Redox Potentials of Methanophenazine and CoB-S-S-CoM, Factors Involved in Electron Transport in Methanogenic Archaea**

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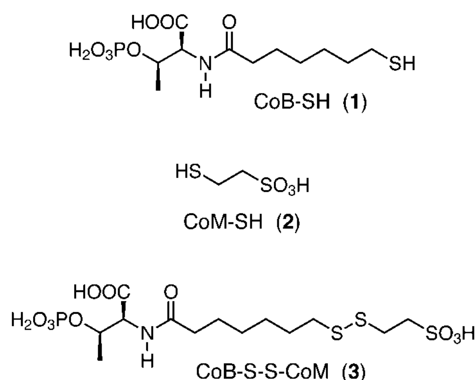
KEYWORDS:

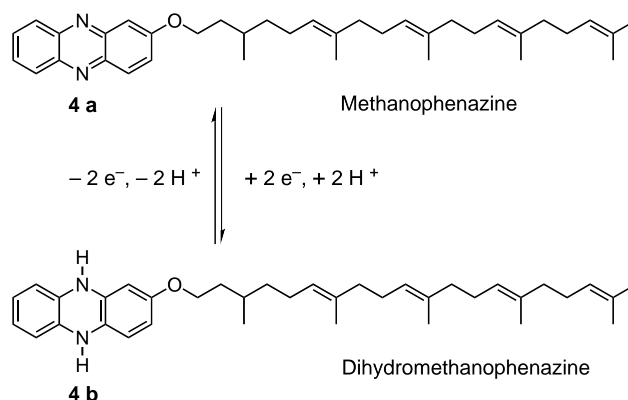
cofactors · cyclic voltammetry · electron transfer · methanogenesis · redox potential

The process of methanogenesis is of utmost importance for the carbon cycle on earth. The metabolic pathways of methanogenic archaea that lead to the formation of methane are unique and comprise a number of unusual enzymes and cofactors. The last step of methanogenesis involves the formation of methane from methyl-*S*-CoM (2-methylthioethane-sulfonate), the central intermediate of all metabolic pathways of methanogens, and includes the reductive demethylation of methyl-*S*-CoM, catalysed by methyl-CoM reductase.[1] The two electrons required for this reductive process come from CoB-SH (**1**) and result in the oxidative formation of the heterodisulfide CoB-S-S-CoM (**3**) from CoB-SH (**1**) and CoM-SH (**2**).[2]



The energy-conserving step in the metabolism of methylotrophic methanogens is the reduction of **3** catalysed by heterodisulfide reductase.[3] In *Methanosarcina* species, the H_2:heterodisulfide oxidoreductase and the $F_{420}H_2$:heterodisulfide oxidoreductase systems are responsible for the reductive regeneration of **1** and **2** from **3**. Both the membrane-bound hydrogenase and the $F_{420}H_2$ dehydrogenase are key enzymes of these electron transport systems.[4] The electrons are transferred to the heterodisulfide reductase and finally to **3**. The most interesting question relates to the structure and function of the electron carriers mediating this electron transfer.

Methanophenazine (**4a**) has recently been isolated from the cytoplasmic membranes of *Methanosarcina mazei* Gö1.[5a] Biochemical experiments have shown that **4a**, which is available



through total synthesis,[5b,c] is able to mediate the electron transport between the membrane-bound hydrogenase, $F_{420}H_2$ dehydrogenase and heterodisulfide reductase enzymes so that the conversion by proton-translocating electron transport systems can be subdivided in two partial reactions (Figure 1).[4, 5b] Most important, these findings indicate that the role of the new cofactor in the energy metabolism of methanogenic archaea corresponds to that of ubiquinone in bacteria and mitochondria.

It is essential to determine the redox potential of **4a** in order to further specify its role in the electron transport of methanogens. According to Figure 1 **4a** must have a redox potential between the values for $F_{420}H_2/F_{420}$ ($E^{\circ\prime} = -360$ mV)[6] and $H_2/2H^+$ ($E^{\circ\prime} = -420$ mV), respectively, and the redox potential of **3**. Whereas we know the $E^{\circ\prime}$ values of $F_{420}H_2/F_{420}$ and $H_2/2H^+$, the redox potential of CoB-S-S-CoM (**3**) still needs to be determined. We assumed that **4a** has a redox potential similar to 2-hydroxyphenazine (**5**) because, as was shown in biological studies, **5** can serve as model for **4a**. The redox potential of **5** was established by Mann as $E^{\circ\prime} = -255$ mV versus the standard hydrogen electrode (vs. SHE) by using conventional polarography with a dropping mercury electrode as the working electrode (WE).[7] Mann conducted his studies with mixtures of phosphate buffer and ethanol as **5** does not dissolve sufficiently in phosphate buffer (at pH 7). We were able to show that the addition of ethanol leads to a considerable shift of the pH value of the solvent system, which in turn causes a shift of the redox potential of **5** in the negative direction. For this reason, we had to find a method allowing measurements to be made in purely aqueous buffer systems without the addition of any organic co-solvents. Cyclic voltammetry with a hanging mercury drop

