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VECTORIAL REDOX REACTIONS OF PHYSIOLOGICAL QUINONES

II. A STUDY OF TRANSIENT SEMIQUINONE FORMATION

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Summary

Transient absorption changes during reduction of quinone in liposomes by external dithionite, in the absence and presence of initially trapped ferricyanide, were matched with absorption spectra of semiquinone and quinone in the blue region. Plastoquinone, ubiquinone-9 and phylloquinone, each having an isoprenoid side chain were compared with trimethyl-*p*-benzoquinone, ubiquinone-9 and menadione, which lack a long side chain.

Semiquinone transients could only be observed by our spectroscopic technique during reduction of quinones lacking the chain. If Triton X-100 was added to the liposomes preparation semiquinone transients were also observed with the isoprenoid quinones. This result is consistent with the view that isoprenoid quinones build domains in the membranes, in which the life time of the semiquinone might be decreased by fast disproportionation, and to which dithionite has limited access.

Introduction

In the preceding papers [1—3] we have shown that the isoprenoid quinones, plastoquinone, ubiquinone and vitamin K, can transport protons and electrons

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Abbreviations: Q-1, Q-9, PQ, TMQ, K-1 and K-3, ubiquinone-1, ubiquinone-9, plastoquinone, trimethyl-*p*-benzoquinone, phylloquinone (vitamin K-1) and menadione (vitamin K-3), respectively; Tricine, *N*-tris-(hydroxymethyl)methylglycine.

through the membrane of lecithin vesicles, from external dithionite to internally trapped ferricyanide. Several differential observations, compared to quinone homologues lacking a long side chain, suggests that the catalytic action is strongly influenced by the isoprenoid side chain. In particular the oxidation of quinol by ferricyanide seems to be facilitated.

Chemical reduction of quinone [4] and oxidation of quinol [5,6] proceed via semiquinone intermediates. Semiquinone intermediates of PQ and Q have been detected in chloroplasts [7,8] and in reaction center complexes of photosynthetic bacteria [9—11], and have been identified as the reduced forms of early electron acceptors from photosynthetic reaction centers. Also during mitochondrial respiration ubisemiquinone is formed [12—14]. Therefore if was of interest to investigate whether semiquinone transients occur during stop-flow measurements with our model system, and whether differences between quinones carrying and lacking an isoprene side chain could be observed also in this respect.

Methods and Materials

The preparation of quinone-containing liposomes and the stop-flow measurements are described in the preceding paper [1]. To study the reduction of the quinones in the membranes by external dithionite, ferricyanide was omitted from the preparation. In this case the lipid/quinone mixture was sonicated in 0.3 M KCl, 50 mM Tricine/NaOH, pH 8.0. The molar ratio of lipid to quinone was 20 in all the experiments reported here.

Spectra of quinone, semiquinone and quinol in the blue absorption region (see Fig. 1) were taken with a spectrophotometer from Aminco, type DW2 UV-Vis. The compounds were dissolved in ethanol to 10^{-4} M. The solutions were made anaerobic in a closed cuvette fitted with a side arm. After recording the spectrum of the quinone, a few grains of sodium borohydride in 10 μl 1 N HCl were added from the side arm. After a few minutes the spectrum of the quinol was recorded. Subsequently 10 μl of 2.5 N NaOH were added, the cuvette was closed quickly and the spectrum recorded as fast as possible. Under these conditions the peaks characteristic for semiquinone [15—17] were seen transiently. Because of excess borohydride, the spectrum of the quinol at alkaline pH appeared after a while. For ultraviolet spectra of physiological quinones consult Refs. 18 and 19.

