Redox Potentials of Certain Vitamins K: Implications for a Role in Sulfite Reduction by Obligately Anaerobic Bacteria

(menaquinone MK-6/Desulfovibrio/terminal redox couples/bioenergetics)

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ABSTRACT Redox potentials of a menaquinone (MK-6), isolated in earlier researches from two species of the obligately anaerobic genus, Desulfovibrio, as well as two other vitamins K—menaquinones (MK-5) and (MK-9)—have been determined polarographically. The measurements have been validated by determination of redox potentials of 1,4-naphthoquinone and vitamin K, which agree with published potentiometric values. E_m for menaquinone (MK-6) is \(-0.067 \pm 0.010\) V. Redox potentials calculated for terminal acceptor couples currently proposed in the mechanisms of sulfate reduction by Desulfovibrio are consistent with the involvement of menaquinone (MK-6) in at least one of the steps postulated during electron transfer with ultimate production of sulfate.

A vitamin K, [menaquinone (MK-6)] has been demonstrated (1, 2) to exist in relatively large concentrations (about 1.7 \(\mu\)mol/g dry weight) in at least two species of the obligately anaerobic genus, Desulfovibrio, which derives metabolic energy by the reduction of sulfate to sulfide. However, the function, if any, of such a naphthoquinone remains undefined. Moreover, the presence of this type of vitamin K in such quantities in these anaerobes poses questions about its possible significance in the evolutionary history of electron transport systems linked to energy conservation. The concurrent existence, also in large concentrations, of \(c\)-type cytochromes ("cytochromes \(c_2\)") in these obligate anaerobes suggests obvious analogies to electron transfer processes in photosynthetic (3, 4), mitochondrial (3, 5), and bacterial (3, 6) systems, wherein quinones and \(c\)-type cytochromes exhibit well-known functions. Whereas, the redox potentials of cytochromes \(c_2\) accord well with a role as intermediates in coupled electron transfer to certain terminal redox couples involved in sulfate reduction (7, 8), those of menaquinones and related vitamins K appear unfavorable (9, 10).

We have measured the redox potentials of Desulfovibrio menaquinone (MK-6) and two related vitamins K—menaquinones (MK-5) and (MK-9)—by a conventional polarographic procedure. This method has been demonstrated as valid for the determination of \(E_m\), as indicated by the consistent values obtained for two reference compounds—1,4-naphthoquinone and vitamin K, for which reliable values of redox potential have been published. In addition, we have prepared new estimates of relevant redox potentials for terminal redox couples thought to be involved in sulfate metabolism in these organisms, including some apparently unpublished hitherto, e.g., \(E_m\) values for the redox couples, sulfite/trithionate, trithionate/thiosulfate-sulfite, and thiosulfate/sulfide-sulfite, which may be of particular interest in considerations of possible functional involvement of menaquinones in biological sulfate reduction.

EXPERIMENTAL

Vitamin K, Tris (Sigma Chemical Co.), and 1,4-naphthoquine (Eastman) were used without further purification. Vitamin K, menaquinone (MK-6) was the sample prepared previously by Maroc et al. (1) from Desulfovibrio gigas. Other vitamins K, (MK-5 and MK-9) were pure samples furnished by Dr. O. Isler (Hoffmann-La Roche Co.). All of these menaquinones dissolved completely in the test solvents used except that a small portion of the menaquinone (MK-6) precipitated on standing and was removed by decantation. All other chemicals used were of the best reagent quality.

Conventional de polarographic measurements were performed with a Sargent polarograph model XV, equipped with a two-compartment H-cell thermostatted in a water bath at 25.0 ± 0.1°. A dropping mercury electrode (DME) was used in the test solution; the reference electrode was a saturated calomel (potassium). All test solutions were de-aerated with a stream of water-saturated argon for 30 min before polarography, and a slow flow of argon was maintained over the test solution during measurements. The solvent system used consisted of 0.15 M Tris·HCl (1.0 M in NaCl), pH 7.3, and 2-propanol (1:2 v/v). The appropriate quinone was added to produce a concentration in the range 10⁻⁴–10⁻³ M. After each determination, the pH of the test solution was measured with an Instrumentation Laboratory model 245 pH meter, equipped with expanded scale and a Fisher combination glass electrode.

