Methanogens outcompete sulphate reducing bacteria for \( \text{H}_2 \) in the human colon

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Abstract

Methanogens and sulphate reducing bacteria compete for \( \text{H}_2 \) in the human colon, and, as a result, faeces usually contain high concentrations of just one of these two organisms. There is controversy over which of these organisms wins the competition for \( \text{H}_2 \), although theoretical data suggest that sulphate reducing bacteria should predominate. To elucidate this question experiments were undertaken in which sulphate enriched homogenates of human sulphate reducing faeces and methane producing faeces were incubated separately or mixed together. Co-incubation of sulphate reducing faeces with methanogenic faeces resulted in a sixfold reduction in the activity of the sulphate reducing bacteria (measured as sulphide production), whereas methane production was not inhibited by co-incubation with sulphate reducing bacteria. Methanogenic faeces also consumed \( \text{H}_2 \) more rapidly and reduced the \( \text{H}_2 \) tension of the homogenate to a lower value than did sulphate reducing faecal samples. In these experiments, methanogens seem to outcompete sulphate reducing bacteria for \( \text{H}_2 \).

If a sulphate reducing flora can utilise \( \text{H}_2 \) more efficiently than a methanogenic flora, one would expect that, in the presence of limited \( \text{H}_2 \), co-incubation of faeces containing each type of flora would result in an inhibition of \( \text{CH}_4 \) production. In a previous report, however, we found that \( \text{CH}_4 \) liberation continued unabated when methanogenic and non-methanogenic faeces were co-incubated, and thus concluded that methanogens predominated. This study has been criticised because no evidence was provided that the non-methanogenic faeces contained sulphate reducing bacteria.

In response to this criticism, we now report \( \text{H}_2 \) competition studies utilising faecal samples in which the presence of strong sulphate reducing activity was documented. These studies indicate that despite their apparent theoretical inferiority, methanogens experimentally outcompete sulphate reducing bacteria for \( \text{H}_2 \).

Methods

EXPERIMENTAL DESIGN

Studies were carried out using freshly passed faeces from six healthy adult volunteers, three of whom were known from previous studies to have faeces with methanogenic activity and three to have strong sulphate reducing activity. The ability of the latter samples to utilise \( \text{H}_2 \) for this reduction reaction was studied by comparing the rates of \( \text{H}_2 \) disappearance from a 10% head space in the presence or absence of 20 mM sodium molybdate, an inhibitor of sulphate reduction. Hydrogen consumption was expressed in units of ml consumed-24 hours-1-log mean [\( \text{H}_2 \)]-1 because of the linear relation between \( \text{H}_2 \) consumption and \( \text{H}_2 \) concentration in the range of \( \text{H}_2 \) tensions observed in these experiments. In the absence of molybdate, the \( \text{H}_2 \) consumption rate of the three samples was 3-0, 2-6, and 3-8 ml-24 h-1-mM-1, falling to, respectively, 0-41, 0-42, and 0-07 ml-24 h-1-mM-1 in the presence of molybdate.

An individual experiment consisted of incubating nine syringes, three containing aliquots of a methanogenic homogenate, three containing a sulphate reducing homogenate, and three a mixture of the two types of homogenates.

Faeces were anerobically homogenised with deoxygenated saline (1:20 w/v) containing 0-2 M \( \text{PO}_4 \) (pH 7-0) and 20 mM \( \text{Na}_2\text{SO}_4 \) in a blender vessel that had been purged with argon. Aliquots (5 ml) of each homogenate were aspirated into 50 ml glass, gas-tight
Methanogens outcompete sulphate reducing bacteria for H2 in the human colon

syringes, and 5 ml of buffer were then added to each syringe. The faecal mixture consisted of 5 ml of a methanogenic and 5 ml of sulphate reducing homogenate. Gas (20 ml) consisting of 10% CO2 and 90% argon was added to each syringe and incubation was carried out at 37°C on a rotating wheel.

After 2, 4, or 24 hours of incubation, the gas space was removed from a syringe for H2 and CH4 measurements, the pH of an aliquot of homogenate was measured via a pH meter. To prevent oxidation of sulphide during storage, 15% (w/v) zinc acetate was then added in sufficient quantity to yield a 3% solution of zinc sulphate in the homogenate.

