Effect of thiosulphate as electron acceptor on glucose and xylose oxidation by Thermoanaerobacter finnii and a Thermoanaerobacter sp. isolated from oil field water

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SUMMARY

During glucose and xylose fermentation, Thermoanaerobacter finnii was observed to produce lactate, acetate, H₂, and CO₂, with ethanol being the major end product. Thermoanaerobacter strain SEBR 5268, an isolate from an oil field, also produced a similar range of end products from glucose and xylose fermentation, with the exception that both ethanol and lactate were the major products of sugar metabolism. Both these strains were able to reduce thiosulphate to sulphide in the presence of these substrates, with acetate being the dominant metabolite in that case. In addition, a faster growth rate and increased cell yield were obtained in the presence of thiosulphate, than in its absence. The higher concentrations of acetate produced in the presence of thiosulphate rather than without any electron acceptor indicated that more ATP was generated from substrate-level phosphorylation. These results have implications for our understanding of the breakdown of carbohydrates present in organic matter found in the natural ecological niches of Thermoanaerobacter species (sulphide-, elemental sulphur- or sulphate-rich thermal hot springs and oil fields).

Key-words: Thiosulphate, Glucose, Xylose, Thermoanaerobacter finnii; Thiosulphate reduction, Anaerobiosis, Glucose and xylose oxidation, Thermal ecosystems, Oil fields.

INTRODUCTION

Several members of the genera Thermoanaerobacter and Thermoanaerobacterium of the "domain" Bacteria and members of the genera Pyrodictium, Thermoproteus and Archeoglobus of the "domain" Archaea are known to reduce thiosulphate, with concomitant production of sulphide (Stetter et al., 1990; Lee et al., 1993). Our recent studies have indicated that this process is also characteristic of all genera of the order Thermotogales (Ravot et al., 1995), one of the deepest branches within the "domain" Bacteria.

It has recently been demonstrated that H₂ can also be oxidized in the presence of thiosulphate by members of the genus Thermoanaerobacter,
which include T. brockii, T. ethanolicus, T. thermohydrodsulfuricus, T. finnii and Thermoanaerobacter strain SEBR 5268, an isolate from an oil-producing well (Fardeau et al., 1993, 1994). In contrast to T. finnii, in T. brockii, thiosulphate reduction coupled with H₂ oxidation participates in energy conservation (Fardeau et al., 1994), as was observed for the hyperthermophilic sulphur-reducing archaeon, Pyrococcus furiosus (Schicho et al., 1993). The addition of thiosulphate as an electron acceptor not only improves the oxidation of peptides, but also improves the oxidation of amino acids, thereby indicating that there is some form of dependency by Thermoanaerobacter species upon this electron acceptor during this process (Faubon et al., 1995).

Most studies of Thermoanaerobacter species have focused on the large-scale growth on carbohydrates as fermentable sources for industrial applications, for example thermostable enzymes and alcohol production (Lowe et al., 1991; Zamost et al., 1991). No study of thiosulphate reduction and its implications for carbohydrate metabolism has been undertaken.

Despite this important and interesting trait of thiosulphate reduction by such a diverse group of microorganisms, more detailed attention has been paid to sulphur rather than to thiosulphate reduction (Adams, 1990; Kelly and Adams, 1994).

In this paper, we report on the effects of thiosulphate as an electron acceptor upon the oxidation of glucose and xylose by T. finnii and Thermoanaerobacter strain SEBR 5268, an oil field isolate. We also discuss the potential importance of this process in the oxidation of carbohydrates present in the organic fraction of the ecological habitats of these microbes, namely, thermal environments.

MATERIALS AND METHODS

Source of organisms

T. finnii (DSM no. 3389) was obtained from the Deutsche Sammlung von Mikroorganismen. Thermoanaerobacter strain SEBR 5268 (DSM no. 9801) was isolated from an oil-producing well and has been deposited within the Sanofi-Recherche Collection of Microorganisms (Labège, France).