RESULTS AND DISCUSSION

Polarographic Measurements. Half-wave potentials (\(E_{1/2}\)) were determined in the usual manner from plots of \(E_{DME}\) against \(\log (i/i_d - i)\), in which the measured potentials \(E_{DME}\) were corrected for cell and recorder resistances, and measured currents, \(i\), for residual current in the solvent system. Cell resistances for the 1,4-naphthoquinone system were ascertained by dilution methods, assuming \(E_{1/2}\), to be independent of concentration, and were then applied to measurements with all other compounds tested. Reported redox potentials, \(E_m\), were calculated from the appropriate \(E_{1/2}\) after correction for the reference electrode potential and the

Abbreviation: DME, dropping mercury electrode.
applicable pH term, namely:

\[ \text{E}_{m7} = \text{E}_{i/1} + \text{E}_{a/mel} + 0.059 \ (pH - 7.00) \]
\[ = \text{E}_{i/1} + 0.245 + 0.059 \ (pH - 7.00). \]  

[1]

Working assumptions were that the ratio of diffusion coefficients and activity coefficients for reduced and oxidized species of test compound were unity, that the electrode process was reversible, and that a two-electron-two-proton transfer was involved. The linearity and slope of the plots of \( \text{EdpE} \) against \( \log (i/i_d - i) \) supported these assumptions, within experimental error, except for menaquinone (MK-5). Measurements were made only at pH 7.3; the dependence of the potentials on pH was taken as \( -\Delta E/\Delta pH = 0.059 \ V \), in accord with a two-electron, two-proton process.

In Table 1, we present results for the various systems tested. It is seen that our values for systems previously reported [1,4-naphthoquinone, vitamin K, and menaquinone (MK-9)] are in good agreement with published values, whether determined polarographically or potentiometrically. The assumption of a two-electron-two-proton process is also supported by agreement of \( \text{E}_{m7} \) values obtained in our experiments and those calculated from the \( \text{E}_{m9} \) values using the pH term applied above.

The \( \text{E}_{m7} \) values for all the vitamins K listed in Table 1 appear to cluster around a potential of about \(-0.072 \pm 0.005 \ V\), except for menaquinone (MK-5). With this vitamin \( K_2 \), no reversible process was noted and so its measured \( \text{E}_{m7} \) cannot be considered reliable. We have no explanation at this time for its anomalous behavior. The dominance of the nuclear chromophore in determination of the overall redox potential and the relative lack of influence exerted by the long hydrophobic side chain has been noted (e.g., see ref. 9).

**Redox Potential Calculations.** The dominant redox couple in the overall process of sulfate reduction (eight-electron transfer) in *Desulfovibrio* sp. is the sulfite/sulfide (six electron transfer) terminal acceptor system (15) for which a calculated value of \(-0.096 \ V\) has been published (7), based on then available thermodynamic data. Our measured value for menaquinone (MK-6) implies that linkage of this vitamin \( K_2 \) to this sulfite/sulfide terminal redox couple involves a steady state of almost complete reduction of the quinone, which, of course, is quite possible in *Desulfovibrio* under actual metabolic conditions for growth of this extremely anaerobic microorganism.

![FIG. 1. Dependence of midpoint potentials on pH. (1) Tri-thionite/thiosulfate-sulfite; (2) menaquinone (MK-6) (dashed portion) indicates that \( \text{pK}_a \) values expected in this region have not been experimentally determined; (3) thiosulfate/sulfide-sulfite; (4) \( H^+ /H_2 \); (5) sulfite/trithionite.](image)

