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Partition coefficients of quinones and hydroquinones and their relation to biochemical reactivity

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Some major effects of ring substituents on the partition coefficients of quinone headgroups are described. Attention is drawn to the large differences in partition coefficients in cyclohexane/water of the two major freely diffusing redox forms, the quinone, Q, and the hydroquinone, QH_2. Methoxy substituents cause a marked increase of the cyclohexane/water partition coefficient of the hydroquinone, but this effect is absent in the quinone and is also not seen in measurements in octanol/water. The relation between partition coefficients and biochemical specificity of quinone binding sites is explored.

Ubiquinone; Plastoquinone; Partition coefficient; Quinone; Quinol; Hydroquinone; Steric specificity; Quinone analogue; Quantitative structure-activity relationship

1. INTRODUCTION

Ubiquinone and plastoquinone are essential components of respiratory and photosynthetic electron transfer chains respectively and they couple electron transfer to proton translocation across the membrane in a way which is now well understood [1]. In many cases, quinone analogues can compete as alternative substrates [2,3] or as inhibitors [4] of the natural reaction. It is of interest to probe the binding sites on the protein for quinone specificity and for clues of biochemical mechanism with analogues with differently substituted quinone rings. This has become of particular medical relevance with possible anti-ageing [5] and other chemotherapeutic uses of ubiquinone and its analogues [6], the carcinogenic and anti-cancer properties of some quinone types, the effects of di-*ter*-butylhydroquinone on calcium homeostasis [7], and the essential nutritional role of the naphthoquinone, vitamin K. For those quinones which are directly involved in energy coupling, a number of studies have related structural features to activity. These include investigations of the quinone specificity of bacterial reaction centres [8–10], chloroplast photosystem II [11–16] and cytochrome *bf* complex [11,15,17], and mitochondrial succinate dehydrogenase, complex I and bc_1 complex of mitochondria [18–21]. Two of the major physical parameters changed by a change of the quinone ring substituents are the partition coefficients and the steric fits of the chemical species involved, and these are in addition to changes of the midpoint potentials and pK values of the electronation and protonation equilibria which have been previously discussed [22].

The polyisoprenyl side chains of the naturally occurring quinones give the molecules partition coefficients which favour the apolar phase by many orders of magnitude, and so make them reside almost exclusively in the lipid bilayer (each isoprenyl unit increases the partition coefficient by approximately 2.5 orders of magnitude [23]). However, the chemically reactive part of the molecule is the headgroup and its microscopic physical properties will contribute to its occupation of different accessible domains of the membrane and its associated proteins and hence will contribute to biological activity and specificity. Any quantitative structure-activity relationship (QSAR) study of quinone headgroup in which the quinone undergoes a redox transformation will require detailed knowledge of partition coefficients of both quinone and hydroquinone (and, ideally, semiquinone) forms of the headgroup and this comparison is made in the present report.

2. MATERIALS AND METHODS

Sources of quinones were: benzoquinone (BQ), hydroquinone (BQH_2), methyl-BQH_2, 2-methyl-6-bromo-BQ, 2,6-dimethyl-BQ, trimethyl-BQH_2, tetramethyl-BQ, phenols, Aldrich; 2,3-dimethyl-BQ, by synthesis from the phenol [24]; methoxy-BQH_2, 2,3-dimethoxy-BQ, 2,6-dimethoxy-BQ, Apin Chemicals; tetramethoxy-BQ, a gift from Professor C.A. Wraight; 2-methyl-1,4-naphthoquinone, Sigma. Syntheses were carried out as described in [24–26]. Where necessary, further purification was

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achieved with preparative thin layer chromatography. Partition coefficients were measured at 23°C by shaking the compound in the appropriate two-phase system. 10 mM HCl was present in the aqueous phase in order to prevent autoxidation of hydroquinone. Concentration ratios were measured spectrophotometrically, assuming an equal absorption coefficient for the compound in both phases (we determined for several compounds that this approximation introduced a possible error to $\log_{10}$ P values of not more than ± 0.2). Partition coefficients are expressed on a molar, rather than a molal, ratio basis.

Beef heart mitochondrial succinate-cytochrome *c* reductase was prepared by the method in [27] and was stored at 77 K. Rate constants for hydroquinone oxidation were determined from the rate of cytochrome *c* reduction at pH 7 and 23°C in 50 mM potassium phosphate, 2 mM EDTA and 1 mM KCN, with high cytochrome *c* and low hydroquinone concentrations so that rates were pseudo-first order in hydroquinone. Second order rate constants were then: k = rate/($QH_2 \times bc_1$).

