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VECTORIAL REDOX REACTIONS OF PHYSIOLOGICAL QUINONES

I. REQUIREMENT OF A MINIMUM LENGTH OF THE ISOPRENOID SIDE CHAIN

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Summary

Physiological quinones carrying isoprenoid side chains have been compared with homologues lacking the side chain, for their ability to carry electrons and protons from dithionite to ferricyanide, trapped in liposomes. Six differential observations were made:

(1) Plastoquinone and ubiquinones, with a side chain of more than two isoprene units, are by far better mediators than their short-chain homologues. Also other benzoquinones lacking a long side chain are poor catalysts, except dimethyl-methylenedioxy-*p*-benzoquinone, a highly autooxidizable compound. Tocopherol is a good catalyst.

(2) Vitamin K-1 and K-2 are poor mediators compared to vitamin K-3.

(3) The reaction catalyzed by quinones carrying long isoprenoid side chains has an about three-fold higher activation energy, irrespective of the catalytic efficiency.

(4) The reaction catalyzed by quinones lacking a long side chain follows

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Abbreviations: Q-*n*, *n* = 1—10, ubiquinone with 1—10 isoprene units in the side chain; PQ, plastoquinone A; K-1, K-2 and K-3, phylloquinone (vitamin K-1), menaquinone-7 (vitamin K-2) and menadion (vitamin K-3), respectively; TMQ, trimethyl-*p*-benzoquinone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; MDQ, 2,3-dimethyl-5,6-methylene-dioxy-*p*-benzoquinone; DQ, 2,3,5,6-tetramethyl-*p*-benzoquinone (duroquinone); DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine (diaminodurol); Tricine, *N*-tris-(hydroxymethyl)methylglycine. Other abbreviations are described in the legends.

pseudo first-order kinetics, while the reaction with quinones carrying a long side chain is of apparently higher order.

(5) The rate with ubiquinone-1 is increasing with increasing pH, while with ubiquinone-9 it is decreasing.

(6) The reaction mediated by short-chain quinones seems to be saturated at lower dithionite concentration.

We conclude that isoprenoid quinones are able to translocate electrons and protons in lipid membranes, and that the side chain has a strong impact on the mechanism. This and the relevance of the model reaction for electron and proton transport in photosynthesis and respiration is discussed.

Introduction

A central issue in current bioenergetics still is the mechanism of proton translocation coupled to electron transport in photosynthesis and respiration [1—3]. An evident possibility is the vectorial redox reaction of a quinone in the membrane, as originally conceived [4] and more recently developed into more complex views [5] by Mitchell. Plastoquinone (PQ) in plant photosynthesis and ubiquinone (Q) in respiration and in bacterial photosynthesis, both present in excess, mobile pools in the membranes [6,7], could fulfill such a role. Both, however, carry isoprenoid side chains, long enough to span the whole double layer of a lipid membrane, if in all-*trans* form. This could restrict the mobility of PQ and Q perpendicular to the plane of the membrane. Indeed, on this basis, arguments against the view that these quinones could act as mobile proton and electron carriers across lipid membranes have been constructed ([8,9], and see Ref. 6 for a review), in spite of the fact that Q had been shown to carry reducing equivalents through lipid barriers [10,11], which has been corroborated recently by the finding that PQ increases the dark and photoconductance of lipid bilayers containing chlorophyll [12].

Employing a liposome model system introduced by Hinkle [13,14], we ourselves have established that PQ and Q per se are efficient translocators of protons and electrons, the isoprene side chain even having a facilitating role [15,16]. The results presented here are an extension of this work with respect to the parameters and the number of compounds tested. The accompanying paper [17] deals with the influence of the isoprenoid side chain on transient semiquinone formation during the model reaction.

Methods and Materials

Quinone-containing liposomes were obtained as previously described [15], with minor modification: 200 mg of soy bean lecithin (Sigma, Type II-s) together with quinone (0.26—13 μmol) were dissolved in 1 ml chloroform in a 5 ml beaker. The solution was evaporated to dryness by a stream of N_2 followed by standing in a vacuum desiccator. Then 5 ml of 0.2 M ferricyanide, buffered at pH 8.0 with 50 mM Tricine/NaOH, were added and the mixture was sonicated with a Branson sonicator using the microtip for 30 min at full output. By cooling the beaker in ice/water the temperature was maintained

below 25°C. Subsequently liposomes were separated from external ferricyanide on a small column of Sephadex G-50, equilibrated with 0.3 M KCl, 50 mM Tricine/NaOH, pH 8.0. Lipid content of the liposome fractions was estimated by comparing the *A* at 550 nm with that of unfractionated liposomes. Fractions with a dilution factor of less than 2 were combined. Eventual loss of quinone during column chromatography was checked by ultraviolet redox difference spectroscopy of quinone-liposomes formed in the KCl instead of the ferricyanide buffer. With most of the quinones loss was negligible. Exceptions are indicated in Table I. In these cases the quinoid compound was added in ethanolic solution to control liposomes which had been formed in the absence of quinone (the final ethanol concentration never exceeded 1%, which was without an effect on control liposomes). To obtain good reproducibility care must be taken to evaporate $CHCl_3$ completely, and to adjust pH and sonic power. These subtle adjustments are probably the reason for some differences to the preliminary results published earlier [16].

