Electrochemistry of ubiquinones

Menaquinones and plastoquinones in aprotic solvents

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First and second half-wave reduction potentials of a series of 1,4-benzo- and 1,4-naphtho-quinones related to the naturally occurring ubiquinones, plastoquinones and menaquinones are correlated with substituent effects. Notably, $E_{1/2}$ of 2,3-dimethoxy-1,4-benzoquinone is positive of the values for the 2,5- and 2,6-dimethoxy isomers, and of the value for methoxy-1,4-benzoquinone. This phenomenon is attributed to steric inhibition of resonance when two methoxy groups occupy adjacent positions, and the significance of this orientation in the ubiquinone series is highlighted.

Reduction potential *Methoxy-1,4-benzoquinone* *Ubiquinone* *Plastoquinone* *Menaquinone*

1. INTRODUCTION

Three prenyl substituted *para*-quinones are widespread in biological systems: ubiquinones [2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinones (**1**)] are found in all mitochondria and in many bacteria; menaquinones [2-methyl-3-polyprenyl-1,4-naphthoquinones (**2**)] occur in many bacteria; and plastoquinones [2,3-dimethyl-5-polyprenyl-1,4-benzoquinones (**3**)] are present in chloroplasts. Some organisms contain only a single type of quinone, but several bacteria possess both ubi- and mena-quinones, whilst green plants have ubiquinones in their mitochondria and plastoquinones in their chloroplasts. One of their main functions is redox electron transfer, addition of one electron giving the semiquinone ($Q^{\cdot -}$, as **4**), and of two electrons giving the dianion (Q^{2-}, as **5**) both of which can be in equilibrium with their protonated forms in suitable media [1,2].

In a comprehensive survey of the electrochemistry of quinones (Q) that might prove useful in biological extraction and reconstitution experiments, we have measured the half reduction potentials for the redox couples $Q \longrightarrow Q^{\cdot -}$, and $Q^{\cdot -} \longrightarrow Q^{2-}$ in aprotic solvents, and observed that the half-reduction potentials of the ubiquinones are markedly more positive than would be expected if the substituents behaved independently. This phenomenon is attributed to steric interaction between the methoxy groups.

2. MATERIALS AND METHODS

Cyclic voltammetry was performed at ambient temperature at a glassy carbon electrode (28 mm^2 area) with a platinum counter-electrode and a saturated calomel reference electrode in dimethyl formamide (dried over 4 Å molecular sieves) containing 50 mM tetrabutylammonium tetrafluoroborate, using a Princeton Applied Research model 173 potentiostat. Measurements were made over the range +1 V to −2 V, and the repeatability of the determinations was within ± 20 mV for $Q/Q^{\cdot -}$ and ± 40 mV for $Q^{\cdot -}/Q^{2-}$. Under these conditions, forrocene has a half-reduction potential of +524 mV.

Benzoquinones were obtained as follows: unsubstituted, 2,3-dimethyl, methoxy and all 3 dimethoxy compounds were prepared in Manchester

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as in [3]; methyl and 2,5-dimethyl from Eastman Kodak (Rochester, NY 14650); 2,6-dimethyl from ICN Pharmaceuticals (Plainview, NY 11803); trimethyl and tetramethyl from Aldrich (Milwaukee, WI 53201); plastoquinones-1 and -9 from Dr P.R. Rich (Department of Biochemistry, University of Cambridge); ubiquinone-0 from Merck, Sharp and Dohme (Rahway, NJ 07065); 6-methylubiquinone-0 from Biochemical Laboratories (San Diego, CA 92112); 6-decylubiquinone-0 from Dr B.L. Trumpower, Dartmouth Medical School (Hanover, NH 03755); and ubiquinones-1, -3 and -10 from Hoffman LaRoche (Nutley, NJ 07110).

The unprenylated naphthoquinones were obtained from Aldrich, and the prenyl substituted naphthoquinones from Hoffman LaRoche.

3. RESULTS

Table 1 lists the half-reduction potentials of the $Q/Q^{\cdot -}$ and $Q^{\cdot -}/Q^{2-}$ couples in dimethylformamide. Parallel results were obtained for solutions in dimethyl sulfoxide and acetonitrile.

3.1. *Plastoquinones (3)*

Each methyl group added to the parent 1,4-benzoquinone lowers the $E_{½}$ of the $Q/Q^{\cdot -}$ couple by 70–80 mV, and of $Q^{\cdot -}/Q^{2-}$ by somewhat less, with, as expected, little positional dependence for the dimethyl compounds. The addition of a prenyl or a polyprenyl group has about the same effect on $Q/Q^{\cdot -}$ as a methyl, although we were unable to detect a clear wave attributable to $Q^{\cdot -}/Q^{2-}$ for any of the prenylated compounds.

