology 1990; 98: A205.

Bjorndal A, Jensen E. Relationships between hydrogen (H2) and methane (CH4) production in man. Scand J Gastroenterol 1982; 17:985–92.

Gibson GR, Macfarlane GT, Cummings JH. Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. J Appl Bacteriol 1988; 65:103–11.

Strucchi A, Levitt MD. Are varying H2 consumption rates in the colon the major determinant of H2 excretion? Gastroenterology 1990; 98: A205.