Reduction of ferricyanide trapped in the liposomes by external dithionite was measured with a stop-flow apparatus, Durrum Model 110, modified by Dr. P. Bartholmes. The set-up was equipped with an Osram-Xe-lamp, XBO 75 W, a monochromator from Schoeffel, GM 100-1, 1180 grooves/nm, and a thermostating water bath. The optical path of the cuvette was 2 cm. The signal was fed to a Datalab transient recorder DL905 connected to a Tectronix 7623A oscilloscope and a recorder. A solution containing the liposomes (2—3 mg lecithin/ml of 0.3 M KCl, 50 mM Tricine/NaOH, pH 8.0) in one syringe was mixed with a dithionite solution (20 mM, except for Fig. 2) from the other syringe within a mixing time of less than 2 ms. Both solutions were sufficiently degassed in vacuum before use. The liposome solution additionally contained valinomycin and nigericin, 1 μg/ml of each. The quinone content varied between 1.3 and 65 nmol/mg lecithin (see Figs. 3 and 6), but in most of the experiments it was 13 nmol/mg lecithin, which corresponds to a molar ratio of lipid/quinone of 100. This value is close to the quinone content of mitochondrial [18] and chloroplast membranes [7]. The ratio of external dithionite (except for Fig. 2) to internally trapped ferricyanide was about 70. The later was estimated from the reductant-sensitive *A* at 420 nm of the liposome preparation, using an ϵ of $1.04 \cdot 10^3\ M^{-1} \cdot cm^{-1}$. The high excess of dithionite should provide for pseudo first order of ferricyanide reduction. At a molar ratio of lipid to quinone of 100, the molar ratio of ferricyanide to quinone was about 10, the quinone thus acting as a catalyst with actual turnover. Measurements were carried out at room temperature.

The series of ubiquinones (Q-1—Q-10) and plastoquinone (PQ) were generous gifts from Dr. Gloor and Dr. Weber, Hoffmann-La Roche, Basel. TMQ, DBMIB, MDQ and DAD were kindly provided by Dr. A. Trebst, Bochum, Menaquinone (vitamin K-2), isolated from *Bacillus megaterium* (ATCC 14581), was generously donated by Dr. A. Kröger, Munich. Phylloquinone (vitamin K-1), menadione (vitamin K-3) and DL-α-tocopherol were purchased from Merck, Darmstadt. Dolichol A from yeast was obtained from Dr. P. Babczinski, Regensburg. Duroquinone (DQ) was prepared from DAD by aeration of an aqueous solution. The water-insoluble, yellow crystals of DQ were recrystallized from ethanol. Q-9sat was obtained by saturation of the iso-

prenoid side chain in Q-9 by hydrogenation with PtO_2 as catalyst. The dimethyl ether of QH_2-9 (= 1-nonaprenyl-2,3,4,5-tetramethoxy-6-methyl benzene) was synthesized with dimethyl sulfate in ethanolid KOH. Both derivatives were purified by thin-layer chromatography, and were checked by NMR spectroscopy. Ubiquinol-9 (QH_2-9) was prepared by the method of Rieske [19]. All quinones were checked for purity by thin-layer chromatography on silicagel plates (Merck, Darmstadt, TLC plates silica gel 60, without fluorescence indicator, precoated, layer thickness 0.25 mm). Either benzene/chloroform (1 : 1), or acetone/paraffin-saturated water (9 : 1) was used as developing solvent. In the later case the silica gel plates had been impregnated with paraffin before use [20]. In addition the purity of the quinone was tested by ultraviolet spectroscopy of the oxidized and reduced forms in ethanol [21]. Reduction was performed by BH_4^-. Occasional impurities were separated by preparative thin-layer chromatography.

Valinomycin and nigericin were obtained from Sigma, St. Louis, and from Eli Lilley, Indianapolis, respectively.

