Methanogens outcompete sulphate reducing bacteria for $H_2$ in the human colon

A Strocchi, J Furne, C Ellis, M D Levitt

Abstract

Methanogens and sulphate reducing bacteria compete for $H_2$ 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 $H_2$, 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 $H_2$ more rapidly and reduced the $H_2$ 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 $H_2$.

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Methanogenic and sulphate reducing bacteria are the major $H_2$ oxidising organisms in the human colon. It is thought that the limited availability of colonic $H_2$ leads to a competition between these two types of organisms; therefore, a given subject tends to harbour high concentrations of only one type of $H_2$ consumer. Attempts to establish which type predominates in this contest for $H_2$ have resulted in a minor continuing controversy. Studies with lake sediments have shown that sulphate reducing bacteria outcompete methanogens for $H_2$, and Gibson et al 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 $CH_4$ 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 $H_2$ consumption via sulphate reduction) reduced the faecal concentration of methanogens and $CH_4$ excretion of three of six $CH_4$ producing subjects.

If a sulphate reducing flora can utilise $H_2$ more efficiently than a methanogenic flora, one would expect that, in the presence of limited $H_2$, co-incubation of faeces containing each type of flora would result in an inhibition of $CH_4$ production. In a previous report, however, we found that $CH_4$ 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 $H_2$ 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 $H_2$.

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 $H_2$ for this reduction reaction was studied by comparing the rates of $H_2$ 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 [H$_2$]$^{-1}$ because of the linear relation between $H_2$ concentration and $H_2$ consumption in the range of $H_2$ tensions observed in these experiments. In the absence of molybdate, the $H_2$ concentration 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 anerobically homogenised with deoxygenated saline (1:20 w/v) containing 0·2 M PO$_4$ (pH 7·0) and 20 mM Na$_2$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
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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).

RESULTS

In each of the experiments performed with a separate pair of methanogenic and sulphate reducing homogenates, sulphate reduction 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 μM.

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

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).
methanogenic homogenates (about 0.1 \( \mu \)M) after 24 hours of incubation was only 1/60 of the 6 \( \mu \)M value cited for the \( K_s \) reported for methanogens.\(^{13}\) A relatively steady state for \( \text{H}_2 \) concentration existed between two and four hours of incubation. During this period the mean \( \text{H}_2 \) concentrations in the head space averaged 250 ppm and 4400 ppm, respectively, for the methanogenic and sulphate reducing homogenates; these values indicate \( \text{H}_2 \) concentrations in the homogenates of about 0-20 \( \mu \)M and 3-6 \( \mu \)M, respectively. During this two hour period, the homogenates produced a mean of 31 \( \mu \)mol/g of \( \text{CH}_4 \) and 7-1 \( \mu \)mol/g of sulphide. Utilising these production rates and published \( K_s \) values for sludge of 6 \( \mu \)M for methanogens and 1 \( \mu \)M for sulphate reducing bacteria,\(^{13}\) equation (1) yields values for \( V_{\text{max}} \) of 480 \( \mu \)mol-g\(^{-1}\)-h\(^{-1}\) and 4.5 \( \mu \)mol-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 \( \text{H}_2 \) consumption, \( \text{CH}_4 \) and sulphide, were measured. The objective was to determine which type of flora most effectively utilised \( \text{H}_2 \). To ensure that the present experiments utilised faeces with a strong sulphate reducing capability, faeces from 20 healthy subjects were preliminary 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 \( \text{H}_2 \) consumption rate of each of these faecal samples was appreciably reduced in the presence of molybdate, a specific inhibitor of sulphate reduction,\(^{6}\) it was clear that \( \text{H}_2 \) 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, \( \text{CH}_4 \) 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 \( \text{H}_2 \) available in homogenates incubated without additional \( \text{H}_2 \) or fermentable substrate.

The greater \( \text{H}_2 \) consuming ability of methanogenic faeces was supported by the observation that less \( \text{H}_2 \) appeared in the head space of the methanogenic than the sulphate reducing homogenates. This \( \text{H}_2 \) represents the net of absolute production minus consumption, and, assuming relatively equal absolute \( \text{H}_2 \) production rates,\(^{6}\) the paucity of \( \text{H}_2 \) in the head space over the methanogenic faeces indicates more rapid \( \text{H}_2 \) consumption. The very low \( \text{H}_2 \) concentration (about 0-1 \( \mu \)M) of the methanogenic homogenates attained at 24 hours of incubation indicates that at a \( \text{H}_2 \) concentration that was only 1/60 of the putative \( K_s \) of 6 \( \mu \)M,\(^{13}\) methanogens were capable of consuming \( \text{H}_2 \) 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 \( \text{H}_2 \) 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 \( \text{O}_2 \)-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 \( \text{H}_2 \) 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 \( \text{H}_2 \) of sulphate reducing bacteria (\( K_s \); 1 \( \mu \)mol/l) is appreciably lower than that of methanogens (\( K_s \); 6 \( \mu \)mol/l). Furthermore, the energetics of \( \text{H}_2 \) oxidation favour sulphate reduction (\( \Delta G^\circ = -152 \) kJ/mol) over methanogenesis (\( \Delta G^\circ = -131 \) 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 raise the aerodynamic lift required, and, hence, continues to fly. Similarly, faecal methanogens, apparently blissfully ignorant of their thermodynamic inferiority, continue to outcompete sulphate reducers for \( \text{H}_2 \).

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 \( \text{H}_2 \) tensions of the homogenates remained relatively constant. While the mean \( \text{H}_2 \) concentration in the methanogenic homogenate (0-20 \( \mu \)M) was only about 1/180 of that of the sulphate reducing homogenate (3-6 \( \mu \)M), \( \text{CH}_4 \) was produced about 4.5 times more rapidly than was
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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 $H_2$ 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, it seems possible that the $K_s$ values cited by Gibson et al for sludge organisms may not accurately reflect the relative $H_2$ affinities of the methanogenic and sulphate reducing bacteria that inhabit the human colon.


6 Chirri SU, Gibson GR, Florin THJ, Cummings JH. The role of dietary sulphate in the regulation of methanogenesis in the large intestine. Gastroenterology 1990; 98: A164.