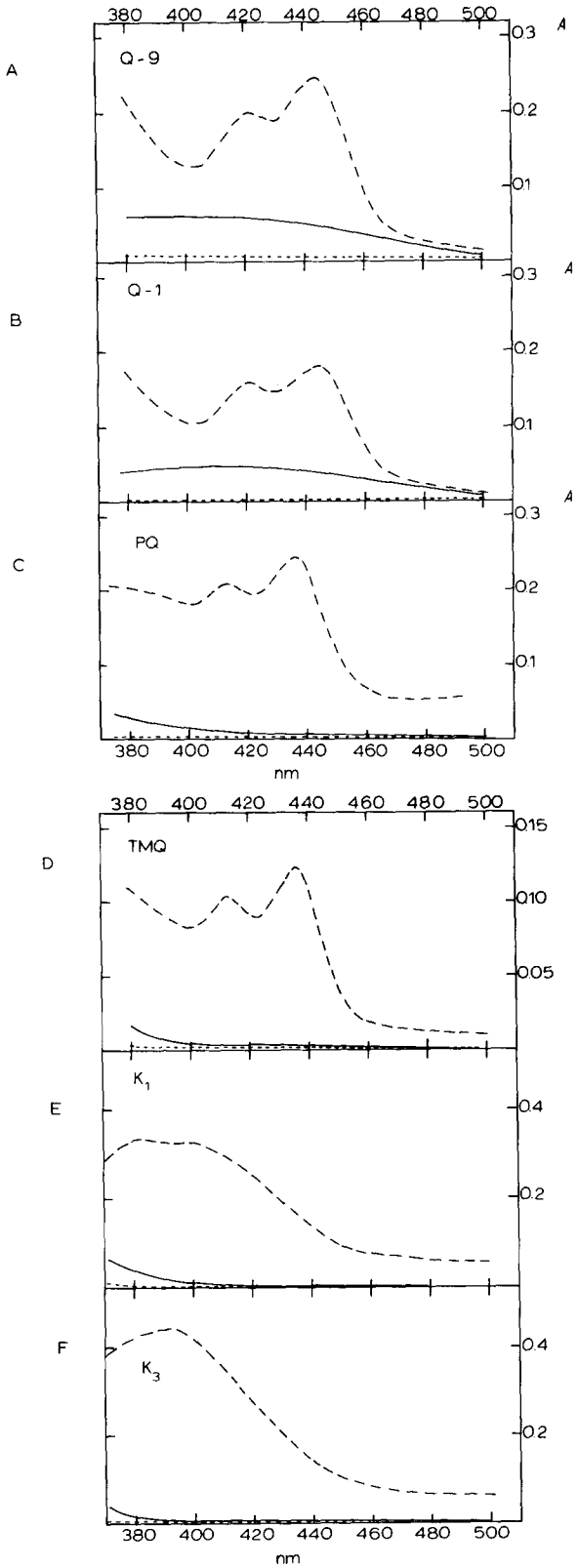
Quinones and other materials were obtained from the sources listed in the preceding paper [1].

Results

Spectra of quinones and semiquinone anions

In Fig. 1A—F, the absorption spectra in the range from 380 to 500 nm are shown for the oxidized and the reduced forms, and for the semiquinone anions

Fig. 1. Absorption spectra in the visible wavelength region, of quinones, quinols and semiquinones anions. The measurement of the spectra is described under Methods and Materials. ———, - - - - - - and · · · · · ·, spectra of quinone, semiquinone anion and quinol, respectively. The symbols for the quinones are explained under abbreviations. All quinone solutions were 10^{-4} M.


A
Q-9
B
Q-1
C
PQ
D
TMQ
E
K1
F
K3
380 400 420 440 460 480 500
nm
A
0.3
0.2
0.1
0.15
0.10
0.05
0.4

of Q-9, Q-1, PQ, TMQ, K-1 and K-3, each 10^{-4} M in ethanol. Absorption of the quinols is negligible in the wavelength range tested, as is the absorption of the oxidized forms of PQ, TMQ, K-1 and K-3. The oxidized forms of Q-9 and Q-1, however, have appreciable absorption [18], the ϵ_{420} being between 500 and 600 $M^{-1} \cdot cm^{-1}$. Thus, under the conditions of the stop-flow measurements in the presence of ferricyanide, the contribution of ubiquinone to the total absorbance change should be about 20%. In fact the change in the absence of ferricyanide is about one-fifth of the change in its presence, as seen in Fig. 7A.

Spectra of semiquinone forms have been published for *p*-benzoquinone [15,17], duroquinone [17], several ubiquinones [16,17] and plastoquinone [16]. Land and coworkers [16,17] most successfully employed the technique of pulse radiolysis to create a high concentration of the unstable semiquinone forms. Anions of *p*-benzosemiquinones are characterized by a double absorption band between 400 and 450 nm, the peak at the longer wavelength having the higher absorbance. This double band is very clearly seen also in our hands. The absorption peaks are at 422 and 445 nm for Q-1 and Q-9, and at 413 and 436 nm for PQ and TMQ, which corresponds well wit the literature [16,17]. The ϵ for the peak at the longer wavelength has been reported to be between 7 and $10 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$, for various *p*-benzoquinones [15,20,21]. For the semiquinone anion of Q-6 a value of $7.2 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ was found [17]. According to this value and to the spectra in Fig. 1A and B, the maximal transient concentration of ubisemiquinone anion we could record by our method was about 30% of the total quinone present. The amount of transient semiquinone increased with increasing concentrations of quinone.