Results

In Fig. 1 traces for the reduction of ferricyanide, trapped in liposomes containing different quinones, by external dithionite are shown. As reported before [15,16], PQ and Q-9, both having a side chain of nine isoprene units,

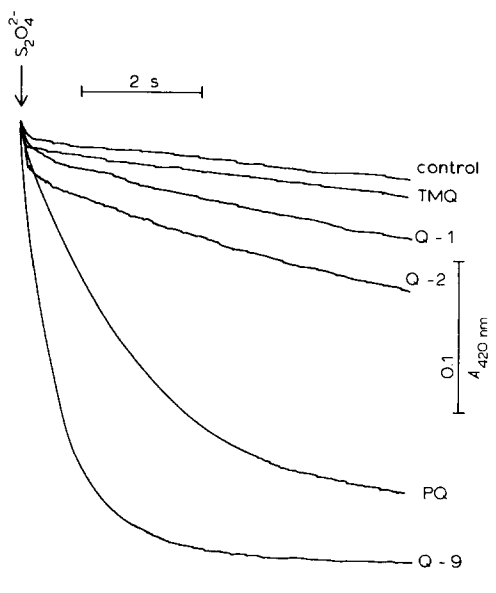

Fig. 1. Reduction of internal ferricyanide by external dithionite in quinone-containing liposomes. The conditions for the stop-flow measurement and the reaction mixtures are given under Methods and Materials. Except for the control, 13 nmol of quinone/mg lecithin were present. The final concentration of dithionite was 10 mM, and valinomycin and nigericin were added to the liposome suspension, 1 μg/ml of each. The piece of trace at the bottom of the figure corresponds to the *A* after about 5 min, and is taken as the baseline.

catalyze the reaction much more efficiently than the short-chain homologues, TMQ and Q-1. From semilog plots of the traces pseudo first-order rate constants can be determined. However, the reactions catalyzed by quinones with long side chains were of higher order, as detailed in Fig. 6. In these cases k relates to the initial rates. The dependence of the reduction on the concentration of external dithionite is shown in Fig. 2. With Q-9 and PQ 10 mM dithionite is almost saturating, while for the control, in the absence of quinone, this is not so. With TMQ and Q-1 already 1 mM dithionite seems to saturate the quinone-dependent part of the reaction (note that the increment above the control stays rather constant). The k value for the reduction of ferricyanide by dithionite without the barrier of the membrane was estimated from measurements with control liposomes dissolved by 2% Triton-X100. It was 70 s^{-1} with 2 mM and higher than 300 s^{-1} with 10 mM dithionite. Ascorbate and sulfite as alternative, impermeable reductants reacted very slowly in our model system.

The reaction is dependent on the amount of the quinone present in the liposomal membrane. In Fig. 3 the initial values are plotted against the quinone/lipid ratio. At a ratio of 1/20 the contribution of ubiquinone to the total absorption change at 420 nm was estimated to be about 20%, and correspondingly less at lower ratios; see Figs. 1 and 10 of the accompanying paper [17]. At the highest quinone/lipid ratio the permeability of the liposomes was somewhat increased, as concluded from a decrease in the half-time of the ferricyanide leak from about a week to one day, and from an increase in the rate of the decay of a pH difference across the membrane, visualized by 9-aminoacridine fluorescence changes [15]. With TMQ a similar concentration dependence was observed as with Q-1, but the k values were too low to be included in Fig. 3.

Reduction of trapped ferricyanide is stimulated by uncouplers which dissipate a proton gradient across the liposome membrane. This was the case for all quinone-catalyzed reactions as well as for the control. In our previous experiments we employed the combination of valinomycin and nigericin as uncou-

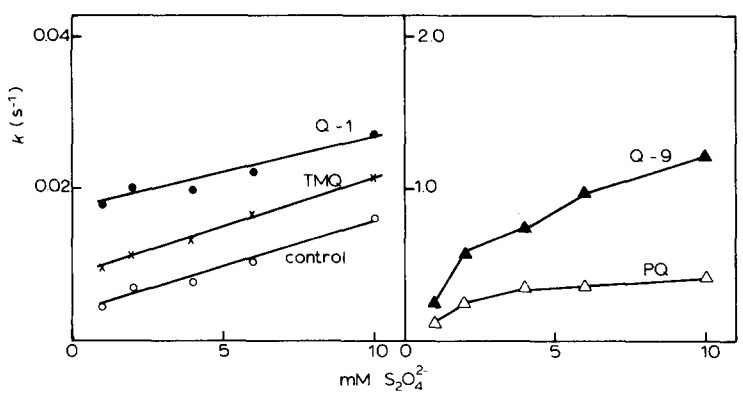

Fig. 2. Dependence of the pseudo first-order rate constant (k) on dithionite concentration. For reaction conditions consult Methods and Materials. The dithionite solution in one of the syringes was varied to yield the final concentrations in the stop-flow cuvette, as indicated in the figure. Except for the control, 13 nmol of quinone/mg lecithin was present.

