THE REDOX POTENTIAL FOR DIMETHYL SULPHOXIDE REDUCTION TO DIMETHYL SULPHIDE

Evaluation and biochemical implications

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1. Introduction

The redox potential for dimethyl sulphoxide reduction to dimethyl sulphide (eq. (1)) is of biochemical interest for several reasons.

$$(CH_3)_2SO + 2\,H^+ + 2\,e^- \longrightarrow (CH_3)_2S + H_2O \quad (1)$$

One is because this interconversion should be closely analogous to that of methionine sulphoxide and methionine; as Ejiri et al. have commented [1], it is reasonable to assume that cells must have mechanisms both to prevent methionine residues in proteins from becoming oxidised (e.g., by H_2O_2) and also to reduce any methionine sulphoxide once formed. Mechanisms for reduction of methionine and biotin sulphoxides are well characterised in microorganisms [1]. A more direct significance stems from observations implying that certain bacteria can use dimethyl sulphoxide as the electron sink for an energy-conserving electron-transport chain, in a manner similar to anaerobic growth of *Escherichia coli* on fumarate or nitrate [2]. Thus, Zinder and Brock have isolated a bacterium for which anaerobic growth on dimethyl sulphoxide was inhibited by azide or uncouplers, and found that a *c*-type cytochrome was prominent in its difference spectra [3]. It has also been shown that some photosynthetic bacteria can grow anaerobically in the presence of dimethyl sulphoxide [4,5], and for certain species transfer to medium containing dimethyl sulphoxide has resulted in synthesis of a characteristic *c*-type cytochrome ([5]; Jones, O. T. G. and Ward, J. A., unpublished). Clearly, a value for the redox potential of reaction (1) would be of great use in working out the electron transport chain of such organisms, and would also enable quantitative measurements of proton pumping, ATP synthesis, and cell growth, to be compared with the total redox energy available. With certain other bacteria, and also for yeasts, anaerobic growth in the presence of dimethyl sulphoxide leads to its reduction without energy being conserved; the reaction is evidently either an incidental consequence of a fairly general sulphoxide reductase activity, or a method of dumping surplus reducing equivalents [6–8]. Dimethyl sulphide makes a significant contribution to the flavour of certain foods and drinks (e.g., lager), and reaction (1) is one of the pathways leading to its formation [9]. The biodegradation of dimethyl sulphoxide is also important because of its presence in effluent from wood-pulp mills, and use as a solvent for drugs.

At least two reference books state that dimethyl sulphide is insoluble in water [10,11]. It is worth considering this point before proceeding further, since the implication is that the effective redox potential of reaction (1) would be perturbed by dimethyl sulphide coming out of solution as a separate liquid phase. In practice, no small molecule is completely insoluble in water, and besides, dimethyl sulphide has a dipole moment [11]. Kushelev et al. have measured the solubility at 20°C, and found that saturation corresponds to 1.8 wt%, or 290 mM [12]. For an organism growing in the wild such a high concentration is unlikely to be attained, and the question of phase separation will therefore not arise under conditions of biochemical interest.

A search through the literature has failed to reveal

Abbreviations. DMS, dimethyl sulphide; DMSO, dimethyl sulphoxide

any direct measurement of the mid-point potential for reaction (1). Any attempted measurement would have to face two problems peculiar to this system. One is the volatility of dimethyl sulphide; at 20°C the 290 mM solution mentioned above would have a vapour pressure of 395 mm Hg (53 kPa) [13], so use of the conventional anaerobic cuvette flushed with nitrogen [14] would lead to it rapidly being blown away. More importantly, dimethyl sulphoxide is thermodynamically unstable with respect to disproportionation into sulphide and sulphone:

$$2\,(CH_3)_2SO \longrightarrow (CH_3)_2S + (CH_3)_2SO_2 \qquad (2)$$

For the pure substances, $\Delta G^{\circ} = -98$ kJ . mol^{-1} for this reaction (from ΔG°_{f} values in [15]), while the redox potentials derived below imply $\Delta G^{\circ}_{aq} = -77$ kJ . mol^{-1}, or $K = 10^{13}$, for dilute aqueous solution. In conventional biochemical redox titrations, interconversion of methionine in proteins with its sulphoxide does not occur (one hopes), reflecting the fact that mechanistically the oxidation requires transfer of [0] (e.g., from a peroxide) rather than 2[H] or mere 2 e^{-}. However, if mediators could be found which enabled reaction (1) to proceed freely in both directions until electrochemical equilibrium was attained, then almost certainly reactions involving the sulphone would be catalysed as well, and only the sulphone/sulphide redox potential would be measurable.