Media

Growth media were prepared using the technique of Hungate (1969). The organisms were cultivated under anaerobic conditions at 60°C. The basal medium contained the following constituents in distilled water (g/l): NH₄Cl, 1.0; K₂HPO₄, 0.3; KH₂PO₄, 0.3; NaCl, 2.0; MgCl₂-6H₂O, 1.3; KCl, 0.2; CaCl₂-2H₂O, 0.1; CH₃COONa, 0.5; yeast extract, 1.0; resazurin, 0.001; cystein. HCl, 0.5; trace mineral solution (Balch et al., 1979), 10 ml; pH was adjusted to 7.0 with 10 M KOH. The medium was boiled under a stream of O₂-free N₂, cooled at room temperature and distributed in Hungate tubes (5 ml) or bottles (50 ml), which were outgassed with N₂-CO₂ (80/20%). After autoclaving (110°C, 40 min), 0.2 ml of 2% Na₂S-9H₂O and 1 ml of 10% NaHCO₃ (sterile, anaerobic conditions) were added for 20 ml of culture medium before inoculation. The basal medium was supplemented with glucose, xylose or thiosulphate at appropriate concentrations from sterile stock solutions. For CO₂ production measurements, an NaHCO₃-free medium was used with N₂ as the gas phase.

Analytical techniques

All experiments were performed in duplicate. Optical density (OD) was measured at 580 nm with a “UV-160A” spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Sulphide was quantified by the method of Cord-Ruwisch (1985). H₂ was measured as previously described (Fardeau et al., 1993). CO₂ was analysed by gas chromatography at 70°C using a “Chrompack CP 9000” gas chromatograph (Chrompack International, Middelsburg, The Netherlands), equipped with a catharometer and two columns filled with silica gel (60/80 mesh) and molecular sieve (60/80 mesh), respectively. Helium was used as a carrier gas. Saguars and fermentation products were quantified by high-performance liquid chromatography using an “Analprep 93” pump (Touzart et Matignon, Vitry-sur-Seine, France) equipped with a refractive index detector and an ORH 801” column (Interaction Chemicals, Inc., Mountain View, CA.). Column temperature was 35°C. Eluant (H₂SO₄ 0.005 N) was used at a flow rate of 0.6 ml/min.

RESULTS

Ethanol (32.7 mM) was the major end product of glucose fermentation by T. finnii at 60°C (table I and fig. 1A). In addition, acetate, lactate, H₂ (table I) and CO₂ (not shown) were also produced. Maximal concentrations of ethanol were
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Table I. End products of glucose and xylose oxidation by *T. finnii* in the presence and in the absence of thiosulphate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sugar consumed (mM)</th>
<th>Acetate (mM)</th>
<th>Lactate (mM)</th>
<th>Ethanol (mM)</th>
<th>H₂S (mM)</th>
<th>H₂ (mM)</th>
<th>Acetate produced/sugar consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu</td>
<td>22.5 ± 2.2</td>
<td>2.4 ± 0.2</td>
<td>17.8 ± 1.4</td>
<td>32.7 ± 0.5</td>
<td>0</td>
<td>2.7 ± 0.3</td>
<td>0.11</td>
</tr>
<tr>
<td>Glu + Thio</td>
<td>16.9 ± 0.2</td>
<td>18.2 ± 0.4</td>
<td>5.9 ± 1.0</td>
<td>14.6 ± 0.0</td>
<td>18 ± 1.2</td>
<td>0.2 ± 0.1</td>
<td>1.08</td>
</tr>
<tr>
<td>Xyl</td>
<td>21.2 ± 2.4</td>
<td>6.2 ± 1.2</td>
<td>7.3 ± 0.8</td>
<td>37.4 ± 0.0</td>
<td>0</td>
<td>3 ± 0.3</td>
<td>0.29</td>
</tr>
<tr>
<td>Xyl + Thio</td>
<td>16.7 ± 0.8</td>
<td>25.5 ± 1.7</td>
<td>7.5 ± 0.4</td>
<td>6.0 ± 0.0</td>
<td>25.9 ± 1.6</td>
<td>0.5 ± 0.2</td>
<td>1.53</td>
</tr>
</tbody>
</table>

*T. finnii* was cultivated at 60°C in the original medium described in “Materials and Methods”. Thiosulphate was added at a concentration of 20 mM. Glu = glucose, Xyl = xylose, Thio = thiosulphate. The results were the mean of two replicates.

produced after 10 h of incubation, whereas lactate concentrations only peaked at 2 days of incubation at 60°C (fig. 1A). Similar end product profiles were obtained during xylose fermentation (table I and fig. 2A).