3. RESULTS

Octanol is often used as a model solvent for understanding partitioning from water into lipid membranes. It is relatively non-polar (dielectric constant of 10.3) but is capable of forming hydrogen bonds. Cyclohexane/water is a reasonable model for partitioning from water into the interior of a lipid bilayer. It has a dielectric constant of 2.0 and cannot form hydrogen bonds.

Fig. 1 and Table I illustrate the effects of methyl group substitution of the quinone ring on the partition coefficient between water and either the nonpolar, hydrogen bonding solvent octanol or the non-polar, non-hydrogen-bonding solvent cyclohexane. The results are very much as predicted by the FRAGMENT method [23]. The parent quinone and hydroquinone partition rather similarly in octanol/water, but the hydroquinone partitions much more strongly into the water phase in cyclohexane/water. The behaviour can be rationalised in terms of two major factors, the polarity of the molecules and their propensity to form hydrogen bonds with the solvents. The hydroquinone, compared to the quinone, is of roughly similar polarity but can form stronger hydrogen bonds with water or octanol. Hence hydroquinone, in comparison to quinone, partitions more into water in the cyclohex-

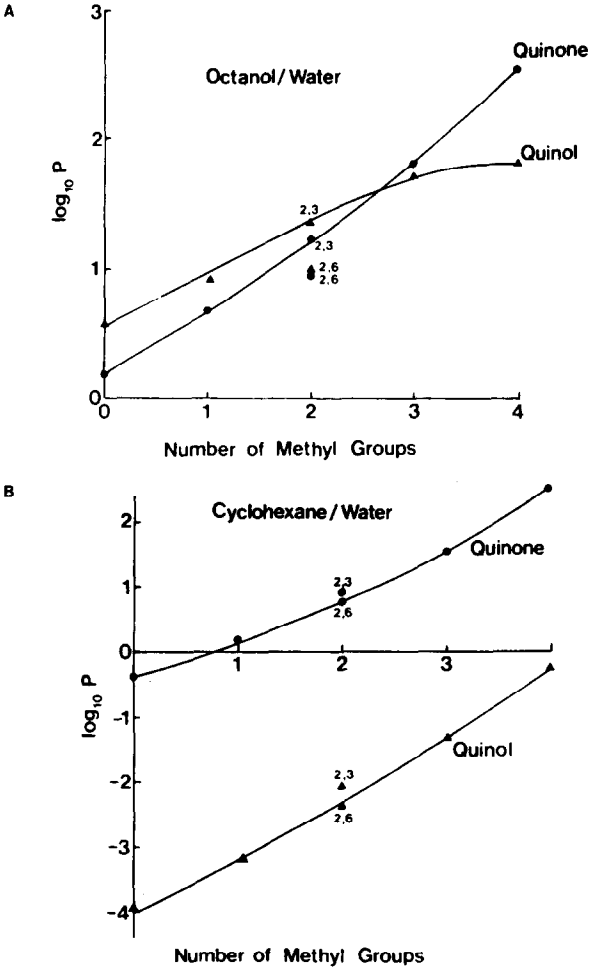

Fig. 1. Partition coefficients of methyl-substituted *para*-benzoquinones/ols. The points for the 2,3-dimethyl or the trimethyl derivative may be considered reasonable models of the headgroup of the naturally occurring plastoquinone.

Table I

Summary of partition coefficients of some quinones and hydroquinones of biochemical interest

Substituents on	Octanol/water		Cyclohexane/water	
	Quinone	Quinol	Quinone	Quinol
1,4-benzoquinones				
None	0.18	0.58	−0.40	−3.89
Methyl-	0.67	0.58	0.19	−3.19
2-Methyl-6-bromo-	1.21	1.38	1.10	−0.78
2,3-Dimethyl-	1.24	1.36	0.92	−2.05
2,6-Dimethyl-	0.94	0.98	0.79	−2.39
Trimethyl-	1.8	1.69	1.58	−1.32
Tetramethyl-	2.52	1.8	2.45	−0.25
Methoxy-	0.27	0.47	1.17	−2.63
2,3-Dimethoxy-	0.25	0.59	−0.36	−1.57
2,6-Dimethoxy-	−0.05	0.17	−1.51	−2.25
Tetramethoxy-	0.37	0.39	−0.54	−1.11
2,3-Dimethoxy-5-methyl- (ubiquinone-0)	0.78	1.02	0.39	−0.39
2,3-Dimethoxy-5,6-dimethyl-	1.26	1.60	0.73	0.51
2,3-Dimethoxy-5-methyl-6-isoprenyl- (ubiquinone-1)	>3	>3	>3	1.6
2,3-Dimethoxy-5-methyl-6-decyl- (*decyl*-ubiquinone)	>3	>3	>3	>3
2,3-Dimethyl-6-isoprenyl- (plastoquinone-1)	>3	>3	>3	0.23
2,3-Dimethyl-6-decyl- (*decyl*-plastoquinone)	>3	>3	>3	2.73
1,4-Naphthoquinones				
2-Methyl- (menaquinone-0)	2.1	1.36	1.86	−0.65