3.2. *Menaquinones (2)*

For the 1,4-naphthoquinones the first methyl on the quinone ring lowers the $E_{½}$-values by about 70 mV whilst a second methyl, or indeed other alkyl substituent, lowers the $E_{½}$ by a little more. Substituents on the adjacent ring have effects which are very position-dependent, and these will be addressed at length elsewhere [4]. In this family a prenyl substituent on the quinone ring is not quite as effective as an alkyl group, and lowers the $E_{½}$ value, at least of $Q/Q^{\cdot -}$, by only 50–60 mV.

3.3. *Ubiquinones (1)*

A single methoxy group lowers the $E_{½}$ of the 1,4-benzoquinone $Q/Q^{\cdot -}$ couple by about 100 mV, and the $Q^{\cdot -}/Q^{2-}$ couple by about half that. The 2,5- and 2,6-dimethoxy compounds exhibit about twice these shifts. However, the effect

Table 1

Half-reduction potentials ($E_{½}$ mV *vs* SCE) for $Q/Q^{\cdot -}$ and $Q^{\cdot -}/Q^{2-}$ in dimethylformamide

	$Q/Q^{\cdot -}$	$Q^{\cdot -}/Q^{2-}$
1,4-Benzoquinones[a]		
Unsubstituted	−401	−1155
Methyl-	−466	−1270
2,3-Dimethyl- (plastoquinone-0)	−543	−1269
2,5-Dimethyl-	−551	−1299
2,6-Dimethyl-	−547	−1257
Trimethyl- (5-methylplastoquinone-0)	−632	−1393
Tetramethyl- (5,6-dimethylplastoquinone-0)	−751	−1422
Plastoquinone-1	−640	
Plastoquinone-9	−619	
Methoxy-	−509	−1213
2,3-Dimethoxy-	−458	−1184
2,5-Dimethoxy-	−670	−1282
2,6-Dimethoxy-	−628	−1282
2,3-Dimethoxy-5-methyl-	−539	−1250
2,3-Dimethoxy-5,6-dimethyl- (ubiquinone-0)	−645	−1303
2,3-Dimethoxy-5-methyl-6-decyl- (6-methylubiquinone-0) (6-decylubiquinone-0)	−622	
Ubiquinone-1	−611	
Ubiquinone-3	−611	
Ubiquinone-10	−602	
1,4-Naphthoquinones[a]		
Unsubstituted	−581	−1310
2-Methyl-	−650	−1384
2,3-Dimethyl- (menaquinone-0)	−746	−1457
2-Methyl-3-undecyl- (3-methylmenaquinone-0) (3-undecylmenaquinone-0)	−730	−1440
Menaquinone-2	−709	
Menaquinone-7	−705	
Menaquinone-10	−709	

[a] Numerals following the names of quinones indicate the number (n in 1–3) of prenyl units

is markedly diminished when both methoxy groups are attached to the same double bond, and indeed the $E_{1/2}$-values of 2,3-dimethoxy-1,4-benzoquinone are positive of those of the mono-substituted compound. This behaviour is retained in the alkyl and prenyl homologues, with the alkyl and prenyl groups lowering the $E_{1/2}$-values in a fashion similar to that observed for plastoquinones and menaquinones.

4. DISCUSSION

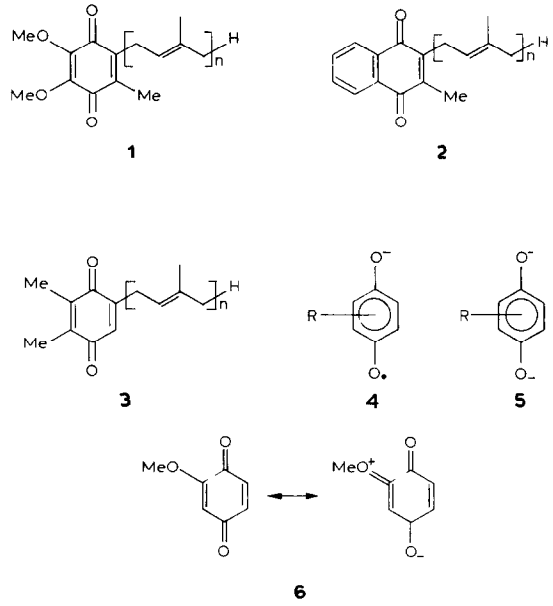
The 3 classes of biological quinones under consideration here are 1,4-benzo- and 1,4-naphthoquinones substituted with methyl, polyprenyl and/or methoxy groups. From measurements on more than 100 benzoquinones and naphthoquinones, we have been able to assign values for both classes of quinone to the reaction constant, ϱ, in the Hammett equation [5]:

$$\Delta E = \varrho \Sigma \sigma_{para}$$

As has been found in [6], appropriate substitution, σ, constants for quinones are those for *para*-substituents in benzene derivatives [7]. As we shall discuss elsewhere [4], in dimethylformamide ϱ = +521 mV for the $Q/Q^{\cdot -}$ couple of 1,4-benzoquinones, and +357 mV for 1,4-naphthoquinones substituted in the 2- and/or 3-positions. The value for the benzoquinones is in excellent agreement with that (+530 mV) determined from the data in [6,8] for solutions in acetonitrile.