The semiquinone anions of K-1 and K-3 have a broad absorption with a maximum around 400 nm. There is some indication for a double peak in the case of K-1, and less in the case of K-3.

Semiquinone transients after stop flow

The absorption of the semiquinone anions in the blue allows us to search for them during reduction of quinones in liposomes by excess dithionite, which constitutes the first half-reaction of vectorial electron transport in our model system. Absorption by dithionite starts below 440 nm. Unfortunately, we did not succeed to find conditions to study the other half-reaction, the oxidation of quinol by ferricyanide, trapped in liposomes. The problem of overlapping absorption of quinones and ferricyanide is complicated by the very high concentration of internally trapped ferricyanide, which is 0.2 M.

Fig. 2A, left part, displays the reduction of Q-9 in liposomes by dithionite at three different time scales. The spectra of the initial, maximal absorption, and of the absorption around half-reduction are shown in the right part of the figure. They follow the spectrum for oxidized Q-9 rather well, and there is no indication for transient semiquinone formation.

In the preceding paper [1] we have shown in Fig. 6, that the kinetics of ferricyanide reduction catalyzed by isoprenoid quinones are of complex, higher order. Also for the reduction of Q-9 in the liposome membrane by dithionite the order is higher than 1, as evident from Fig. 3, suggesting that in the case of catalysis by quinones with long isoprenoid side chains, the overall reaction from external dithionite to internal ferricyanide is governed by the first half-