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[**] *CoB = coenzyme B, CoM = coenzyme M.*

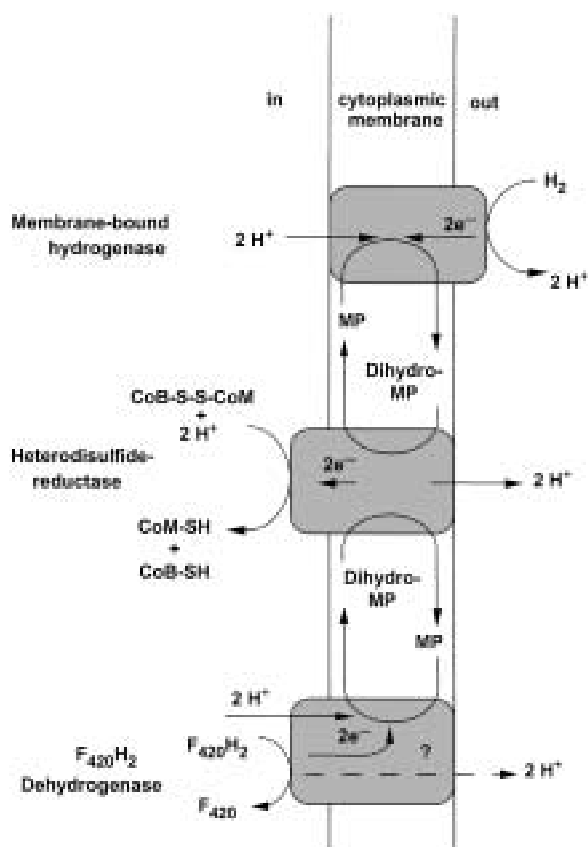

Figure 1. Model of the membrane-bound electron transfer of Methanosarcina mazei *Gö1 according to ref. [5b]. CoB-SH = coenzyme B (**1**); CoM-SH = coenzyme M (**2**); F_{420} = coenzyme F_{420}; $F_{420}H_2$ = reduced form of coenzyme F_{420}; MP = methanophenazine (**4a**); dihydro-MP = dihydromethanophenazine (**4b**).*

electrode (HMDE) as WE was best suited to this purpose.[8a] Compound **5** in phosphate buffer at pH 7 gave a redox potential of $E^{\circ\prime} = -191 \pm 8$ mV vs. SHE. More interestingly though, measurements of methanophenazine (**4a**) gave a redox potential of $E^{\circ\prime} = -165 \pm 6$ mV vs. SHE under the same conditions (Figure 2).

We assume that strong adsorption of phenazines onto mercury is the reason why electrochemical measurements of highly water-insoluble phenazines such as **4a** are possible in purely aqueous buffer systems with an HMDE.[8] Two findings support this assumption: on the one hand, the almost identical mirror-image position of the anodic and cathodic peaks on the potential axis, and on the other hand, an increase of the anodic and cathodic peak currents with a growing number of voltammetric cycles (Figure 2). The differences between the redox potential of **5** recorded by Mann and our own finding may partially be attributed to adsorptive interactions that occur between the analyte and the mercury electrode and shift the redox potentials in the positive direction.[8a] On the other hand, these differences may be due to the lower pH value of the buffer solutions we used, which results from the avoidance of ethanol as a co-solvent. In this context we examined the pH dependency of the redox potential of **8** (Table 1). We found that increasing the pH value by one unit causes a shift of the redox potential by -64 ± 0.6 mV (Figure 3).

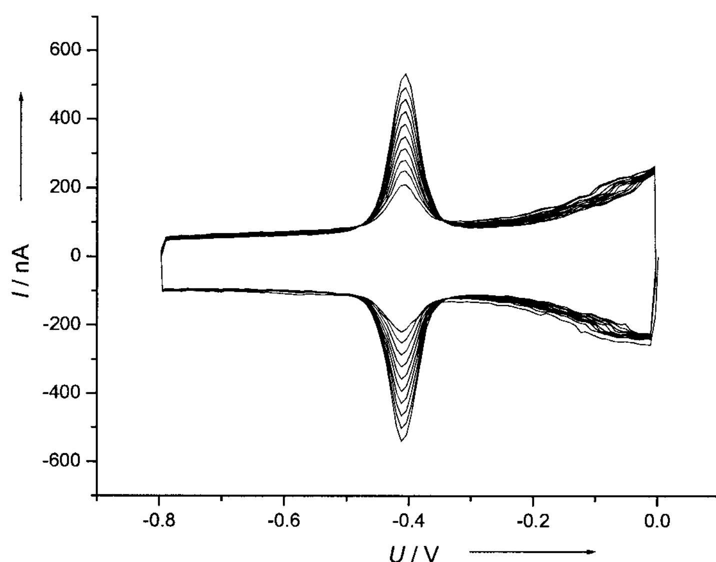

*Figure 2. Cyclic voltammogram of methanophenazine (**4a**) in phosphate buffer at pH 7 measured by using an HMDE (scan rate: -100 mV s^{-1}; 10 cycles).*