Values are given as $\log_{10}$P

ane/water system, but in octanol/water is rather similar in behaviour to the quinone. Addition of each methyl group causes further partitioning into the hydrophobic phase in all cases as expected.

Addition of methoxy, rather than methyl, groups is in general predicted to cause little change in partition coefficient compared to the parent compound [23]. This is roughly borne out experimentally (Fig. 2) for partitioning of methoxy-substituted quinones and hydroquinones in octanol/water and of methoxy-substituted quinones in cyclohexane/water. However, the partition coefficients of the methoxy-substituted hydroquinones in cyclohexane/water increase by roughly an order of magnitude for each group added for the mono- and 2,3-di-substituted compounds. This is equivalent to almost 1.5 kcal/mol/methoxy and so is a significant factor in determination of the measured partition coefficients of the hydroquinone structures. The effect presumably arises from a weakening of the hydrogen bonding in water of the hydroquinone by the methoxy substituents. This might be caused by intramolecular hydrogen bonding between the methoxy and hydroxy substituents, as occurs in 2-methoxybenzoic acid [28], but this seems unlikely to be strong because of the electron donating property of the methoxy group and because of the angles in any resulting hydrogen bond. Alternatively, it might arise from the electron donating effect of the methoxy groups, since the ring can act as a donor in charge transfer complexes or as a hydrogen bond acceptor.

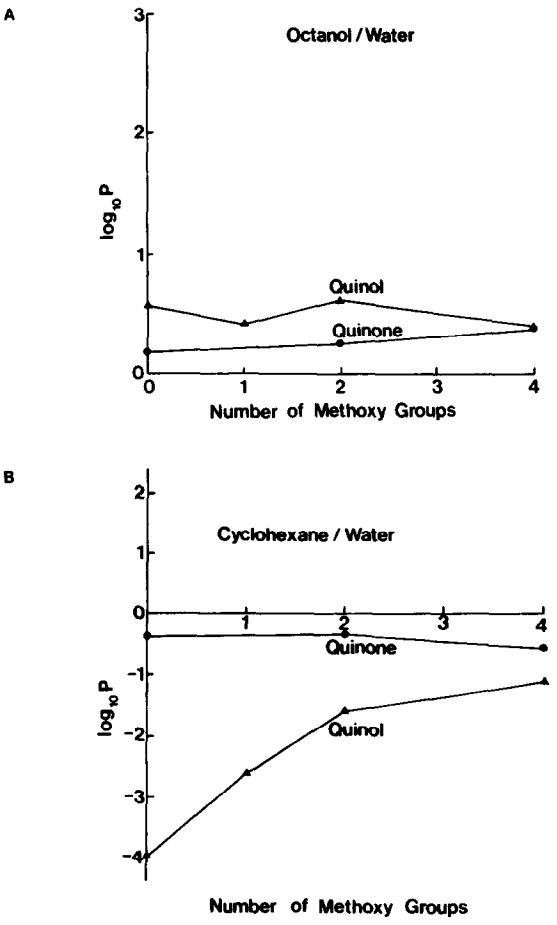

Fig. 2. Partition coefficients of methoxy-substituted *para*-benzoquinones/ols.

The origin of the 2-methoxy effect was further explored with monomethoxyphenols as model compounds (Table II). The elevation of the cyclohexane/water partition coefficient can again be seen for the methoxy group in the 2-position, but was not apparent for *meta* or *para* substitutions, a result indicating that the effect originates from the proximity of the methoxy and hydroxy groups. The measured partition coefficient of 2-methoxyphenol was unchanged over a concentration range of solute of three orders of magnitude, a result arguing against an effect caused by polymerisation in one or both solvents. The result could also not be explained by large variations in extinction coefficient in different solvents. The partition coefficients were unchanged by substituting H_2SO_4 or phosphoric acid for the HCl in the aqueous phase, so that a specific interaction with other ions could also be ruled out. However, we could not detect any significant difference (<0.25 pH units) between the aqueous pK values of 2-methoxyphenol and phenol. A measurable pK change might have been expected if there were a tendency for internal hydrogen bonding between methoxy and hydroxy substituents. Hence, we are at present unable to positively identify the molecular origin of the 2-methoxy effect in either phenols or hydroquinones. Although we have excluded several trivial explanations, we cannot completely rule out polymerisation, or the possibility that water itself might form a complex with the 2-methoxy and hydroxy groups in cyclohexane. Whatever the cause, an understanding may shed new light on the physical biochemistry of ubiquinone in the lipid membrane.