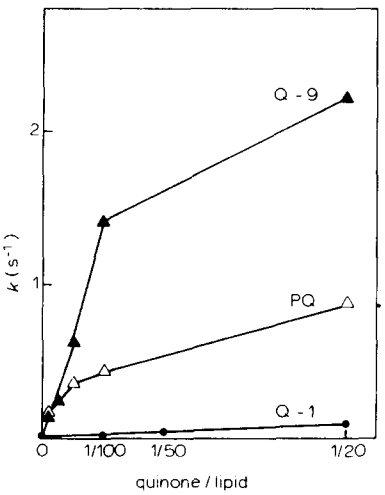

Fig. 3. Dependence of the reaction an the molar ratio of quinone to lipid. For reaction conditions see Methods and Materials. The quinone content of the liposomes was varied between 1.3 and 65 nmol/mg lecithin, which corresponds to a range of molar ratios of lipid to quinone from 1000 to 20. The contribution of ubiquinone to the absorption at 420 nm was about 20% at the highest concentration [17]. The dithionite concentration after mixing in the stop-flow apparatus was 10 mM.

pling ionophores [15]. However, nigericin alone, or amines, gave similar stimulatory effects. Valinomycin alone yielded hardly any stimulation. This agrees with old results of Hinkle [14] and suggests that quinones translocate electron and protons across the lipid membrane in an electroneutral way.

Pseudo first-order rate constants and activation energies are summarized in Table I for the quinone compounds tested so far. The series of ubiquinones reveals that a minimum length of three isoprene residues in the side chain is required for efficient catalysis of the reaction. Stressing the data one might see a tendency for lower k values in the case of nine and ten isoprene units, compared to somewhat shorter side chains. However, the small differences are probably more likely reflecting experimental variation. Thus we could not confirm our older finding [16] that Q-4 is a substantially more effective catalyst of the reaction than Q-9. The sudden increase in catalytic efficiency going from Q-2 to Q-3 is striking. The double bonds in the isoprenoid side chain do not contribute to its role in the reaction, since saturation of these bonds in Q-9 did not significantly change catalytic efficiency. PQ is less effective than the higher homologues of Q, but still much more than TMQ, which can be regarded as a short-chain homologue of PQ, and than other *p*-benzoquinones with small substituents (DQ and DBMIB). An exception is MDQ which catalyzes a rather fast reaction. This compound is highly autooxidizable [22]. DL-α-Tocopherol, which can be oxidized by ferricyanide in a rather complex manner [23], is also catalyzing the reaction very well. So far, what the *p*-benzoquinone series is concerned, the facilitating role of a side chain with a minimum length of about 12 C-atoms, for quinone catalysis of the transmembrane reaction seems well established. However, this is not holding for the vitamin K series, where menadione is found to be about ten times more effective than phyllo- or

TABLE I

RATE CONSTANTS AND ACTIVATION ENERGIES FOR THE REACTION WITH VARIOUS QUINONES

The conditions for the reaction were as described in Methods and Materials, as specified in the legend for Fig. 1. The k values in the first column were obtained for liposomes formed in the presence of quinones, the values in the second column belong to liposomes to which quinones were added in ethanolic solution after they had been formed and separated from excess ferricyanide by gel chromatography (see Methods and Materials). Symbols not found under abbreviations are: E_a, activation energy; k, pseudo first-order rate constant; α-T, *DL-α-tocopherol*; Q-9sat, *ubiquinone-9 saturated in the side chain*; QH_2-9, ubiquinol-9.

Compound	k (s^{-1})		E_a ($kJ \cdot M^{-1}$)
	1	2	
Q-1	0.0283	0.0284	17.7
Q-2	0.0622	—	35.7
Q-3	1.498	—	71.0
Q-4	1.732	—	64.6
Q-5	1.502	—	74.4
Q-7	1.853	—	59.1
Q-9	1.439	0.0172	61.7
Q-10	1.277	—	58.1
Q-9sat	1.267	—	59.6
QH_2-9	1.477	0.0134	—
PQ	0.293	—	57.8
α-T	0.724	—	—
K_1	0.0522	—	55.3
K_2	0.0449	—	—
K_3	0.580	—	17.5
TMQ	0.0205	0.0326	—
DQ	0.0340	0.0485	16.4
DBMIB	0.0493	0.0698	—
MDQ	0.0194	0.518	8.3
DAD	—	51.3	15.9
Control	0.0132		16.5

menaquinone. An additional observation to be emphasized in this context is the very high rate of the reaction catalyzed by DAD, a compound lacking a long side chain.