The addition of thiosulphate altered the concentrations of metabolites during glucose and xylose oxidation, indicating that thiosulphate modified the metabolic pathway of *T. finnii*. The changes observed were as follows: (i) thiosulphate was reduced to sulphide with concomitant reduction of H₂ to barely detectable levels (table I); (ii) the acetate concentration increased to such an extent that it became the major end product of glucose (table I and fig. 1B) and xylose (table I and fig. 2B) oxidation. A concomitant decrease in the production of ethanol was observed for glucose and xylose oxidation, whereas the lactate concentration decreased only when *T. finnii* was grown on glucose.

Modifications in the oxidoreductive pathway due to the presence of thiosulphate during glucose and xylose oxidation were observed to be beneficial for *T. finnii*: higher ODs were measured, indicating that (i) higher cell yield (table III) and (ii) a faster growth rate were achieved (table III).

*Thermoanaerobacter* strain SEBR 5268 produced an end product profile similar to that of *T. finnii*, but with the exception that ethanol and lactate were the major end products during glucose and xylose fermentation (table II). Cell yield increased and a faster growth rate was achieved (table III), with acetate becoming the major end product of carbohydrate metabolism in the presence of thiosulphate (table II), a phenomenon similar to that observed for *T. finnii*.

DISCUSSION

Thiosulphate reduction to sulphide is a common trait of sulphate-reducing bacteria (Barrett and Clark, 1987; Le Faou *et al.*, 1990). This feature is also observed amongst the carbohydrate-fermenting thermophilic anaerobic members of the genera *Thermoanaerobacter* and *Thermoanaerobacterium* (Lee *et al.*, 1993) and the order *Thermotogales* (Ravot *et al.*, 1995). We recently demonstrated that thiosulphate is used as an electron acceptor for oxidation of H₂, and it also improves the oxidation of peptides and amino acids by *Thermoanaerobacter* species (Fardeau *et al.*, 1993, 1994; Faudon *et al.*, 1995). We have extended these observations in this paper and have provided evidence that thiosulphate can be used as an electron acceptor, and dramatically modifies glucose and xylose metabolism in *T. finnii* and *Thermoanaerobacter* strain SEBR 5268, a strain isolated from a French oil field. Increased cell yield, faster growth rate and higher acetate production per mole of carbohydrate utilized in the presence of thiosulphate than in its absence suggest that this reductive process leads to a more favourable energetic reaction. We hypothesize that the use of thiosulphate as an electron
acceptor causes a shift in the flow of electrons, favouring H₂S production. This results in channeling of the electrons away from lactate and ethanol to acetate, thereby increasing its concentration. Such a shift in metabolism was previously reported, and was attributed to interspecies H₂ transfer (McNemey and Bryant, 1981; Mountfort et al., 1982; Laube and Martin, 1981; Winter and Wolfe, 1979). Here, for the first time, we evidence this type of sugar metabolism in the presence of thiosulphate within members of the genus Thermanaerobacter. In contrast to T. finnii, the archaeal Thermoproteus tenax used a limited amount of glucose in the presence of thiosulphate (or sulphur) and completely oxidized it to CO₂ and H₂S (Selig and Schönheit, 1994).
Table II. End products of glucose and xylose oxidation by strain SEBR 5268 in the presence and in the absence of thiosulphate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sugar consumed (mM)</th>
<th>Acetate produced (mM)</th>
<th>Lactate (mM)</th>
<th>Ethanol (mM)</th>
<th>H₂S (mM)</th>
<th>H₂ (mM)</th>
<th>Acetate produced/sugar consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu</td>
<td>7.5 ± 0.4</td>
<td>ND</td>
<td>7.7 ± 2.5</td>
<td>7.2 ± 0.3</td>
<td>0</td>
<td>0.7 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>Glu + Thio</td>
<td>7.4 ± 0.6</td>
<td>11.4 ± 1.9</td>
<td>5.4 ± 0.9</td>
<td>1.9 ± 0.2</td>
<td>2.7 ± 0.3</td>
<td>0.1 ± 0.1</td>
<td>1.54</td>
</tr>
<tr>
<td>Xyl</td>
<td>10.6 ± 0.5</td>
<td>1.1 ± 1.0</td>
<td>11.7 ± 2.1</td>
<td>9.6 ± 1.2</td>
<td>0</td>
<td>1.1 ± 0.2</td>
<td>0.10</td>
</tr>
<tr>
<td>Xyl + Thio</td>
<td>15.0 ± 1.5</td>
<td>18.0 ± 2.5</td>
<td>5.8 ± 1.6</td>
<td>4.3 ± 0.0</td>
<td>5.8 ± 0.4</td>
<td>0.3 ± 0.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

