OXIDATION–REDUCTION PROPERTIES OF COENZYME M (2-MERCAPTOETHANE SULPHONATE) AT THE MERCURY ELECTRODE

Douglas B. KELL and J. Gareth MORRIS

Dept. Botany and Microbiology, The University College of Wales, Aberystwyth, Dyfed SY23 3DA, Wales

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1. Introduction

Coenzyme M, 2-mercaptoethane sulphonate ($HSCH_2CH_2SO_3^-$), is a recently discovered, heat-stable cofactor implicated in the methane-forming reactions of methanogenic bacteria [1–5]. It is believed [3,4] that the reduced form of the cofactor may be methylated by methylcobalamin in an enzyme-catalysed methyltransferase reaction, the methyl-CoM so formed being reduced to methane in a reaction catalysed by a methylreductase [5]. In addition, it has been shown [6] that if the coenzyme is present in its dithio-form, $(CoM)_2$, it must be reduced either enzymatically in the presence of NADPH or directly by sodium borohyride, according to eq. (1), before it can act as a methyl carrier:

$$\begin{matrix} S{-}CH_2CH_2SO_3^- \\ | \\ S{-}CH_2CH_2SO_3^- \end{matrix} + 2\,e^- + 2\,H^+ \rightleftharpoons 2HSCH_2CH_2SO_3^- \qquad (1)$$

$(CoM)_2$ CoM

However, the cellular location [4] and the pathways of electron transfer necessarily implicated in methanogenesis [3,4] and the involvement [8] or otherwise [9] of ATP in this process, remain matters of uncertainty. We have therefore undertaken a study of the thermodynamic oxidoreduction properties of the CoM couple (eq. (1)), the better to inform speculations regarding its role in methanogenic bacteria. Here we demonstrate that CoM is quasi-reversibly electroactive at the mercury electrode, and report the standard potential of the 2-mercapto-ethane sulphonate/2,2′-dithiodiethane sulphonate couple (eq. (1)) so measured.

Abbreviations: CoM, 2-mercaptoethane sulphonate; $(CoM)_2$, 2,2′-dithiodiethane sulphonate; Methyl-CoM, 2-(methylthio)-ethane sulphonate

2. Materials and methods

Polarograms were run on a PAR model 174A polarographic analyser connected to a 3-electrode cell containing a model 303 mercury electrode, an Ag/AgCl reference electrode (3 M KCl bridge) and a Pt counter electrode. Current–voltage curves were displayed on a Hewlett-Packard 7035B X–Y recorder. Unless otherwise stated, all potentials are given versus the Ag/AgCl electrode and all experiments were performed at room temperature (18°C). Other polarographic conditions are given in the legends to the figures. All solutions were 3.3 mM in CoM, dissolved in 'buffer KM3' titrated to the appropriate pH with HCl or KOH. Buffer KM3 contained the following components: 40 mM malonic acid; 75 mM malic acid; 80 mM di-potassium oxalate; 25 mM tripotassium citrate; 75 mM maleic acid; 25 mM disodium-β-glycerophosphate; 100 mM dipotassium hydrogen phosphate; 40 mM *N*-2-hydroxy-ethylpierazine-*N*′-2-ethane sulphonate; 25 mM triethanolamine hydrochloride; 75 mM Tricine; 100 mM glycylglycine; 25 mM 2-amino,2-methylpropanediol; 80 mM sodium metaborate; 75 mM 2-amino,2-methylpropanol. All solutions were bubbled with oxygen-free nitrogen for at least 15 min prior to their

polarographic behaviour being determined under an atmospheric of nitrogen. pH values were measured with an Orion 901 Ionalyser.

CoM was obtained from Pierce and Warriner, Chester Cheshire. Trebly-distilled mercury was supplied by Alexander Pickering Ltd, Slough, Berks and water was doubly distilled in an all-glass apparatus. All other chemicals were of the highest quality commercially available and were obtained from Sigma Chemical Company, Poole, Dorset or from BDH Chemicals, Poole, Dorset.

3. Results

Figure 1 shows the differential pulse polarogram [10] displayed by solutions of CoM at two different pH values. At pH values less than pH 8 (fig.1a) only a single peak (peak I) was obtained in the potential range −0.1 to −0.85 V (versus Ag/AgCl). At more alkaline pH values a second, more anodic, peak appeared (peak II, fig.1b), although the peak current of peak I was undiminished (data not shown). This, and other evidence (fig.2) suggested that peak II was very possibly a kinetic or catalytic peak [11], and