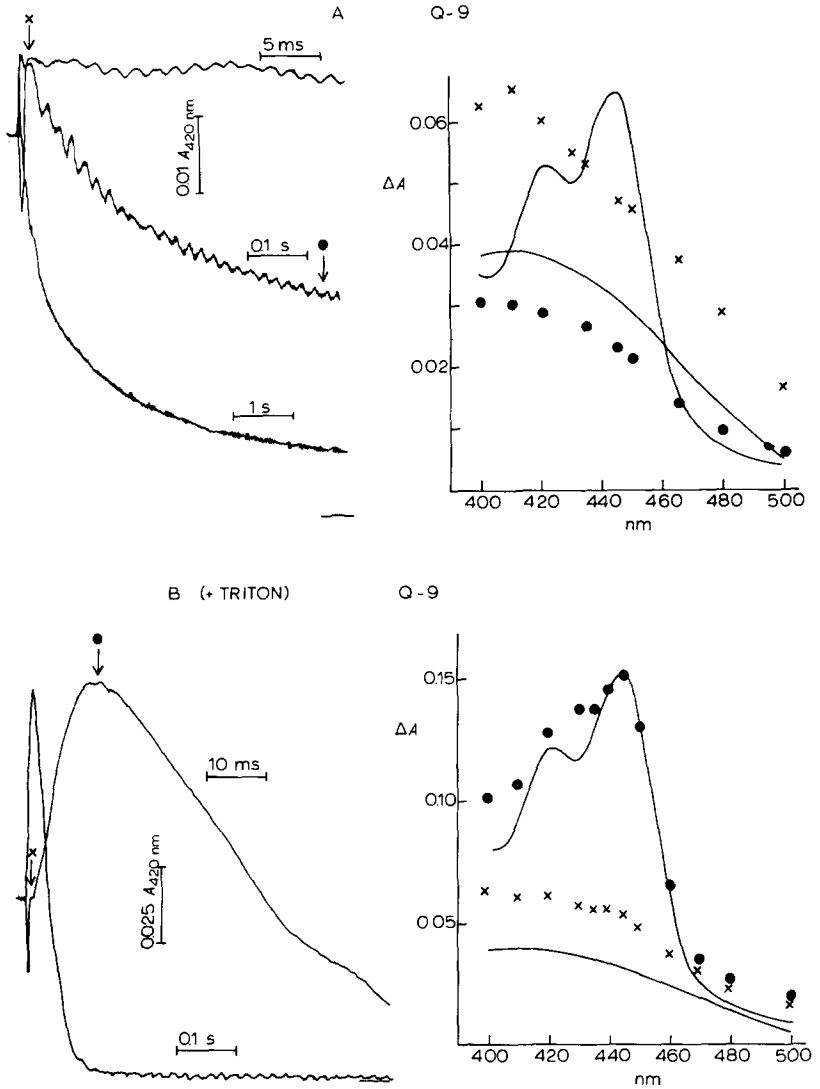


Fig. 2. Stop-flow traces and spectra of transients for the reduction of Q-9 in liposomes by external dithionite. Liposomes were prepared without ferricyanide. For this preparation and the recording procedure consult Methods and Materials. The left part of the figure shows absorption traces at 420 nm. The right part depicts spectra for certain transients along these traces, which are indicated by arrows and corresponding symbols. For comparison the right part contains the spectra of quinone ($6.5 \cdot 10^{-5}$ M) and of the semiquinone anion (arbitrary scale), as measured for Fig. 1 (———). For the measurement in part B Triton X-100 was added to the liposomes, 2% final concentration, before mixing with dithionite. The molar ratio of lipid to quinone was 20, corresponding to 10^{-4} M quinone present in the final mixture, which contained 1.5 mg lipid/ml.

reaction. The pseudo first-order rate constant for the initial phase is 2.5 s^{-1}, matching well with 2.3 s^{-1}, the value for the initial phase of the overall reaction, which extends beyond the maximally possible contribution of the quinone to the total absorption change (Ref. 1, Figs. 3 and 7A; see also Fig. 8A of this paper). Also the pH dependence of the half-reaction resembles the one

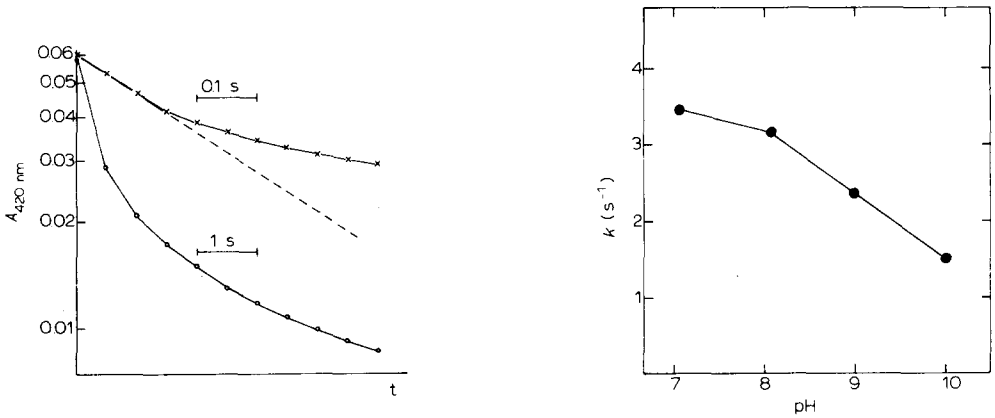


Fig. 3. First-order plot of the traces at the two slower time scales in Fig. 1A.

Fig. 4. pH dependence of the initial rate for reduction of Q-9 by dithionite in liposomes. Liposomes were prepared without ferricyanide as described under Methods and Materials, and as specified in the legend to Fig. 2. The pH of the two solutions was carefully adjusted before mixing in the stop-flow apparatus. This was helped by the presence of Tricine and NH_4Cl, 50 mM each, in the liposome suspension. The pH values correspond to the pH of the mixture, which was measured after stop flow.

of the overall reaction. The rate constants decrease with increasing pH (compare Fig. 4 with [1], Fig. 6).

If the liposome membrane is dissolved by detergent before reduction of Q-9 by dithionite, a transient absorption change is observed which largely follows the spectrum of the semiquinone anion of Q-9, as seen in Fig. 2B. Around 20% of the total Q-9 were present as the semiquinone anion at the peak of the transient, if an ϵ_{420} of $6 \cdot 10^3\ M^{-1} \cdot cm^{-1}$ is assumed [17].

