Methanogens outcompete sulphate reducing bacteria for H₂ in the human colon

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Abstract

Methanogens and sulphate reducing bacteria compete for H₂ 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 H₂, 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 H₂ more rapidly and reduced the H₂ 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 H₂.

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Methanogenic and sulphate reducing bacteria are the major H₂ oxidising organisms in the human colon.¹ It is thought that the limited availability of colonic H₂ leads to a competition between these two types of organisms; therefore, a given subject tends to harbour high concentrations of only one type of H₂ consumer. Attempts to establish which type predominates in this contest for H₂ have resulted in a minor continuing controversy. Studies with lake sediments have shown that sulphate reducing bacteria outcompete methanogens for H₂,^{2,3} and Gibson *et al*^{4,5} have marshalled a strong theoretical argument to support the concept that a similar situation exists in the human colon. Thus, these investigators propose that the ability of some subjects to consistently excrete appreciable CH₄ while others do not reflect the absence or presence respectively of a sulphate reducing flora. Additional data to support this concept was provided by a study showing that dietary supplementation with sulphate (the rate limiting substrate for H₂ consumption via sulphate reduction) reduced the faecal concentration of methanogens and CH₄ excretion of three of six CH₄ producing subjects.⁶

If a sulphate reducing flora can utilise H₂ more efficiently than a methanogenic flora, one would expect that, in the presence of limited H₂, co-incubation of faeces containing each type of flora would result in an inhibition of CH₄ production. In a previous report, however,⁷ we found that CH₄ 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.⁵

In response to this criticism, we now report H₂ 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 H₂.

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 H₂ for this reduction reaction was studied by comparing the rates of H₂ disappearance from a 10% head space in the presence or absence of 20 mM sodium molybdate, an inhibitor of sulphate reduction.⁸ Hydrogen consumption was expressed in units of ml consumed·24 hours⁻¹·log mean [H₂]⁻¹ because of the linear relation between H₂ consumption and H₂ concentration in the range of H₂ tensions observed in these experiments.⁹ In the absence of molybdate, the H₂ consumption rate of the three samples was 3·0, 2·6, and 0·38 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 anaerobically homogenised with deoxygenated saline (1:20 w/v) containing 0·2 M PO₄ (pH 7·0) and 20 mM Na₂SO₄ in a blender vessel that had been purged with argon. Aliquots (5 ml) of each homogenate were aspirated into 50 ml glass, gas-tight

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

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

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% CO_2 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 H_2 and CH_4 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.⁴

ANALYSES

Gas samples were analysed for H_2 and CH_4 concentration by gas chromatography (Beckman Instruments, Fullerton, CA) using a gas sampling valve, a molecular sieve column (3 ft×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 H_2 , and a flame ionisation detector for CH_4 . Sulphide measurements were performed using the method described by Cline for analysis of water,¹⁰ modified to permit accurate assessment of sulphide in faeces.¹¹

CALCULATIONS

The volumes of H_2 and CH_4 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 V_{max} of faecal productions of CH_4 and sulphide were calculated from the standard equation describing the reaction rate with Michaelis-Menten kinetics:

$$\text{Reaction rate} = V_{max} \cdot [\text{substrate}] / (K_s + [\text{substrate}]) \quad (1)$$

where [substrate] concentration refers to the H_2 concentration in the homogenate estimated from the partial pressure of H_2 in the head space and an H_2 partition coefficient in water:

gas of 0.018,¹² and K_s is the H_2 concentration that yields 1/2 the maximal reaction rate.

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, CH_4 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 H_2 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 P_{H_2} 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 H_2 concentration of the

Figure 3: Net H_2 production by sulphate reducing faeces (open triangles), methanogenic faeces (solid triangles) or the mixture of methanogenic and sulphate reducing faeces (solid circles).

methanogenic homogenates (about 0.1 μM) after 24 hours of incubation was only 1/60 of the 6 μM value cited for the K_s 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 concentrations 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 $\mu\text{mol/g}$ of CH_4 and 7.1 $\mu\text{mol/g}$ of sulphide. Utilising these production rates and published K_s 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 $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ and 4.5 $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ 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_s 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 al*⁵ 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_s : 1 $\mu\text{mol/l}$) is appreciably lower than that of methanogens (K_s : 6 $\mu\text{mol/l}$). Furthermore, the energetics of H_2 oxidation favour sulphate reduction ($\text{DGo}' = -152$ kJ/mol) over methanogenesis ($\text{DGo}' = -131$ 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

sulphide. If the values cited in published reports for the K_s of methanogenesis (6 μM) and sulphate reduction (1 μM) correctly represent the kinetics of the human colonic organisms, the maximal H_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_2 tension,⁹ it seems possible that the K_s values cited by Gibson *et al*⁵ for sludge organisms may not accurately reflect the relative H_2 affinities of the methanogenic and sulphate reducing bacteria that inhabit the human colon.

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