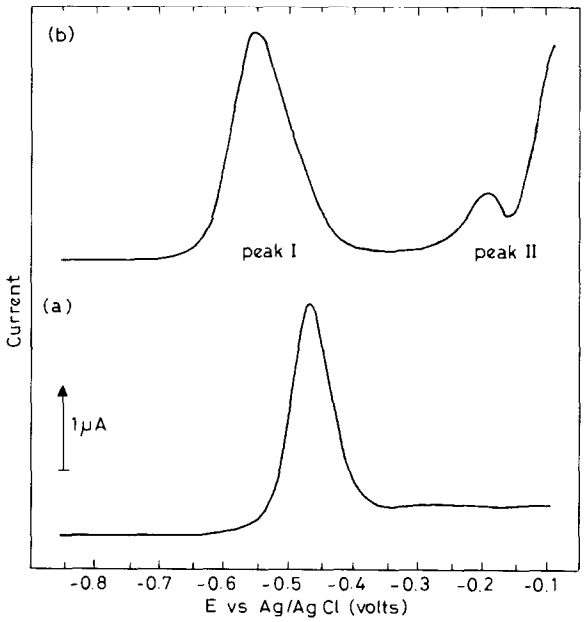

Fig.1. Differential pulse polarograms of 3.3 mM CoM in KM3 buffer. Initial potential, −0.1 V; final potential, −0.85 V; scan rate, 2 mV/s; modulation amplitude, 25 mV; Droptime, 1 s. (a) pH 7.74, (b) pH 10.24. The asymmetry of peak I is caused by the difference in the diffusion coefficients of CoM and $(CoM)_2$.

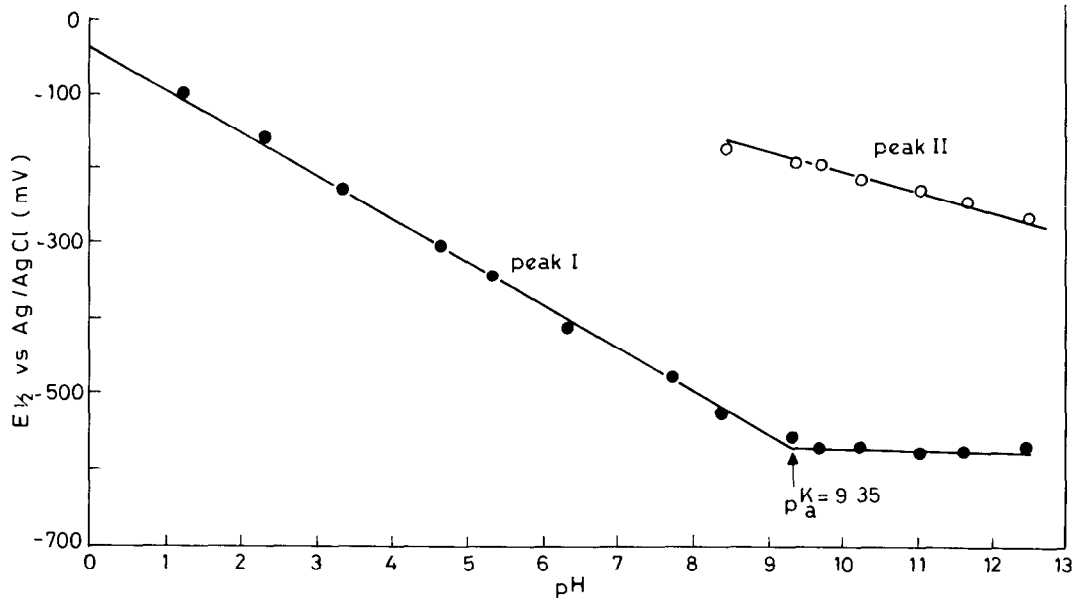

Fig.2. Effect of pH on polarographic half-wave potentials of CoM. Polarographic conditions as in fig.1. The lines were fitted by a least squares method on a Commodore PET computer. (●——●) Peak I; (○——○) peak II.

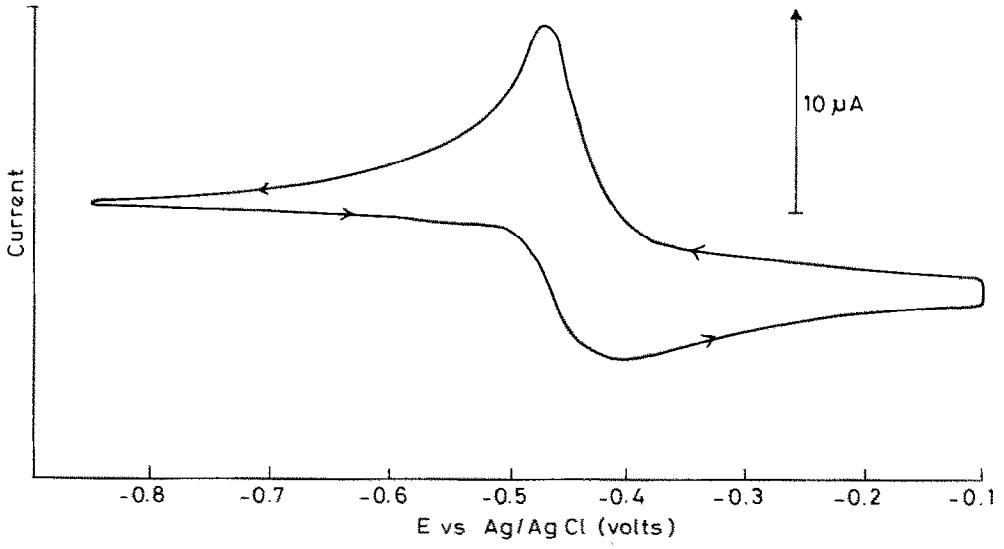

Fig.3. Cyclic voltammogram of 3.3 mM CoM in KM3 buffer (pH 7.33) at the hanging mercury drop electrode. Scan rate 50 mV/s. Direction of scan as indicated by arrows.