In Fig. 5A and B analogous data for reduction of Q-1 are shown. In this case, in contrast to Q-9, a fast, complex transient, which might reflect semiquinone, is observed, even in the absence of Triton X-100. In addition a slow component is seen. In the presence of Triton X-100 the slow component is lost and the $Q^{\cdot -}$ transient has a different shape. Its decay is about ten times faster than the one with Q-9. In the absence or presence of Triton X-100, the maximal amount of $Q^{\cdot -}$ present is only a few percent of the total Q-1.

In Fig. 6A and B results similar to Fig. 2 with Q-9, are shown for PQ, but the changes are smaller and the correlation with the spectra of PQ and $PQ^{\cdot -}$ are poorer. No transient absorption increase is observed unless Triton X-100 is present. With TMQ the absorption changes were very small, and the necessary higher amplification caused too much scattering of the data for a correlation with the spectra. However, a transient increase in absorption was observed in the presence and absence of Triton X-100, resembling the results with Q-1 in Fig. 5.

The differential behavior of the pairs Q-9/Q-1 and PQ/TMQ is again observed with the pair K-1-K-3. With K-3 in the absence (Fig. 8B, dotted traces) and in the presence of triton X-100 (data not shown), while with K-1 only in the presence of detergent (data not shown) a transient absorption increase is observed, which approximately follows the spectra in Fig. 1E and F. The

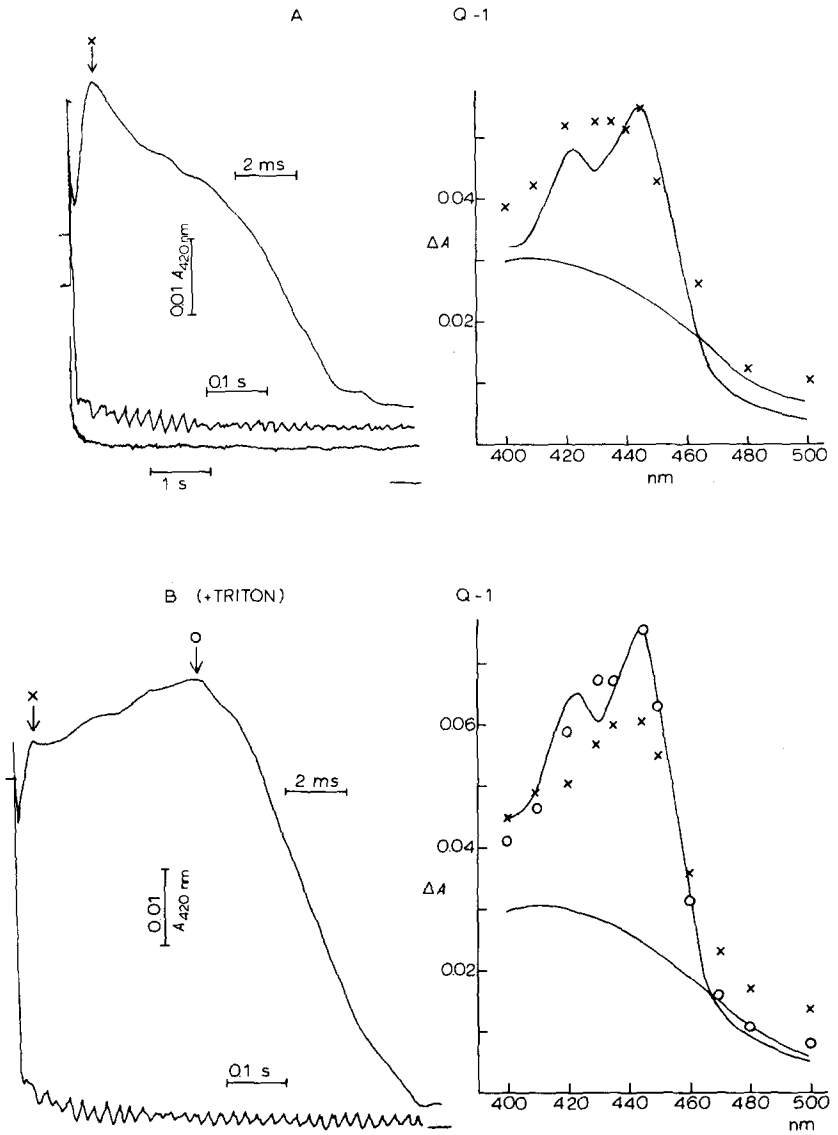


Fig. 5. Stop flow traces and spectra of transients for the reduction of Q-1 in liposomes by external dithionite. The conditions for measurement and the organization of the figure are analog to Fig. 2.

transient with K-3 is complex, with a lag before the peak is reached, in the absence of Triton X-100.