A comparison of the activation energies (last column in Table I) clearly shows that about three times higher values are found for the compounds with long side chains, which is not correlated to the rate of the reaction. DAD with the highest k value shows an activation energy as low as the *p*-benzoquinones with small substituents, and phylloquinone, a rather poor catalyst, has the same high T dependence as the efficient higher homologues of ubiquinone. Q-2 is the only compound which shows an intermediate value for the activation energy. The reaction catalyzed by MDQ has an exceptionally low T dependence.

The effect of the isoprenoid side chain is not detergent like as one might suspect, increasing membrane permeability for dithionite or ferricyanide. This can be concluded from the findings that phyllo- and menaquinone having long isoprenoid side chains are ineffective catalysts and that compounds with long, linear C-skeletons, like carotenoids, chlorophyll *a*, or fatty acids, do not appreciable accelerate the reaction when added in corresponding molar ratios to liposomes (Hurt, E. and Hauska, G., unpublished results). More significantly,

also dolichol A from yeast, which is a mixture of polyprenyl alcohols, the one with 17 isoprene units predominating, and even 1-nonaprenyl-2,3,4,5-tetramethoxy-6-methyl benzene, which is the dimethyl ether or QH_2-9, are not catalyzing the reaction.

Except for MDQ all quinones are firmly bound by liposomal membranes, as seen from a comparison of the *k* values in the first and second column in Table I, which belong to quinones incorporated either before or after column chromatography on Sephadex G-50. Only MDQ seems to be lost on the column. This could also be confirmed by ultraviolet spectroscopy of liposomes see Methods and Materials). Quinones with long side chains, on the other hand could only be efficiently incorporated when added before liposome formation (see values for Q-9 in Table I). Added to preformed liposomes in ethanolic solution the *k* value was hardly higher than for the control, and increased to 0.0408 after letting Q-9 and liposomes incubate for 20 h. Similar results were obtained with ubiquinol-9 (QH_2-9 in the table), for which a *k* value of 0.0374 was obtained after incubation with preformed liposomes for 20 h. Presumably quinones with a long side chain, when added in ethanol to aqueous solutions, form precipitates and extremely low concentrations of soluble monomers.

It should be mentioned that DBMIB, which by itself is a rather poor mediator of the reaction, does not inhibit the action of Q-10 ot PQ. This might be of interest in view of the inhibitory action of DBMIB on physiological reactions of PQ in chloroplasts [24] and of Q-10 in mitochondria [25] and bacteria [26].

Also the pH dependence is characteristically different for the reaction mediated by quinones with long and short side chains. This is shown for Q-9 and Q-1 in Fig. 4. The rate with Q-1 increases, but with Q-9 decreases with increasing pH. At a molar ratio of 20 for lipid/quinone, at pH 10, Q-1 is even more efficient than Q-9 in catalyzing the reaction. The dramatic increase of catalytic efficiency with pH suggests that the oxidation of ubiquinol-1 anion is rate limiting. If this is so indeed, the question whether the monoanion or the dianion is active can be answered from the power with which the reaction depends on OH^- concentration [27,28]. It should be 2 for the dianion and 1 for the monoanion. For our reaction with Q-1 it is less than 1, as seen from the slope in the plot of log *k* against pH in Fig. 5, suggesting that quinol monoanion as well as some undissociated quinol is oxidized by ferricyanide. A very similar dependence on pH was found for the reaction with the more active K-3 and MDQ, both also lacking a long side chain. At pH 10 the reaction with K-3 was extremely fast. MDQ was not stable at the higher pH values. In both cases the slope of log *k* against pH was again less than 1. The rate of the control ratio is somewhat decreasing with increasing pH, except for the highest value (Fig. 4).

We have shown before for measurements on a slower time scale with lower concentrations of dithionite, that the reaction with quinones carrying a long isoprene side chain is multiphasic, while with quinones lacking a long side chain it is monophasic [16]. This is seen again in Fig. 6 for measurements on the faster time scale of stop flow experiments, for different concentrations of Q-1 and Q-9. At the highest quinone concentration the total absorption change at 420 nm was increased by contribution from the quinone itself. A maximum of

