Volume 263, number 1, 57–60 FEBS 08309 April 1990

# ATP synthesis coupled to electron transfer from $H_2$ to the heterodisulfide of 2-mercaptoethanesulfonate and 7-mercaptoheptanoylthreonine phosphate in vesicle preparations of the methanogenic bacterium strain Gö1

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Received 7 February 1990

Crude vesicle preparations of the methanogenic strain Gö1 were able to couple the reduction of the heterodisulfide of 2-mercaptoethanesulfonate and 7-mercaptoheptanoylthreonine phosphate (CoM-*S*-*S*-HTP) by $H_2$ with ATP formation. The rate of ATP synthesis was 1 nmol/min mg protein. ATP synthesis and disulfide reduction were only observed with CoM-*S*-*S*-HTP, but not with CoM-*S*-*S*-CoM or HTP-*S*-*S*-HTP. The methylreductase inhibitor 2-bromoethanesulfonic acid had no effect on ATP synthesis induced by CoM-*S*-*S*-HTP reduction with $H_2$. ATP synthesis was completely inhibited by the uncoupler SF 6847 whereas the concomitant CoM-*S*-*S*-HTP reduction was stimulated. The ATP synthase inhibitors DCCD and DES also inhibited ATP formation completely and decreased the CoM-*S*-*S*-HTP reduction rate to 35% of the control. These inhibitory effects were abolished by addition of the uncoupler. From these results it is concluded that energy coupling between the electron transfer from $H_2$ to the heterodisulfide and ATP synthesis occurs via a transmembrane proton gradient.

ATP synthesis; Heterodisulfide reductase; Methanogenic bacterium; Vesicle preparation

## 1. INTRODUCTION

The universal methanogenic reaction from all substrates, the reductive demethylation of methyl-CoM by the methyl-coenzyme M methylreductase system, has always been considered as the reaction coupled with ATP synthesis. Investigations performed with whole cells of *Methanosarcina barkeri* [1] and whole cells and protoplasts of the methanogenic bacterium strain Gö1 [2] led to the conclusion that the reductive demethylation of methyl-CoM by $H_2$ gives rise to an electrochemical gradient of protons which is subsequently used for ATP formation by a $H^+$-translocating ATP synthase. It was recently shown, that methanogenesis from methyl-CoM and $H_2$ as catalyzed by the methylreductase system proceeds in two steps: methane is formed by the methylreductase (eq. 1) and the reduced coenzymes are generated by the heterodisulfide reductase (eq. 2) [3,4]:

$$CH_3\text{-}S\text{-CoM} + \text{HTP-SH} \longrightarrow CH_4 + \text{CoM-}S\text{-}S\text{-HTP} \quad (1)$$

$$\text{CoM-}S\text{-}S\text{-HTP} + H_2 \longrightarrow \text{CoM-SH} + \text{HTP-SH} \quad (2)$$

Looking at these reactions it was proposed that the second one, the electron transfer from $H_2$ via unknown electron carriers to the heterodisulfide of CoM-SH and HTP-SH (CoM-*S*-*S*-HTP) gives rise to the electrochemical proton gradient observed [3].

Based on this proposal, it was desirable to study energy conservation during CoM-*S*-*S*-HTP reduction by $H_2$ at the subcellular level. This was possible by taking advantage of a crude vesicle preparation of the methanogenic bacterium strain Gö1, which consists to 90% of vesicles with an inside-out orientation [5]. These vesicles are able to synthesize ATP in response to methanogenesis from $H_2$/methyl-CoM [6]. Here we report on a CoM-*S*-*S*-HTP reduction-dependent ATP synthesis by this crude vesicle preparation.

## 2. MATERIALS AND METHODS

### 2.1. *Organism and cultivation*

The methanogenic bacterium strain Gö1 was obtained from the German Culture Collection (DSM), Braunschweig, FRG. Strain Gö1 was grown with methanol (final concentration 150 mM) on a medium described previously [7] supplemented with 1 g/l sodium acetate.