Also included in Table I is the effect of bromination on the partition coefficients of methyl-1,4-benzoquinone. The increase in $\log_{10}P$ of 0.5–1 in favour of the hydrophobic phase is generally as expected. As in the case of methoxy substituents, however, the increase of partition coefficient of the hydroquinone from water into cyclohexane is much greater, with $\log_{10}P$ being raised by more than 2 units. This should arise from the

Table II

Partition coefficients of methoxy-substituted phenols

Substituents	$\log_{10}P$ in octanol/water	$\log_{10}P$ in cyclohexane/water
None	1.57	−0.68
2-Methoxy-	1.21	0.48
3-Methoxy-	1.44	−1.05
4-Methoxy-	1.38	−0.99

electron withdrawing property of the halogen which will tend to weaken the external hydrogen bonding ability of one or both hydroxy groups. The figures allow us to predict the $\log_{10}P$ values of the important quinone-binding site inhibitor 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB), if we assume that a second bromo group has the same effect as the first and using an average value of +1.72 for the difference in hydrophobic fragment constant of isopropyl and hydrogen [29]. Such extrapolations give $\log_{10}P$ values for the quinone and hydroquinone forms respectively of 3.5 and 3.9 (octanol/water) and 3.7 and 3.4 (cyclohexane/water).

The partition coefficients of 2-methyl-1,4-naphthoquinone have also been included in Table I since this can be considered to be a parent compound from which partition coefficients of a variety of derivatives of biological importance may be estimated.

4. RELEVANCE TO BIOLOGICAL SYSTEMS

The measured partition coefficients for the ubi(hydro)quinone analogue 2,3-dimethoxy-5-methyl-benzo(hydro)quinone are much as expected from comparison with the behaviour of the other derivatives. An important point is that the methoxy groups have the effect of bringing closer together the partition coefficients of the quinone and hydroquinone forms in a non-hydrogen bonding solvent, as would be the case in the interior of the mitochondrial lipid bilayer. Hence the distribution of headgroups within the membrane can be similar for both redox forms of ubiquinone. In the case of plasto(hydro)quinone derivatives, such as 2,3-dimethyl- or trimethyl-benzo(hydro)quinone, the partition coefficients of quinone and hydroquinone in the non-hydrogen bonding hexane/water system are widely separated. This implies that the headgroups of plastoquinone in the membrane will tend to occupy different microenvironments in the quinone and hydroquinone forms. This behavior represents the most clear physical difference between the ubiquinone and plastoquinone systems which has yet been noted. A partition coefficient difference of 10^4 between the quinone and hydroquinone forms of plastoquinone is expected to lower the measured $n = 2$ midpoint potential of the membrane (QH_2/Q) couple by 120 mV from its aqueous solution value of about +109 mV [22]. This appears not to be the case, however, and implies that microenvironments are available to the quinone headgroup within the membrane environment which is not modelled by cyclohexane. This might be consistent with the plastohydroquinone headgroup spending proportionally more time close to the membrane surfaces than does the plastoquinone headgroup.

In addition to the hydroquinone ring electrons acting as possible donors in charge transfer complexes, a