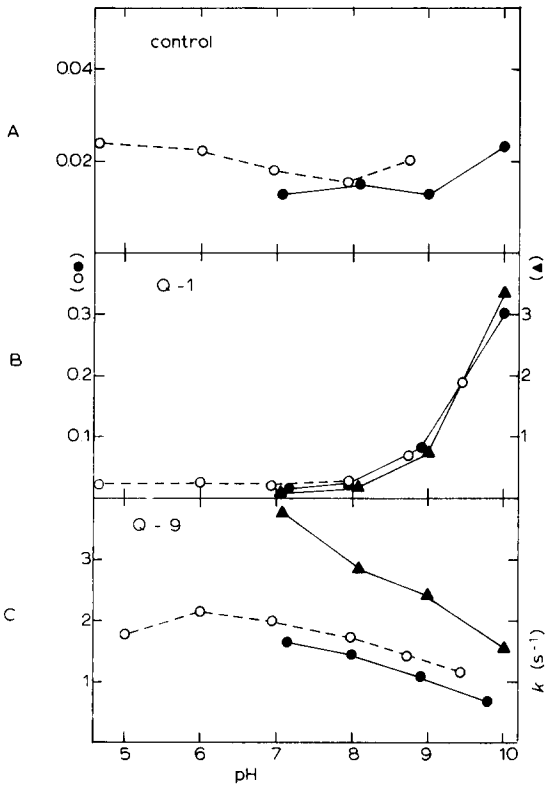

Fig. 4. pH dependence of the reaction with Q-1 and Q-9, and of the control. The conditions for the reaction are described in Maethods and Materials, and are specified in the legend to Fig. 1. The pH of the two solutions was carefully adjusted before mixing in the stop-flow apparatus. ○, Tricine, Mes (2-(*N*-morpholino)ethanesulfonic acid), Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and NH_4Cl were present as buffers, 20 mM of each in the liposome suspension. ●, ▲, only Tricine and NH_4Cl, 50 mM each, were included. The pH values correspond to the pH of the mixture, which was measured after stop flow. Valinomycin and nigericin were present, 1 μg of each/ml, to equilibrate the pH outside and inside the vesicles. (A) Control reaction. (B) Q-1; lipid/quinone molar ratio was 20 (▲), to which the *k* values on the right ordinate belong; the other values correspond to a molar ratio of 100 (○, ●). (C) Q-9; ▲, a molar ratio lipid/quinone of 20; ○, ●, a ratio of 100.

20% can be calculated for this contribution which in part might be responsible for the fast transient observed with Q-1 (see the accompanying paper [17]). The major change clearly follows pseudo first-order kinetics in the case of Q-1. With Q-9 the order of the reaction is apparently even higher than 2. We would like to explain this by the assumption that Q-9 is distributed heterogenously among the individual lipid vesicles, and that the course of the reaction results from superposition of several different pseudo first-order rates. The extent of the faster reactions, however, is too large to explain the difference of the reaction with Q-9 and Q-1 by a mere concentration effect in a heterogeneous vesicle population. From the other quinones listed in Table I, Q-4, Q-7, PQ and K-1 behaved like Q-9, while TMQ, MDQ and K-3 catalyzed a pseudo first-order reaction, like Q-1. With Q-3 the higher order of the reaction was less pronounced.

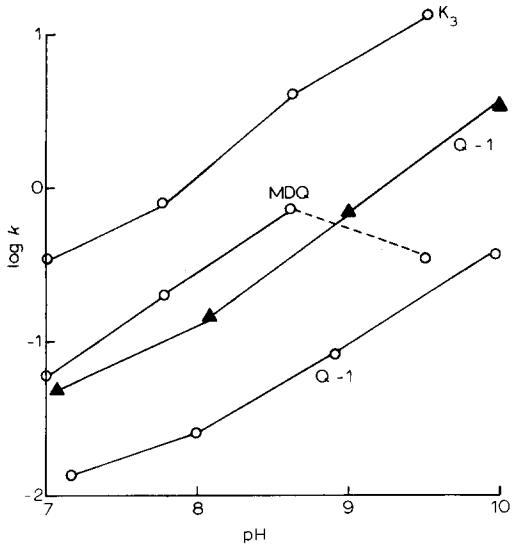

Fig. 5. pH dependence of the reaction with Q-1, K-3 and MDQ on a logarithmic scale. Data from experiments as described for Fig. 4B were replotted on a logarithmic scale. Numbers stand for the slope of the curves in the higher pH region. ▲ and ○, molar ratios of lipid to quinone of 20 and 100, respectively.