**TABLE 1. Redox potentials for 1,4-naphthoquinone and related vitamins K**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Experimental ( \text{E}_{m7} ) (V)*</th>
<th>Published ( \text{E}_{m7} ) (V)†</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-naphthoquinone</td>
<td>+0.084 ± 0.010</td>
<td>+0.064 (PT) (9)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>+0.058 (PL) (11)</td>
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<tr>
<td></td>
<td></td>
<td>+0.074 (PT) (12)</td>
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<tr>
<td></td>
<td></td>
<td>+0.075 (PT) (9)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>+0.043 (PT) (13)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>+0.086 (PL) (14)</td>
<td></td>
</tr>
<tr>
<td><strong>Vitamin K_1</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>-0.078 ± 0.010</td>
<td></td>
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<tr>
<td><strong>Vitamin K_2 (MK-6)</strong> isolated from <em>Desulfovibrio</em> sp.</td>
<td>-0.067 ± 0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vitamin K_3 (MK-9)</strong></td>
<td>-0.070 ± 0.010</td>
<td>-0.075 (PT) (9)</td>
<td></td>
</tr>
<tr>
<td><strong>Vitamin K_4 (MK-5)</strong></td>
<td>-0.108 ± 0.010</td>
<td></td>
<td></td>
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</tbody>
</table>

* Polarographic determinations at 25.0 ± 0.1°; solvent system consisted of 0.15 M Tris, 1.0 M NaCl, pH 7.3:2-propanol (1:2, v/v).
† \( \text{E}_{m7} \) values listed were calculated from published values using relation \( -\Delta E/\Delta pH = 0.059 \ V \) at 25°. PT and PL indicate potentiometric or polarographic methods, respectively. The experimental conditions used were as follows: For ref. 9, \( T = 25^\circ \), solvent system was 0.2 M LiCl, 0.2 M HCl-n-propanol (50% v/v). For ref. 11, \( T = 25^\circ \), solvent system was 0.1 M Britton buffer, pH 7.20, and ethanol (10% v/v). For ref. 12, \( T = 25^\circ \), 0.2 M LiCl, 0.1 M HCl, and ethanol (50% v/v). For ref. 13, \( T = 20^\circ \), 0.2 M LiCl, 0.2 M HCl, and ethanol (50% v/v). For ref. 14, none specified.

**TABLE 2. Standard potentials for terminal redox couples in sulfate reduction**

<table>
<thead>
<tr>
<th>Electrode reaction</th>
<th>Calculated ( \text{E}_{m9} ) (V)*</th>
<th>Published value†</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 3\text{SO}_4^- + 6\text{H}^+ + 2e^- \rightleftharpoons \text{S}_3\text{O}_4^2- + 3\text{H}_2\text{O} )</td>
<td>-0.355 (R)</td>
<td>-0.179 (L)</td>
<td></td>
</tr>
<tr>
<td>( \text{S}_3\text{O}_4^2- + 2e^- \rightleftharpoons \text{S}_2\text{O}_3^3- + \text{S}_2\text{O}_4^3- )</td>
<td>+0.381 (R)</td>
<td>+0.253 (L)</td>
<td></td>
</tr>
<tr>
<td>( \text{S}_2\text{O}_3^3- + 2e^- \rightleftharpoons \text{S}^- + \text{S}_2\text{O}_4^3- )</td>
<td>-0.390 (R)</td>
<td>-0.424 (L)</td>
<td></td>
</tr>
<tr>
<td>( \text{S}_2\text{O}_3^3- + 2\text{H}^+ + 2e^- \rightleftharpoons \text{S}^- + \text{H}_2\text{O} )</td>
<td>-0.442 (R)</td>
<td>-0.486 (8)</td>
<td></td>
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<tr>
<td>( \text{SO}_3^2- + 8\text{H}^+ + 8e^- \rightleftharpoons \text{S}^- + 4\text{H}_2\text{O} )</td>
<td>-0.201 (R)</td>
<td>-0.188 (7)</td>
<td></td>
</tr>
<tr>
<td>( \text{SO}_3^2- + 6\text{H}^+ + 6e^- \rightleftharpoons \text{S}^- + 3\text{H}_2\text{O} )</td>
<td>-0.120 (R)</td>
<td>-0.120 (8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.116 (L)</td>
<td>-0.096 (7)</td>
<td></td>
</tr>
</tbody>
</table>

* \( R \) and \( L \) indicate values based on appropriate free energies of formation listed by Rossini et al. (22) and Latimer (23), respectively.
† Details of methodology used in studies cited can be ascertained from references.
nism. However, it is instructive to examine all other relevant terminal redox couples involved in the sequence of step-wise sulfite reduction postulated (16-19) for *Desulfovibrio* metabolism, namely:

$$\text{SO}_4^{2-} \rightarrow \text{SO}_4^{2-} \rightarrow \text{SO}_4^{2-} \rightarrow \text{S}^{-} \quad [2]$$

The two-electron reduction of sulfate to sulfite which initiates this sequence is also of interest because the actual form of sulfate involved is as the adenylate derivative, APS, (20, 21). The $E_{\text{m}}$ for the APS $\rightarrow$ SO$_4^{2-}$ + AMP couple has been estimated as about 0 V (8).