Table 1. *Redox potentials of phenazine ethers **4a**, **6–12**.[a]*

5 + RX —base→ **4a, 6–12**

Compound	R	$E^{\circ\prime}$ vs. SHE [mV]
4a		−165
6		−168
7		−165
8		−170
9		−164
10		−166
11		−163
12		−169

[a] *Phenazine ethers **4a**, **6–12** were synthesised by etherification of 2-hydroxyphenazine (**5**) with RX and their redox potentials were determined by cyclic voltammetry in phosphate buffer at pH 7 by using an HMDE. X = OMs*

It was previously assumed[5b] that the terpenoid side chain of **4a** mainly serves to anchor the cofactor in the cytoplasmic membrane. For this reason, the structure of the side chain should exert only a minor influence on the redox potentials of the respective phenazine ethers. A variety of phenazine ethers (**6–12**) were synthesised by Williamson ether synthesis and their redox potentials determined (Table 1).[9] Our assumption is indeed corroborated by these experiments.

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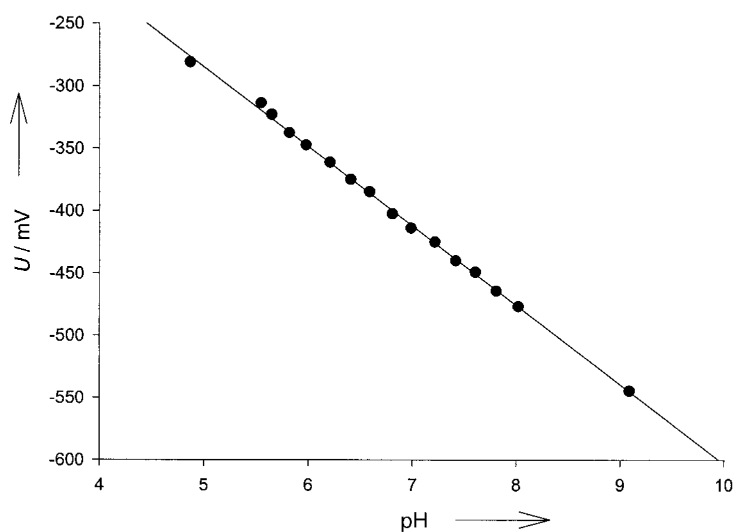
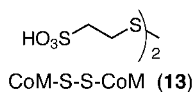
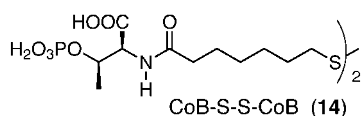


*Figure 3. pH dependence of the redox potential of **8**. Measurements were performed in phosphate buffer at different pH values by cyclic voltammetry with an HMDE.*

Finally we determined the $E^{\circ\prime}$ value of CoB-S-S-CoM (**3**), which was synthesised according to known procedures.[3, 10] The redox potential of this sensitive compound could be established by using the same experimental set-up as for the phenazines. It was also possible to conduct measurements in phosphate buffer at pH 7 in this case. We followed Heyrovský's procedure[8] and took the cyclic voltammograms after presweep polarisation at 0.20 V vs. SCE for 1 min with a scan rate of $100\,mV\,s^{-1}$. The redox potential of CoB-S-S-CoM (**3**) was found to be $E^{\circ\prime} = -143 \pm 10$ mV vs. SHE. This result indicates a much more positive redox potential for **3** than for typical disulfides such as glutathione and cysteine, for which values of $E^{\circ\prime} = -204 \pm 6$ mV vs. SHE and $E^{\circ\prime} = -202 \pm 3$ mV vs. SHE, respectively, have been established. This deviation is probably due to the presence of sulfonate and phosphate groups in **3**.[11] This assumption was confirmed by measurements of the redox potentials of the synthetically accessible homodisulfides CoM-S-S-CoM (**13**) ($E^{\circ\prime} = -139 \pm 7$ mV vs. SHE) and CoB-S-S-CoB (**14**) ($E^{\circ\prime} = -177 \pm 5$ mV vs. SHE).





This is the first time that the redox potentials of methanophenazine (**4a**) and CoB-S-S-CoM (**3**) have been reported. These values allow both the reduction of methanophenazine (**4a**) to dihydromethanophenazine (**4b**) by $F_{420}H_2$ and H_2, respectively, and the oxidation of **4b** to **4a** by **3**, which strongly supports the hypothesis that methanophenazine plays a central role as an electron carrier in the electron transport system of methane-producing organisms.

Methods

Measurements were performed by using an electrochemical workstation IM6 (Zahner-elektrik) equipped with a hanging mercury drop electrode as working electrode (drop diameter: approx. 0.52 mm; surface area: 0.86 ± 0.03 mm), a saturated calomel electrode (SCE) as reference electrode and a Pt electrode as counter electrode. All the potentials given refer to the standard hydrogen electrode. The difference between the SCE and SHE electrodes was taken to be 244.4 mV. All experiments were conducted in phosphate buffer at pH 7 under argon at room temperature.

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Received: August 13, 2002
Revised version: February 13, 2003 [Z 473]