Discussion

The results presented in this paper reveal six characteristic differences between quinones lacking and carrying a long side chain in catalyzing the reduction of ferricyanide by dithionite through a lipid membrane: 1, In benzoquinones the presence of the side chain substantially aids the catalytic action, if it is longer than two isoprene units, a very sudden transition to effective catalysis is observed going from Q-2 to Q-3 (Table I). This discontinuity in the series of ubiquinones has not been found by other investigators [29], studying a different model system. Specificities for the length of the side chain in reconstituted mitochondrial electron transport systems are complex, and also do not show such a large difference between Q-2 and Q-3 [6]. 2, In the naphthoquinone series the precence of the isoprenoid side chain hinders catalytic action (Table I). It should be noticed, however, that K_3 is about 50 times more effective than chain-lacking benzoquinones, with the exception of MDQ, which like K_3 is highly autooxidizable [22]. Perhaps the comparatively low redox potential of K_3 is responsible for a faster reaction with ferricyanide, like it is for the reaction with O_2 [28]. 3, All quinones with long side chains catalyze a reaction with about three times higher activation energy, independently from the efficiency of catalysis (Table I). 4, The reaction catalyzed by quinones lacking the side chain is of pseudofirst order under our conditions, but the quinones with a long side chain catalyze reactions of apparently higher order (Fig. 6). 5, The rate of the reaction with Q-9 decreases with increasing pH, while with Q-1 it inreases dramatically (Fig. 4). Around pH 10 at high concentration, Q-1 is a more efficient catalyst than Q-9, which might resemble the more efficient action of K-3 compared to K-1 at pH 8.0. 6, The reaction with Q-1 and TMQ

seems to be saturated at much lower concentrations of dithionite than the reactions with Q-9 and PQ (Fig. 2). A seventh differential observation, which is dealt with in the subsequent paper [17], is that the semiquinone anion is detectable as a transient during the reaction with quinones lacking the long side chain, but is indetectable for quinones with the side chain. Obviously, on the basis of this multiple evidence, the reaction mechanism is different in the presence and in the absence of the isoprenoid side chain. Consequently, a first lesson we can learn is that the use of short chain analogs to study the reactions of physiological quinones is of limited value.

The catalytic action of the quinone in the liposomes can be divided into three parts, reduction by external dithionite, oxidation by internal ferricyanide and the transport of electrons and protons across the membrane, either by the permeation of individual quinol and quinone molecules in opposite directions, or by intermolecular hopping of electrons and protons along some supermolecular organisation of the quinone. Each part could be rate limiting. From the very high catalytic efficiency of DAD, a compound even more hydrophilic than the benzoquinones with small substituents, we can infer that the transport of reducing equivalents through the membrane per se is not rate limiting, but rather the redox interactions at the membrane surfaces. Autooxidation of benzoquinols preferentially proceeds via the quinol dianion, as inferred from the observation that the rate increases with the square of OH^- concentration [27,28]. The reaction with Q-1 in our system depends on a power of OH^- concentration of less than 1. Therefore, we suggest that oxidation of the quinol and its monoanion by ferricyanide occurring at the inner surface of the lipid vesicles, is rate limiting in the case of Q-1, K-3, MDQ, and probably also in the case of all other quinones lacking the long side chain. In contrast, the reaction with Q-9 is even faster at lower pH. Except for the highest pH value, the

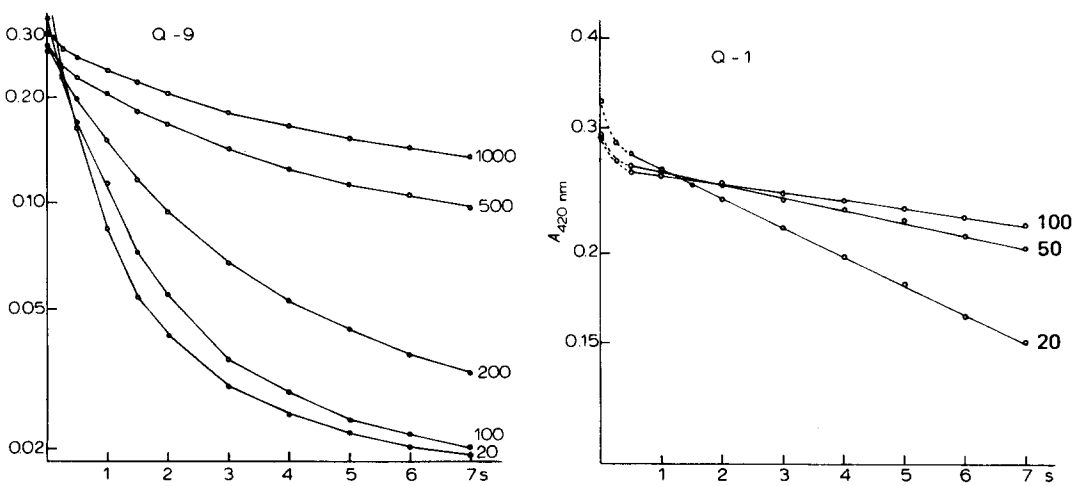

Fig. 6. Pseudo first-order plots of the reaction catalyzed by different concentrations of Q-1 and Q-9. The reaction conditions were as described under Methods and Materials, as specified in the legend to Fig. 1, except that the quinone content was varied between 13 and 65 nmol Q-1, and between 1.3 and 65 nmol Q-9/mg lecithin, which corresponds to the molar ratios of lipid/quinone given as a number for each trace in the figure.