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*Abbreviations:* BES, 2-bromoethanesulfonic acid; CoM-SH, 2-mercaptoethanesulfonate; CoM-*S*-*S*-HTP, heterodisulfide of CoM-SH and HTP-SH; DCCD, *N,N'*-dicyclohexylcarbodiimide; DES, diethylstilbestrol; HTP-SH, 7-mercaptoheptanoylthreonine phosphate; $CH_3$-*S*-CoM or methyl-CoM, 2-(methylthio)ethanesulfonate; SF 6847, 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalononitrile; sulfobetaine, *N*-tetradecyl-*N,N*-dimethylammonio-3-propanesulfonate

*Published by Elsevier Science Publishers B.V. (Biomedical Division)*
00145793/90/$3.50 

2.2. *Preparation of the vesicles*

Vesicles of strain Göl were prepared from protoplasts as described previously [6]. The vesicle buffer had the same composition (20 mM potassium phosphate, 20 mM $MgSO_4$, 0.4 M sucrose, 1 mg/l resazurin, pH 6.8) except it was not reduced with 10 mM dithioerythritol but by stepwise addition of a few $\mu$l of 100 mM Ti(III) citrate [8] until the resazurin turned colorless. The crude vesicle preparation contained approximately 25–30 mg protein/ml, which was determined according to [9]. Until use the vesicles were stored under a hydrogen atmosphere at −70°C.

2.3. *Experimental conditions for the determination of ATP and of the thiol compounds*

The experiments were performed at 25°C under a hydrogen atmosphere in 3 ml glass vials containing vesicle preparation suspended in the above phosphate buffer to a final volume of 0.5 ml (protein content: approximately 1 mg/ml). The experiments were started by adding 2 mM CoM-*S*-*S*-HTP. To determine the ATP concentration aliquots of 10 $\mu$l were withdrawn by syringe and the ATP content was measured immediately by using the luciferin/luciferase assay [1]. For following the reduction of CoM-*S*-*S*-HTP aliquots of 10–15 $\mu$l were withdrawn by syringe and analyzed for thiols with Ellman's reagent as described previously [10]. Methane was monitored by gas chromatography according to [1].

Additions were made as indicated. DCCD, DES and SF 6847 were added as ethanolic solutions. The controls received ethanol only. CoM-*S*-*S*-HTP (only the L form was used) and the homodisulfides of CoM-SH (CoM-*S*-*S*-CoM) and of HTP-SH (HTP-*S*-*S*-HTP) were synthesized as in [3].

2.4. *Chemicals and gases*

All chemicals were reagent grade and were purchased from Sigma (Taufkirchen, FRG). SF 6847 was obtained from Wako Chemie (Neuss, FRG). Gases were purchased from Messer Griesheim (Kassel, FRG).

## 3. RESULTS

### 3.1. *ATP formation coupled to CoM-S-S-HTP reduction by $H_2$*

Vesicle preparations of the methanogenic bacterium strain Göl were incubated under $H_2$. Upon addition of CoM-*S*-*S*-HTP, ATP was formed at a rate of 1 nmol/min mg protein (Fig. 1). Reduction of CoM-*S*-*S*-HTP as determined from the thiol formation rate proceeded at a rate of 47 nmol/min mg protein (Fig. 1) indicating a ratio of 1 mol ATP formed per 47 mol CoM-*S*-*S*-HTP reduced. In the absence of CoM-*S*-*S*-HTP neither ATP was synthesized nor thiol components were formed. Under none of these conditions methane formation was observed (data not shown).

ATP formation was strictly specific for CoM-*S*-*S*-HTP reduction. Addition of CoM-*S*-*S*-CoM or HTP-*S*-*S*-HTP did not lead to ATP synthesis. Accordingly, none of the two compounds was reduced (data not shown).

### 3.2. *Effect of various inhibitors on ATP formation coupled to CoM-S-S-HTP reduction by $H_2$*

If ATP formation coupled to CoM-*S*-*S*-HTP reduction was driven by a protonmotive force it should be possible to uncouple ATP synthesis from CoM-*S*-*S*-HTP reduction. As is apparent from Fig. 2 this was indeed possible. Addition of the uncoupler SF 6847 inhibited ATP formation completely whereas CoM-*S*-*S*-HTP reduction was not only unaffected but was even stimulated to a rate of 72 nmol/min mg protein (Fig. 2B). This uncoupler effect is in agreement with previous results obtained in whole cells of *Ms. barkeri* [1] and whole cells and protoplasts of the methanogenic bacterium strain Göl [2].