ANALYSES
Gas samples were analysed for H2 and CH4 concentration by gas chromatography (Beckman Instruments, Fullerton, CA) using a gas sampling valve, a molecular sieve column (3 ft x 1/8"), an oven temperature of 100°C, and argon (30 ml/min) as the carrier gas. A reduction detector (Trace Analytical, Menlo Park, CA) was used for detection of H2, and a flame ionisation detector for CH4. Sulphide measurements were performed using the method described by Cline for analysis of water, modified to permit accurate assessment of sulphide in faeces.

RESULTS
In each of the experiments performed with a separate pair of methanogenic and sulphate reducing homogenates, sulphate reduction as measured by sulphide production was considerably reduced during co-incubation with methanogenic faeces (see Fig 1). In contrast, CH4 production was not influenced by co-incubation with sulphate reducing faeces (Fig 2). To determine if methanogenic faeces metabolised sulphate, methanogenic homogenates were spiked with sodium sulphide to yield a faecal concentration of 39 μM. After 24 hours of incubation at 37°C, the sulphide concentration of the homogenates averaged 42 μmol.

At each time point, the quantity of H2 appearing in the head space of the homogenates containing methanogenic faeces was appreciably less than was observed with sulphate reducing faeces (Fig 3). At 22 hours, the average PH2 of the methanogenic homogenates had been reduced to about 0·076 torr (100 ppm), or about 20 times lower than that of the sulphate reducing homogenates (1·4 torr or 1800 ppm). The H2 concentration of the

CALCULATIONS
The volumes of H2 and CH4 were determined from the total volume of gas in the syringe (to the nearest 0·5 ml) and the concentration of the specific gas. Results for the various types of incubations are presented as mean (SEM).

The relative Vmax of faecal productions of CH4 and sulphide were calculated from the standard equation describing the reaction rate with Michaelis-Menten kinetics:

\[ \text{Reaction rate} = \frac{V_{\text{max}} \times \text{[substrate]}}{K_s + \text{[substrate]}} \]  

where [substrate] concentration refers to the H2 concentration in the homogenate estimated from the partial pressure of H2 in the head space and an H2 partition coefficient in water.

![Figure 1: Sulphide production by sulphate reducing faeces incubated alone (open triangles) and sulphate reducing faeces co-incubated with methanogenic faeces (solid circles).](image1)

![Figure 2: Methane production by methanogenic faeces incubated alone (solid circles) or co-incubated with sulphate reducing faeces (solid circles).](image2)

![Figure 3: Net H2 production by sulphate reducing faeces (open triangles), methanogenic faeces (solid triangles) or the mixture of methanogenic and sulphate reducing faeces (solid circles).](image3)
methanogenic homogenates (about 0·1 μM) after 24 hours of incubation was only 1/60 of the 6 μM value cited for the \( K_a \) reported for methanogens.13

A relatively steady state for \( H_2 \) concentration existed between two and four hours of incubation. During this period the mean \( H_2 \) concentrations in the head space averaged 250 ppm and 4400 ppm, respectively, for the methanogenic and sulphate reducing homogenates; these values indicate \( H_2 \) concentration in the homogenates of about 0·20 μM and 3·6 μM, respectively. During this two hour period, the homogenates produced a mean of 31 μmol/g of \( CH_4 \) and 7·1 μmol/g of sulphide. Utilising these production rates and published \( K_a \) values for sludge of 6 μM for methanogens and 1 μM for sulphate reducing bacteria,13 equation (1) yields values for \( V_{max} \) of 480 μmol·g\(^{-1}\)·h\(^{-1}\) and 4·5 μmol·g\(^{-1}\)·h\(^{-1}\) for methanogenesis and sulphate reduction, respectively.

The \( pH \) values of the two types of homogenates and the mixture were similar and averaged 7·0, 6·9, 6·8, and 6·8 respectively at 0, 2, 4, and 24 hours of incubation.

**Discussion**

In the present study methanogenic and sulphate reducing faeces were incubated separately and in combination, and the production of the respective metabolic products of \( H_2 \) consumption, \( CH_4 \) and sulphide, were measured. The objective was to determine which type of flora most effectively utilised \( H_2 \). To ensure that the present experiments utilised faeces with a strong sulphate reducing capability, faeces from 20 healthy subjects were preliminarily screened for sulphide production during a 24 hour incubation with excess sulphate. The three subjects with the greatest faecal sulphide production then provided faeces for the present study. Since the \( H_2 \) consumption rate of each of these faecal samples was appreciably reduced in the presence of molybdate, a specific inhibitor of sulphate reduction,8 it was clear that \( H_2 \) was being oxidised in the sulphate reducing reaction.