We can conclude that formation of semiquinone anion in measurable amounts is prevented by the isoprenoid side chain, during reduction of quinone in liposome membranes by dithionite, the first half of our model reaction. Fig. 7 shows that this is also true for the overall reaction. In the case of Q-1 a transient is seen, very similar to that during reduction by dithionite, but with Q-9 it is again absent. From a value of $6 \cdot 10^3\ M^{-1} \cdot cm^{-1}$ for ϵ_{420} of the semiquinone anion [17] it can be calculated that less than 10% of the total Q-1 are

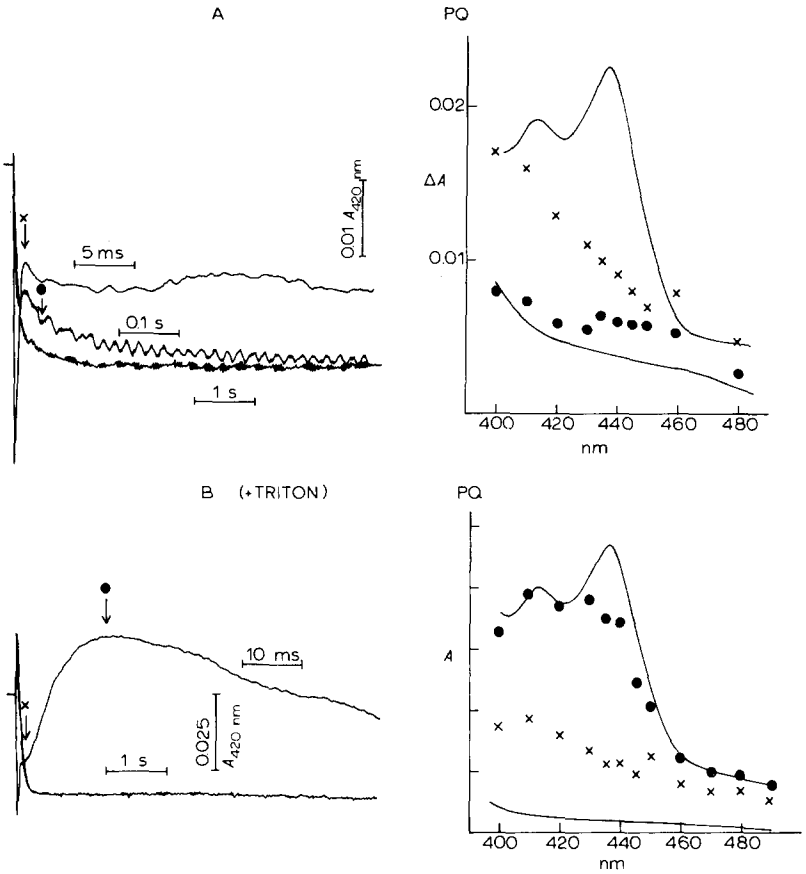


Fig. 6. Stop flow traces and spectra of transients for the reduction of PQ in liposomes by external dithionite. The conditions for measurement and the organization of the figure are analog to Fig. 2.

in the form of $Q^{\cdot -}$ at the peak of the transient. Analogous results for K-1 and K-3 are presented in Fig. 8. Only with K-3 semiquinone anion formation is observed for the half-reaction as well as for the overall reaction. Note again that the rate of ferricyanide reduction is much faster with Q-9 than with Q-1, but is much slower with K-1 than with K-3 (Figs. 7 and 8).