that for our present purposes we confined out attention to peak I. For differential pulse polarography the half-wave potential $E_{1/2}$ is given by $E_p - (\Delta E/2)$ [10] where E_p is the measured peak potential and ΔE the modulation amplitude. The half-wave potentials of the two peaks at different pH values are displayed in fig.2. At pH values more acidic than 9.35 the $E_{1/2}$ value of peak I was accurately described by the relationship $E_{1/2} = -0.033 - (0.058 \times \text{pH})$ V, indicating (eq. (1)) that the number of protons and electrons involved in the oxidoreduction were indeed equal, whilst above pH 9.35 the $E_{1/2}$ was independent of pH. The subsituent involved in this behaviour is undoubtedly the thiol group, with an app. pK value which is slightly lower (pK 9.35) than that of other aliphatic thiols such as cysteine (pK 10.46), a result of the electron-withdrawing action of the sulphonate group. No peak was observed when the thiol group was reacted with twice the molar concentration of permanganate or of iodoacetate (data not shown). The anomalous slope of the $E_{1/2}$/pH plot for peak II (23 mV/pH unit, fig.2) was additional evidence that it was not related to the reaction described by eq. (1).

The half-wave potentials measured by the polarographic method are not in general equal to the thermodynamic mid-point potentials (E_m) of the couple involved. Only in the case of a reversible reaction at the mercury electrode can $E_{1/2}$ and E_m values be equated. To determine whether or not the oxidoreduction of CoM at the mercury electrode was reversible, cyclic voltammetric studies were undertaken. A typical cyclic voltammogram for CoM is shown in fig.3. The magnitude of the separation of the cathodic (upper) and anodic (lower) peaks, ~64 mV (fig.3), and its independence from scan rate (data now shown), indicated that the oxidoreduction of CoM at the mercury electrode was indeed an essentially reversible reaction. Further, the peak currents were directly proportional to the square-root of the scan rate, indicating [12,13] that no follow-up chemical reactions were involved in the polarographic process (data not shown). Thus, since cyclic voltammograms indicated reversible redox behaviour of CoM even at scan rates of 100 mV/s (the maximum scan rate feasible with our X–Y recorder) it may justifiably be concluded that the half-wave potentials recorded using the differential pulse polarographic method at a scan rate of 2 mV/s (fig.1,2) may be equated with the thermodynamic mid-point potential of the CoM couple. Although the cathodic and anodic peaks of the cyclic voltammogram were of unequal heights (fig.3), a phenomenon sometimes taken to indicate irreversible behaviour [14], their ratio (1.4) is easily explained if it is

assumed that the diffusion coefficient of $(CoM)_2$ is twice that of CoM, since [15] the peak heights in cyclic voltammetry are proportional to the square root of the diffusion coefficient of the species involved. It would thus seem that adsorption phenomena, such as those observed for cysteine at the Hg electrode [16], do not constitute a problem in the present study.

4. Discussion

Since the standard EMF of the Ag/AgCl electrode at 18°C is equal to +0.246 V [17], at pH values more acidic than pH 9.35, we may describe the $CoM/(CoM)_2$ couple by the equation [18]:

$$E_h = 0.213 - 58.06 \times pH + \frac{RT}{nF} \ln \frac{(CoM)_2}{CoM}$$

such that the modified standard potential, E'_0 at pH 7 and 18°C is −193 mV. Of those electron transport components known to be possessed by methanogenic bacteria [3,4] only the NADP/NADPH couple possesses well-authenticated thermodynamic properties, with an E'_0 of −0.32 V [18]. Passage of two electrons between NADPH and $(CoM)_2$ poised at their mid-point potentials would be accompanied by a free energy change of −24.5 kJ/mol, only ~50% of that required to drive ATP synthesis in vivo [3]. Since the other known membrane-associated electron carriers, such as factor F_{420} [19,20], are thought to lie on the reducing side of the pyridine nucleotide couple it must be concluded that it is most unlikely that the $CoM/(CoM)_2$ couple donates electrons to a carrier involved in ATP synthesis. One way consequently speculate that whilst methyl-CoM indisputably acts as a methyl carrier the $CoM/(CoM)_2$ couple itself, present in methanogenic bacterial cells at 0.2–2 mM [21], may act as a soluble electron sink.

Finally, we may point out that the present polarographic study might form the basis of an assay for CoM that would be much more convenient than the current bioassay [21].

Acknowledgements

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