This paper shows how a completely different approach can be used, in which literature data is deployed to evaluate $\Delta G^{\circ}_{f,aq}$, the standard molar free energy of formation in aqueous solution, for each of the components in eq. (1).

2. Results and discussion

For each component in a solution formed from two volatile liquids:

$$\mu_i = \mu_i^{\circ} + RT \ln(x_i f_i) \qquad (3)$$

where μ_i is the partial molar free energy of component i, x_i is its mole fraction, μ_i° is the partial molar free energy for the pure component ($x_i = 1$), and f_i is its activity coefficient, referred to a value of unity for pure i [16]. It should be noted that with this convention, $f_i \to 1$ as $x_i \to 1$, but $f_i \not\to 1$ as $x_i \to 0$. (A different convention is usually adopted for involatile solutes.)

In evaluating $\Delta G^{\circ}_{f,aq}$, some care is needed over the choice of standard states. By convention, the standard state for a solute in aqueous solution is taken as the hypothetical solution of unit molality having activity coefficients for the solute and solvent as for an infinitely dilute real solution [15]. In forming a solution of this description, 1000/18.015 = 55.51 moles of water are converted from a mole fraction of 1.0 to one of 55.51/56.51 = 0.9823, for every 1 mole of solute at a mole fraction of 1/56.51 = 0.0177. Under the convention described above, the activity coefficient for the water will be 1.0. Hence, from eq. (3), if the solute is regarded as component 2, and its molar free energy of formation is written as $\Delta G^{\circ}_{f,liq}$:

$$\Delta G^{\circ}_{f,aq} = \Delta G^{\circ}_{f,liq} + RT \ln \left\{ 0.0177 \right.$$

$$\left. (f_2)_{x_2 \to 0} \right\} + 55.51\, RT \ln 0.9823 \qquad (4)$$

Consider first dimethyl sulphoxide. The binary system dimethyl sulphoxide – water was studied in some detail by Philippe and Jambon [17]. They found that experimental results for the departure of such solutions from ideality could be fitted very closely by an equation derived by Redlich and Kister [18]. This equation involves three temperature-dependent coefficients, denoted A_1, A_2 and A_3 in [17]. As demonstrated [18], these parameters are related in a simple way to the ratio of the activity coefficients at infinite dilution:

$$(\ln f_{DMSO}/f_{H_2O})_{x_{DMSO} \to 0} = A_1 - A_2 + A_3$$

At 25°C, $A_1 = -2.0605$, $A_2 = 0.9023$ and $A_3 = -0.3348$ [17]; and with the convention adopted here, $f_{H_2O} = 1$ at $x_{DMSO} \to 0$. Therefore, in very dilute solutions, $\ln f_{DMSO} = -3.298$, and $f_{DMSO} = 0.0370$. We can now make use of eq. (4), since $\Delta G^{\circ}_{f,liq} = -99.16$ kJ . mol^{-1} [15]. For $T = 298$ K, it follows that $\Delta G^{\circ}_{f,aq} = -119.8$ kJ . mol^{-1} for dimethyl sulphoxide.

For dimethyl sulphide a slightly different approach is required, reflecting the much lower polarity of this molecule. At 20°C a saturated solution contained 1.8% dimethyl sulphide by weight [12]. Now, in general, if p_i is the vapour pressure of a volatile component in a solution, and p_i° is the vapour pressure of this component when pure [16]:

$$p_i = p_i^o x_i f_i$$

For a saturated solution, $p_i = p_i^o$; and therefore $f_i = 1/x_i$. In this case, the composition of the saturated solution corresponds to $x_{DMS} = 0.0053$. It is reasonable to suppose that f_{DMS} at this very low mole fraction is close to the value for $x_{DMS} \to 0$ (cf. [19]). Therefore, $f_{DMS} \approx 190$, at $x_{DMS} \to 0$. Simple experiments with a stoppered cuvette show that the solubility of dimethyl sulphide at 25°C is similar to that at 20°C, and therefore since f_{DMS} is only used in the calculation as its logarithm, it should be safe to regard the 20°C value as applicable to 25°C. We can now proceed as before; Wagman et al. give $\Delta G^o_{f,liq} = +6.07$ kJ . mol^{-1} for dimethyl sulphide [15], and hence use of eq. (4) yields $\Delta G^o_{f,aq} = +6.6$ kJ . mol^{-1}.