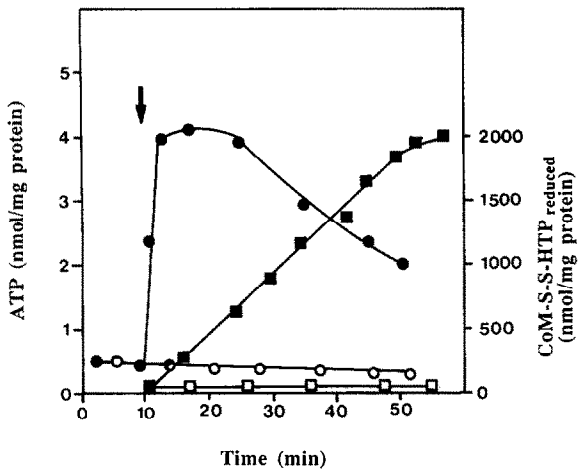


Fig. 1. ATP synthesis coupled to CoM-*S*-*S*-HTP reduction by $H_2$ in vesicle preparations of the methanogenic strain Göl. Crude vesicles were suspended in a final volume of 0.5 ml of 20 mM phosphate buffer, pH 6.8 (protein content: 1 mg/ml) and preincubated on a rotary shaker at 25°C under a $H_2$ atmosphere. At the time indicated by the arrow the reaction was started by addition of 2 mM CoM-*S*-*S*-HTP. ATP, CoM-SH and HTP-SH were determined as described in section 2. Circles, ATP concentration; squares, reduction of CoM-*S*-*S*-HTP; filled symbols, CoM-*S*-*S*-HTP added; open symbols, CoM-*S*-*S*-HTP omitted.

The participation of an ATP synthase in CoM-*S*-*S*-HTP reduction-dependent ATP formation was tested by adding the ATP synthase inhibitors DCCD (75 nmol/mg protein) and DES (65 nmol/mg protein). Both compounds had the same effect and, therefore, only the experiment with DCCD is depicted in Fig. 2. DCCD inhibited ATP formation completely (Fig. 2A) and decreased the rate of CoM-*S*-*S*-HTP reduction to 16 nmol/min mg protein. Addition of the uncoupler SF 6847 abolished the inhibitory effect of DCCD on CoM-*S*-*S*-HTP reduction indicating a stringent coupling between the electron transport from $H_2$ to CoM-*S*-*S*-HTP and ATP synthesis. Such a stringent coupling was not observed with methyl-CoM as electron acceptor [6].

2-Bromoethanesulfonic acid inhibits methanogenesis and ATP formation in the latter system completely [6]. CoM-*S*-*S*-HTP reduction (Fig. 2B) and ATP synthesis (Fig. 2A) were not affected by this compound.

In contrast, sulfobetaine, a detergent which interferes with membrane structure and function, did not only inhibit ATP synthesis (Fig. 2A) but also CoM-*S*-*S*-HTP reduction (Fig. 2B); methanogenesis from methyl-CoM and $H_2$ as well as concurrent ATP formation are likewise affected by sulfobetaine [6].