control reaction in the absence of quinone also is slightly favored at lower pH, as reported before [15]. This can be interpreted by the action of neutral $H_2S_2O_4$ or singly charged $HS_2O_4^-$, as the membrane-permeable species. Possibly the reduction by dithionite is rate limiting in the case of Q-9, and also of the other quinones carrying long isoprenoid side chains, which to a higher extent than their homologues lacking the chain are confined to the hydrophobic core of the membrane, into which dithionite has to permeate. The observation that comparatively high concentrations of dithionite are required to saturate the reaction with Q-9 is corroborating our conclusion. The relative inefficiency of K-1 and K-2 in our model reaction can accordingly be explained by the fact that their hydrophobic nature is even higher compared to corresponding benzoquinones. For the organisation of the Q pool and the evidence that the heads of Q and K-2 are located in the hydrophobic core of biological membranes consult the review by Crane (Ref. 6, p. 453 ff.). By a spin label photodesctruction technique [30], Oettmeier et al. could more specifically infer that the head of Q-6 is preferentially located around C_5 of the lipid acyl chains [30]. However, the argument immediately arises, that what holds for the reaction of quinone with dithionite should apply even more for the reaction of quinol with the highly charged ferricyanide ion, although the quinol is more hydrophylic than the quinone. It is known indeed that ubiquinol in mitochondrial membranes is not oxidized appreciably by ferricyanide in a direct way [31]. On the other hand, one should note that ferricyanide is 0.2 M in our experiments.

Whatever the rate limit might be, it seems clear that the presence of a side chain longer than two isoprene units profoundly changes the reaction mechanism. But what is this effect of the side chain? The view of an anchoring effect, leaving the more hydrophilic quinone head close to the membrane surface ([8,9], and see Ref. 6) does not explain our observations, unless one assumes a direct participation of the side chain in the redox reaction via its double bonds. Janzen and Bolton indeed, based on photoelectric studies of molecular layers of chlorophyll and plasto- or ubiquinone, recently suggested the isoprenoid side to constitute 'natures molecular wire' [32]. Unfortunately, this beautiful concept is not supported by our own studies, since quinones with saturated side chains are equally effective catalysts (see Table I). Also in the mitochondrial membrane the perhydro side chain can substitute for the native chain during redox reactions of ubiquinone (see Table XIV in Ref. 6), although to a somewhat decreased extent. From the results presented in Fig. 6 we conclude that the isoprenoid side chain exerts a tendency to form clusters of higher molecular structure, or domains rich in quinone, within the lipid membrane, which leads to heterogeneous occupancy of the individual lipid vesicles responsible for the observed high order of the reaction. Thus the reaction could be considered as the superposition of many first-order reactions with different rate constants. The catalytic action of these quinone domains would require some particular thermal mobility as reflected by the comparatively high T coefficient. In some unknown manner it should facilitate the reduction of ferricyanide. Possible locations of isoprenoid quinones in biological membranes have been discussed by Crane recently [6]. An ordering effect of Q-3, particularly when reduced, on phospholipid bilayers has been described [33—35]. The view of quinone domains is consistent with the notion that quinone pools exist

in photosynthesis and respiration in high excess of electron transport chains (see Ref. 6). Recently, however, the significance of a mobile pool for the function of ubiquinone in electron transport has been questioned [36,37]. Takamiya and Dutton [36] concluded from their studies of quinone-extracted membranes from photosynthetic bacteria, that only a small fraction of ubiquinone, presumably all bound to membrane protein, is fully competent for rapid turnover of electron flow. It remains to be demonstrated that their conclusion, based on flash-relaxation experiments, also holds for steady-state conditions.

The relevance of our model reaction for the action of isoprenoid quinones in biological membranes depends in the first place on its rate in comparison to the rates of biological electron flow. A rough estimation of quinone turnover reveals that our reaction might be fast enough [16].

The relevance of our investigation is limited, on the other hand, by the fact that biological redox reactions of isoprenoid quinones are enzymatic processes of certainly higher complexity. Furthermore special forms of plastoquinone [7,38] and ubiquinone [36] have been discovered in photosynthetic electron transport with certainty, and also for respiratory electron transport the ubiquinone pool is known to be heterogeneous (see Ref. 6 for a recent review). However, we have established that protons and electrons can be translocated through lipid membranes by quinones having a long, isoprene side chain, which greatly influences the reaction mechanism and in the case of the benzoquinones increases catalytic efficiency at physiological conditions.

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