Equation (1) includes a water molecule as one of the products. For liquid water (the standard state), $\Delta G^o_f = -237.18$ kJ . mol^{-1} [15]. The remaining entities in eq. (1) are the solvated proton and electron. For both these species, the convention is that $\Delta G^o_f = 0$, in order to give $E_m = 0$ for the standard hydrogen electrode [15].

$\Delta G^o_{f,aq}$ values have now been obtained for every component in eq. (1). By summation, for this reaction $\Delta G^o = -110.8$ kJ . mol^{-1}. This refers to pH 0; at pH 7 we have:

$$\Delta G^o_{pH\ 7} = \Delta G^o + RT \ln (10^{-7})^{-2}$$

or $\Delta G^o_{pH\ 7} = -30.9$ kJ . mol^{-1}. Therefore, for the 2 e$^-$ reduction:

$$E_{m,pH\ 7} = -\Delta G^o_{pH\ 7}/2F$$

where F is Faraday's constant, 96487 C . mol^{-1}, and hence $E_{m,pH\ 7} = +160$ mV.

It is difficult to give precise error limits to this value, since the accuracy of the figures used to derive it is in most cases not stated. A slightly different value for $\Delta G^o_{f,liq}$ for dimethyl sulphide of +5.77 kJ . mol^{-1} is given in [20], but this only alters the final answer by 1 mV. The errors are likely to amount <5 mV, so the answer should be quite precise in biochemical terms. The redox potential will decline by 59 mV . 1 unit rise^{-1} in pH, as for O_2/H_2O, fumarate/succinate.

The system is unusual in the enormous difference in polarity between reactant and product. This is reflected in the activity coefficients, 0.0177 and 190, as against 1.0 for an ideal solution. One consequence is that the redox potential will be unusually sensitive to other substances present in the solution. It could also be raised at high cell densities by an appreciable fraction of the dimethyl sulphide becoming dissolved in the lipid phase of cell membranes. Another process leading to a raising of the [DMSO]:[DMS] ratio would be loss of dimethyl sulphide by evaporation. However, dimethyl sulphoxide reduction is only an important process under anaerobic conditions, and anaerobiosis generally implies only restricted contact with the atmosphere.

Dimethyl sulphone has physical properties similar to those of the sulphoxide. If its activity coefficient in dilute aqueous solution is assumed to equal that for dimethyl sulphoxide, then given $\Delta G^o_f = -302.50$ kJ . mol^{-1} [15], it follows that $\Delta G^o_{f,aq} \approx -323$ kJ . mol^{-1}. An approximate value can now be found for the redox potential of its 2 e$^-$ reduction:

$$(CH_3)_2SO_2 + 2\ H^+ + 2\ e^- \longrightarrow (CH_3)_2SO + H_2O$$

Proceeding as before, $\Delta G^o \approx 34$ kJ . mol^{-1} for this reaction, giving $\Delta G^o_{pH\ 7} \approx +46$ kJ . mol^{-1}, and $E_{m,pH\ 7} \approx -240$ mV. This value should be fairly accurate, since, a factor of 10 error in the activity coefficient only affects the final result by 30 mV.

Some of the implications of these values for bacterial growth are as follows. The $E_{m,pH\ 7}$ value for dimethyl sulphoxide reduction lies between those for fumarate/succinate and nitrate/nitrite, and thus the coupling of dimethyl sulphoxide reduction to ATP synthesis presents no energetic difficulty [2]. One would also expect that certain aerobic bacteria would be able to use dimethyl sulphide as reductant for an electron-transport chain ending with O_2 reduction (cf. use of succinate, nitrite and H_2S) and indeed growth on dimethyl sulphide has been demonstrated for *Thiobacillus*-like bacteria [21]. As for dimethyl sulphone, the very low redox potential for its reduction explains why no organism capable of using this reaction to support anaerobic growth was found [3] (cf. $SO_4^{2-} \to H_2S$, $E_{m,pH\ 7} = -207$ mV, yielding only ~1 ATP (4 e$^-$)$^{-1}$ for *Desulfovibrio* [22]). On the other hand, the aerobic oxidation of dimethyl sulphoxide should provide a very favourable energy source for some enterprising bacterium.

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