SEBR 5268 was cultivated at 60°C in original medium described in "Materials and Methods". Thiosulphate was added at a concentration of 20 mM. Glu = glucose, Xyl = xylose, Thio = thiosulphate. The results were the mean of two replicates. ND = not detected.

Table III. Effect of thiosulphate on growth of Thermoanaerobacter strains cultivated on glucose or xylose.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Treatments</th>
<th>Generation time (hours)</th>
<th>Maximal OD (580 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. finnii</td>
<td>Glucose</td>
<td>4</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>Glucose + thiosulphate</td>
<td>2.6</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>Xylose</td>
<td>5.9</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Xylose + thiosulphate</td>
<td>3.6</td>
<td>1.08</td>
</tr>
<tr>
<td>Thermoanaerobacter</td>
<td>Glucose</td>
<td>6.9</td>
<td>0.85</td>
</tr>
<tr>
<td>SEBR 5268</td>
<td>Glucose + thiosulphate</td>
<td>4.7</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>Xylose</td>
<td>15.4</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Xylose + thiosulphate</td>
<td>5.5</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Thermoanaerobacter strains were cultivated at 60°C. Glucose, xylose and thiosulphate were added at 20 mM to the original medium described in "Material and Methods".

The higher concentrations of acetate obtained from carbohydrate oxidation when thiosulphate is provided indicate that growth improvements result from more ATP generated from substrate-level phosphorylation. In the case of T. finnii and Thermoanaerobacter strain SEBR 5268, energy conservation via thiosulphate reduction as an alternative energy-yielding reaction can be ruled out, since we recently showed that no growth improvement resulted from concomitant oxidation of H₂ and reduction of thiosulphate to sulphide (Fardeau et al., 1993). On the other hand, T. brockii might generate ATP during this oxidoreductive process (Fardeau et al., 1994). This situation is analogous to that observed for the sulphur-reducing hyperthermophilic Archaea. Indeed some members of Archaea produced energy from elemental sulphur reduction, whereas others did not (Adams, 1990; Schicho et al., 1993). Questions have been raised concerning the enzymatic and energetic systems involved in the reduction of sulphur by members of the Archaea (Kelly and Adams, 1994). On this basis, similar questions about the enzymatic and energetic systems involved in thiosulphate reduction can now be asked concerning members of the genus Thermoanaerobacter.

We recently demonstrated that thiosulphate reduction by members of the genus Thermoanaerobacter greatly improved the oxidation of
proteinaceous compounds, e.g. peptides and amino acids (Faudon et al., 1995). Acetate, iso-butyrate, isovalerate, small amounts of propionate and sulphide were detected as the end products of this metabolism. In the present paper, we have reported on modifications in the metabolism of carbohydrates in the presence of thiosulphate.

Thiosulphate is known to be formed chemically from sulphide in the presence of oxygen (Cypionka et al., 1985; Jorgensen, 1990) or from elemental sulphur, anaerobically, at temperatures above 80°C (Pryor, 1962; Belkin et al., 1985). Furthermore, it has recently been demonstrated that thiosulphate acts as a shunt in the sulphur cycle of marine sediments and that sulphate- and sulphide were detected as the end products of this metabolism. In the present paper, we have reported on modifications in the metabolism of carbohydrates in the presence of thiosulphate.

In this respect, it has recently been demonstrated that thiosulphate acts as a shunt in the sulphur cycle of marine sediments and that sulphate- and sulphide were detected as the end products of this metabolism. In the present paper, we have reported on modifications in the metabolism of carbohydrates in the presence of thiosulphate.

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