The data for the alkyl- and methoxy-substituted compounds (with the exception of the 2,3-dimethoxy compounds) fit the Hammett equation very well, allowing us to estimate a σ_{para}-value for the prenyl substituent of about −0.16 compared with −0.17 for a methyl group [7]. No significant differences were observed between polyprenyl chains having from 1–10 prenyl units, or between alkyl chains from 1–11 carbons in length.

A single methoxy group has a σ_{para}-value of −0.27, but when two methoxy groups are adjacent, the apparent σ_{para} for the pair is only −0.10. This can be explained by consideration of the two separate electronic effects of a methoxy substituent. One, the inductive electron-withdrawing effect of the oxygen, always operates, regardless of steric effects; this, if it operated alone, would make, for



example, methoxy-1,4-benzoquinone easier to reduce than 1,4-benzoquinone itself. The other, the resonance or mesomeric effect, is electron donating and normally predominates: it results from delocalisation (as **6**) of the lone pair of electrons on the methoxy oxygen over the enone system of the quinone nucleus, with the net result that methoxy-1,4-benzoquinone is more difficult to reduce than the unsubstituted quinone. This delocalisation requires that the methoxy group can adopt a conformation in which the *p*-orbital carrying the lone pair is parallel to the π system of the ring, a requirement which may be sterically impaired by an adjacent substituent such as the second methoxy group in 2,3-dimethoxy-1,4-benzoquinone. Molecular orbital calculations [9] for 2,3-dimethoxy-5,6-dimethyl-1,4-benzoquinone suggest a preferred conformation in which the O–CH_3 bonds of the methoxy groups lie on opposite sides of the plane of the quinone ring, although an X-ray crystallographic study [10] of 2,3-dimethoxy-5-methyl-1,4-benzoquinone shows that only one of the methoxy groups adopts a conformation which deviates significantly from coplanarity with the ring. Thus, although definitive data relating to the preferred conformation of a 2,3-dimethoxy-1,4-benzoquinone in solution are not available, it is to be expected that the mesomeric effects of the methoxy groups will not be additive. Their inductive effects

should be essentially undiminished. 2,3-Dimethoxy-1,4-benzoquinone is therefore more easily reduced than methoxy-1,4-benzoquinone. Notably, the half-reduction potentials of the ubiquinones (1) are similar to those of the plastoquinones (3) despite the presence of the additional methyl group in the former. The menaquinones (2), which differ from the plastoquinones in having a 1,4-naphthoquinone nucleus instead of a 2,3-dimethyl-1,4-benzoquinone one, are more difficult to reduce by about 100 mV.

We were unable to detect the reduction of $Q^{\cdot -}$ to Q^{2-} in any of the prenylated compounds in dimethyl formamide. This may be a consequence of the relatively low solubility of these compounds, together with the observation that the waves due to the second reduction are usually less pronounced than those for the Q to $Q^{\cdot -}$ reaction. If this explanation is correct, we may estimate $E_{1/2}$ for the second reduction using our estimate for σ_{para} for the prenyl group, and our measured value of the reaction constant ϱ for other quinones. We find ϱ to be +418 mV for the benzoquinone $Q^{\cdot -}/Q^{2-}$ couple, and +288 mV for the naphthoquinones substituted in the 2- or 3-positions [4]. The $E_{1/2}$-values for the second reduction steps may then be estimated as −1336 mV for plastoquinones 1–9, −1317 mV for ubiquinones 1–10 and −1430 mV for menaquinones 1–10 in dimethyl formamide.

In addition to raising the reduction potential above that expected from simple predictions, the presence of two adjacent methoxy groups on the 1,4-benzoquinone nucleus results in a significant bathochromic shift relative to 2,6- and 2,5-dimethoxy-1,4-benzoquinone [11]. This is particularly noticeable for the solid quinones and their concentrated solutions in aprotic solvents: 2,3-dimethoxy-1,4-benzoquinone is orange-red, and the 2,6- and 2,5-dimethoxy isomers are, respectively, orange-yellow and yellow. The branched donor-acceptor (dimethoxy-enedione) chromophore present in 2,3-dimethoxy-1,4-benzoquinone may lead to a particularly small HOMO–LUMO splitting energy, and hence to the marked bathochromic shift [12].

Whilst the absolute values of the reduction potentials and electronic absorption spectra of the quinones presently under discussion would be expected to be significantly dependent on environment, the relative differences between the variously substituted quinones will probably parallel those now reported on. These highlight the importance of the 2,3-dimethoxy-1,4-benzoquinone moiety in biological systems.

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