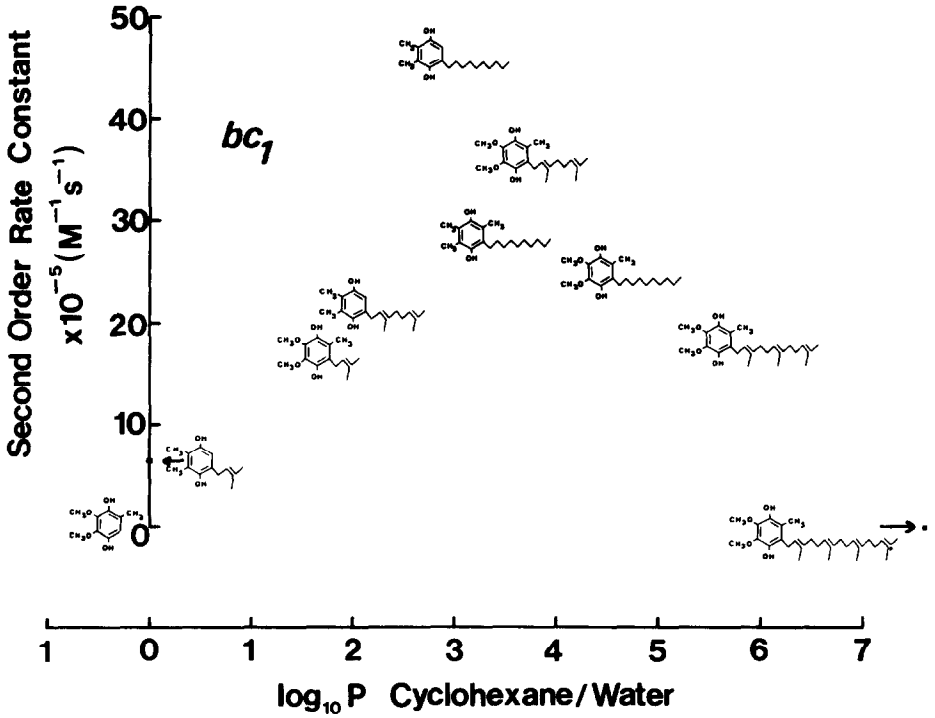

Fig. 3. Second order rate constants for oxidation of hydroquinones by beef heart mitochondrial bc_1 complex and their relation to cyclohexane/water partition coefficients. Partition coefficients of the hydroquinones in cyclohexane/water were estimated by addition of an offset for the hydrophobic side chain, obtained by FRAGMENT estimation, to the measured values of the parent compounds. The offset compared to hydrogen substitution was taken to be 10^2 for each isoprenyl and $10^{4.8}$ for *n*-decyl. These figures were obtained by differences of measured values and are slightly lower than FRAGMENT estimation.

dominant factor for binding of the headgroups into a proteinaceous quinone binding site will presumably be the formation of hydrogen bonds, as is the case with the known structure of the Q_A and Q_B sites of bacterial reaction centres [30]. A function of the very long hydrophobic tail of the natural quinones will be to counteract these enthalpic binding forces with the entropic forces associated with re-entry into the fluid membrane interior. In this way rapid exchange with the binding site is possible. In the case of the hydroquinone form of ubiquinone, the methoxy groups should also aid re-entry back into the lipid phase. It might be pointed out that hydrophilic (hydro)quinones will generally be poor substrates not only because their polarity is not suited to the relatively apolar binding sites, but also because they are already hydrogen bonded into the aqueous solvent, so that no net gain is made in a 'hydrogen bond inventory' [31] on binding.

When attempting to determine *steric* specificity of a quinone binding site, it is important to consider the influence of partition coefficient. For example, a plot of rate constant for reaction of hydroquinone with the mitochondrial bc_1 hydroquinone oxidation site versus $\log_{10}P$ of hydroquinone in cyclohexane/water (Fig. 3) shows that the partition coefficient is a major factor in reactivity determination, with steric specificity for sidechain or for methoxy- or methyl-substituents playing little role. A peak in the profile at around $\log_{10}P$ of 3 is seen. The fall in rate constant at higher values probably represents an artefact caused by the very limited aqueous solubility of the hydroquinones involved, or possibly a very low dissociation constant of quinone product which would then hinder multiple turnovers of the enzyme. It is likely that some substituent specificity effects which have been observed in the past (e.g. [20,21,32]) are predominantly caused by partitioning and thermodynamic factors, rather than by steric factors. In an extensive series of studies, Oettmeier et al. [11–15] have demonstrated the dominant influence of partition coefficient on the effectiveness of a range of halogenated benzoquinones as alternative acceptors of photosystem II. Low steric specificity of a site for the substituents on the parent compound is unusual in biology. A similar low steric specificity of the Q_A site of bacterial reaction centres has been found by Gunner et al. [33], who made allowance for partitioning effects so that the relation between thermodynamics of electron transfer and rates could be assessed. Presumably, the results indicate some flexibility of the binding sites and the dominance in the binding forces of hydrogen bonding to the quinone oxygens and bonding with the ring orbitals. Such low specificity is by no means necessarily expected to be the case for all quinone binding sites. Indeed, in experiments with the chloroplast cytochrome *bf* complex (data not shown) we have found a limited specificity for plastoquinone-like quinones. Some biomedical effects of quinones are likely to be caused by their chemical redox mediating properties. For example, they may catalyse the oxidation of reductants by molecular oxygen to produce superoxide radicals, or may act by removal of radical intermediates. In such cases, partition coefficients might play a less dominant role, and potency will be mostly determined by the midpoint potentials and pK values of electronation and protonation.

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