When sulphate reducing faeces were incubated with methanogenic faeces, the sulphide concentration at 24 hours fell to about 15% of that observed when sulphate reducing faeces were incubated alone. Since the methanogenic homogenates showed no ability to catabolise sulphide, the reduced concentration of sulphide in the homogenate mixture apparently represented inhibition of sulphide production. In contrast, \( CH_4 \) production by the faecal mixtures persisted at the same rate as that observed in the methanogenic homogenates. Thus, under the conditions of this study, methanogenic bacteria seemingly outcompeted sulphate reducers for the limited \( H_2 \) available in homogenates incubated without additional \( H_2 \) or fermentable substrate.

The greater \( H_2 \) consuming ability of methanogenic faeces was supported by the observation that less \( H_2 \) appeared in the head space of the methanogenic than the sulphate reducing homogenates. This \( H_2 \) represents the net of absolute production minus consumption, and, assuming relatively equal absolute \( H_2 \) production rates,9 the paucity of \( H_2 \) in the head space over the methanogenic faeces indicates more rapid \( H_2 \) consumption. The very low \( H_2 \) concentration (about 0·1 μM) of the methanogenic homogenates attained at 24 hours of incubation indicates that at a \( H_2 \) concentration that was only 1/60 of the putative \( K_a \) of 6 μM,13 methanogens were capable of consuming \( H_2 \) at least as rapidly as this gas was being produced.

This apparent predominance of methanogens cannot be attributed to non-availability of sulphate, since homogenates were supplemented with 20 mM sulphate, a concentration far in excess of that required to oxidise all the \( H_2 \) released during the incubation. In addition, this result cannot be explained by a \( pH \) that favoured methanogenesis since both reactions are near maximal at the neutral \( pH \) of the homogenates.1 Lastly, since sulphate reducing bacteria are less \( O_2 \)-sensitive than methanogens, the predominance of methanogenesis in our experiments cannot be attributed to failure to maintain anaerobic conditions. While the possibility remains that some unknown aspect of the intracolonic environment was not simulated in our homogenates, for the present we conclude that human colonic methanogens more rapidly and efficiently consume \( H_2 \) than do sulphate reducing bacteria. Thus, the presence or absence of methanogens in the colon should determine the numbers of sulphate reducing bacteria rather than vice versa.

Based on thermodynamic calculations, Gibson et al5 have suggested that sulphate reduction should predominate over methanogenesis in the human colon since studies with sediment organisms have shown that the affinity constant for \( H_2 \) of sulphate reducing bacteria (\( K_a \) : 1 μmol/l) is appreciably lower than that of methanogens (\( K_a \) : 6 μmol/l). Furthermore, the energetics of \( H_2 \) oxidation favour sulphate reduction (\( \Delta G_o^{\prime\prime} = -152 \) kJ/mol) over methanogenesis (\( \Delta G_o^{\prime\prime} = -131 \) kJ/mol). Thus, we seem to have a situation that is analogous to the classic case of the bumble bee which, according to all laws of aerodynamics, should not be able to fly. However, the bumble bee is unable to read the aerodynamic literature, and, hence, continues to fly. Similarly, faecal methanogens, apparently blissfully ignorant of their thermodynamic inferiority, continue to outcompete sulphate reducers for \( H_2 \).

A possible explanation for the paradoxical predominance of our methanogenic homogenates is provided by an analysis of the data obtained during the two to four hour period of incubation when the \( H_2 \) tensions of the homogenates remained relatively constant. While the mean \( H_2 \) concentration in the methanogenic homogenate (0-20 μM) was only about 1/180 of that of the sulphate reducing homogenate (3·6 μM), \( CH_4 \) was produced about 4·5 times more rapidly than was
Methanogens outcompete sulphate reducing bacteria for H\textsubscript{2} in the human colon

sulphide. If the values cited in published reports for the $K_s$ of methanogenesis (6 $\mu$M) and sulphate reduction (1 $\mu$M) correctly represent the kinetics of the human colonic organisms, the maximal H\textsubscript{2} consuming ability per g of methanogenic faeces would be more than 100 times that of sulphate reducing faeces. Since differences of this magnitude have not been observed in studies carried out at very high H\textsubscript{2} tension, it seems possible that the $K_s$ values cited by Gibson et al\cite{1} for sludge organisms may not accurately reflect the relative H\textsubscript{2} affinities of the methanogenic and sulphate reducing bacteria that inhabit the human colon.


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Gut 1994 35: 1098-1101
doi: 10.1136/gut.35.8.1098

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