In connection with evaluation of the standard potentials, $E_{\text{m}}$, for the relevant terminal redox couples, we used the calculation procedures described in detail by Clark (10). Calculations of $E_{\text{m}}$ values were made with use of the relation defining a standard potential, $E$, according to Clark (10), namely:

$$E = -\Delta G^o/nF \quad [3]$$

where the symbols on the right side of relation 3 have their usual significance. From thermodynamic data compiled by Rossini *et al.* (22) or by Latimer (23) and proton dissociation constants provided by Yui (24), the $E_{\text{m}}$ values for the first electrode reaction, sulfite to trithionate, obtained from Eq. 3 and the appropriate Nernst equation were $-0.179$ V and $-0.355$ V, depending on whether free energies of formation for sulfite and trithionate were those provided by Rossini (indicated as $R$ in Table 2) or by Latimer (indicated as $L$ in Table 2). In like manner, $E_{\text{m}}$ values for other half-reactions in the metabolic sequence of relation 2 were calculated and are summarized in Table 2. It should be noted that although bisulfite, rather than sulfite, is the dominant form in solution at physiological pH, this fact in no way alters the $E_{\text{m}}$ values calculated for these couples. Likewise, these values take into account the dominant forms of all components involved.

The positive $E_{\text{m}}$ value for the reductive disproportionation of trithionate to sulfite and thiosulfate was remarkable in the series of redox couples which otherwise all exhibited negative values. In this connection it was requisite to examine the pH dependence of these couples, as well as to compare redox potentials at a given pH, because of involvement of protons in the balanced half-reactions and associated proton equilibria. We have exhibited in Fig. 1 the variations in $E_{\text{m}}$ with pH, as calculated in the conventional manner with the appropriate Nernst relation for each couple, using available thermodynamic data compiled by Latimer (23). Two important features emerge. First, the $E_{\text{m}}$ for reduction disproportionation of trithionate is more positive than that of the menaquinone (MK-6) couple at all pH values. Secondly, the couple for trithionate formation exhibits a comparatively large pH dependence, approaching $-0.09$ V/pH unit at pH values $< pK_a$ for bisulfite and $-0.18$ V/pH unit at pH values $< pK_a$ for bisulfite. The expected variation for the menaquinone couple is $-0.06$ V/pH unit over most of the acid range of pH. Hence, the curves calculated for $E_{\text{m}}$ of the menaquinone and trithionate formation couple intersect at acid pH.

The finding from these calculated values of the various terminal redox couple potentials of the positive $E_{\text{m}}$ values for the reductive disproportionation of trithionate to sulfite and thiosulfate obviously suggests involvement of the menaquinone (MK-6) in this process, which would be greatly favored on the basis of energetics, and suggests search for an enzyme system mediated by this naphthoquinone at this step of the metabolic sequence. However, the redox potential value for menaquinone (MK-6) is also close enough to one of the calculated values for the sulfite-trithionate couple to invite inquiries as to a possible intervention of a menaquinone (MK-6) reductase in sulfite reduction to trithionate.

There also is the possibility of menaquinone involvement in electron transfer and phosphorylation processes consequent upon fumarate reduction to succinate (28), associated in these bacteria with growth on combinations of fumarate and sulfate. Specifically, a $b$-type cytochrome—apparently a component of a particulate system that mediates fumarate reduction by hydrogen—has been characterized in *Desulfovibrio gigas* (26). In addition, there is evidence for participation of menaquinone (MK-6) in electron transport between hydrogenase and fumarate reductase in the same microorganism (26). Another suggestion pictures the menaquinone interposed as an electron carried between cytochrome $c_1$ and the bound $b$-type cytochrome (27). The $E_{\text{m}}$ determined in our work for the *Desulfovibrio* vitamin K$_2$ derivative is consistent with such a role in sulfite reduction.

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