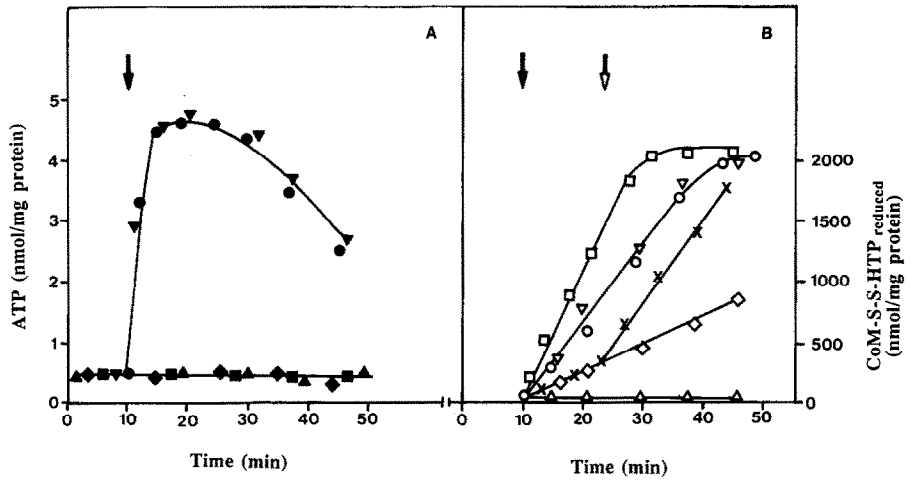


Fig. 2. Effect of various inhibitors on ATP formation (A) and CoM-*S*-*S*-HTP reduction (B) by vesicle preparations of the methanogenic strain Gö1. Vesicle preparations (protein content: 1.3 mg/ml) were preincubated under $H_2$ at 25°C with various inhibitors. At the time indicated by the closed arrow the reactions were started by addition of 2 mM CoM-*S*-*S*-HTP. Open symbols, ATP concentration; closed symbols, reduction of CoM-*S*-*S*-HTP; circles, no further additions; triangles pointing down, preincubation with 10 mM BES; triangles pointing up, with 0.15% sulfobetaine; squares, with 6 nmol SF 6847/mg protein; diamonds, with 75 nmol DCCD/mg protein; times-signs, with 75 nmol DCCD/mg protein plus 6 nmol SF 6847/mg protein at the time indicated by the open arrow.

## 4. DISCUSSION

Everted vesicles of the methanogenic strain Gö1 are able to couple $CH_4$-formation from $CH_3$-*S*-CoM and $H_2$ with ATP synthesis via an electrochemical transmembrane proton potential [6]. Here we report on a CoM-*S*-*S*-HTP reduction-induced ATP synthesis mediated by this type of vesicle preparation. Investigations with inhibitors indicate the same coupling mechanism. However, the coupling between CoM-*S*-*S*-HTP reduction and ATP formation is more stringent than between methyl-CoM reduction and ATP synthesis. This is for example seen in the effect of the uncoupler SF 6847, which inhibited ATP formation completely but stimulated CoM-*S*-*S*-HTP reduction by 50%. Such an uncoupler-mediated increase in electron flow was also seen during methanogenesis from $H_2$/methanol in whole cells and protoplasts of strain Gö1 [2], but not during methanogenesis from $H_2$/$CH_3$-*S*-CoM by the crude vesicle preparation [6]. Furthermore, the inhibitory effect of the ATP synthase inhibitors DES or DCCD on electron transfer was reversed by uncouplers in the case of CoM-*S*-*S*-HTP but not in the case of methyl-*S*-CoM as electron acceptor. A possible explanation for these differences may be that the reduction of $CH_3$-*S*-CoM is more complex than the CoM-*S*-*S*-HTP reduction in that it needs the methylreductase reaction in addition to the heterodisulfide reductase reaction. The methylreductase is known to lose much of its specific activity upon cell breakage [3]. This was explained by the inability to adjust in vitro the proper reducing conditions [11] and may explain why much higher protein concentrations are needed to observe ATP synthesis in response to the reduction of methyl-*S*-CoM [6] than in response to the reduction of CoM-*S*-*S*-HTP.

If CoM-*S*-*S*-HTP reduction by $H_2$ is coupled to ATP formation via a transmembrane proton gradient the heterodisulfide reductase can be expected to be membrane-bound. In *Methanobacterium thermoautotrophicum* a $H_2$-dependent and a benzyl viologen-dependent heterodisulfide reductase was found in the cytoplasmic fraction [10,12] whereas in the methanogenic strain Gö1 a $F_{420}H_2$ -dependent [13] and a $H_2$-dependent (unpublished results) CoM-*S*-*S*-HTP reductase were membrane-bound. It remains now to be shown how the electron transfer from $H_2$ to the heterodisulfide leads to the generation of an electrochemical transmembrane proton gradient. Previous experiments led to the conclusion that at least one additional membrane-bound electron carrier is needed [14] but the nature of the component must await further investigations.

*Acknowledgement:* This work was supported by a grant of the Deutsche Forschungsgemeinschaft (Bonn-Bad Godesberg).

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