Discussion

Several differential observations between quinones carrying and lacking an isoprene side chain, in catalyzing the reduction of ferricyanide trapped in liposomes by external dithionite, led us to conclude in the preceding paper [1] that oxidation by ferricyanide of chain lacking, and reduction by dithionite of chain-carrying quinones is rate limiting, respectively. The transport of reducing equivalents through the membrane is not rate limiting. This has been extensively discussed [1]. With the results of the present paper we can add the following in support of this conclusion: (1) Q-1 and K-3, both lacking an isoprene side chain, are reduced by dithionite within a few milliseconds, even

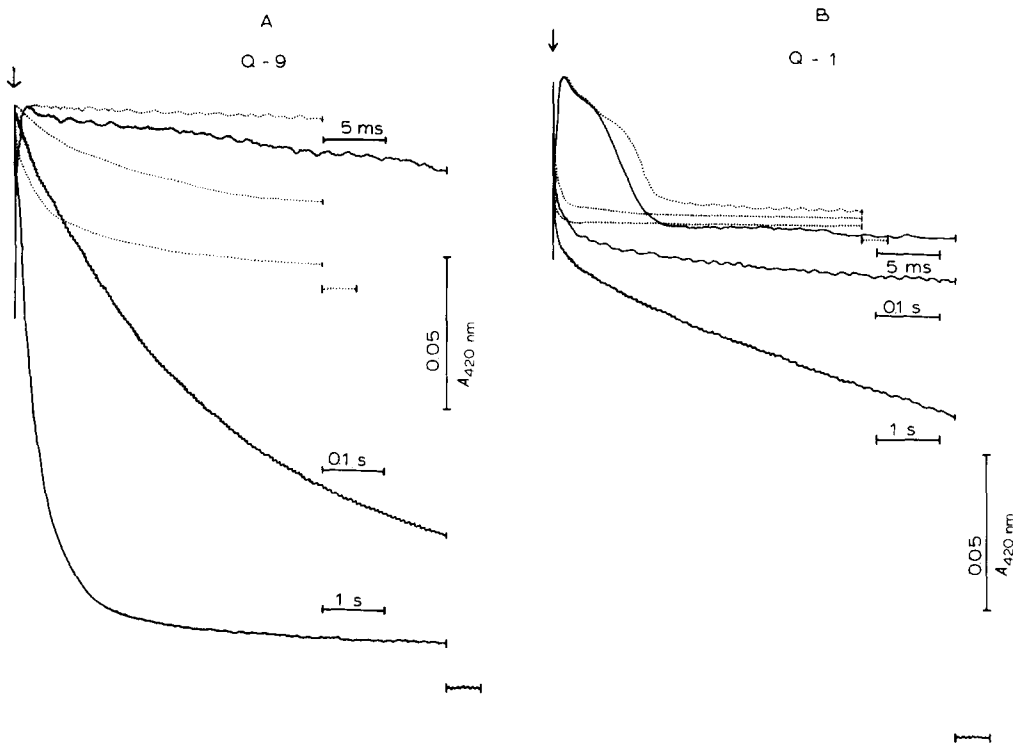


Fig. 7. Transient absorption changes during quinone-catalyzed ferricyanide reduction in liposomes by external dithionite. Liposomes containing 65 nmol Q-9 (A) or Q-1 (B)/mg lecithin were prepared according to Methods and Materials. For comparison the transients in the absence of ferricyanide, transformed from Figs. 2A and 5A to the proper *A* scale, are also presented (· · · · · ·). The course of the absorption change is shown at three time scales, which are indicated by bars for the solid traces. The same order of time scales holds for the dotted traces, going from bottom to top. At the end of the solid and dotted traces, small, separate bars indicate the baseline of the reaction, corresponding to the absorption after 5 min. For the conditions of the reaction consult Methods and Materials.

before reduction of ferricyanide has started to an appreciable extent, as seen by the fast, transient formation of the semiquinone anions in Figs. 7B and 8B. Q-9 and K-1, on the other hand, do not show such a transient, their reduction being a matter of seconds (Figs. 7A and 8A). (2) The rate of reduction of Q-9 is about the same as the rate of reduction of ferricyanide catalyzed by Q-9. (3) Reduction of Q-9 reveals the same complex reaction order as ferricyanide reduction. Also the pH dependence is similar for the first half-reaction and the overall reaction.

The reasons why no semiquinone anion transient is observed in the case of isoprenoid quinones could be many. One could be that disproportion of $Q^{\cdot -}$ is speeded up by the side chain either directly, or indirectly be being responsible for quinone domain formation. The latter posibility gains some support from the fact that if the organisation of isoprenoid quinones in the liposome membrane is disrupted by detergent, $Q^{\cdot -}$ transients can be observed. Another reason could be that other forms of semiquinone intermediates are built under the influence of side chain, which cannot be detected by our spectroscopic means.

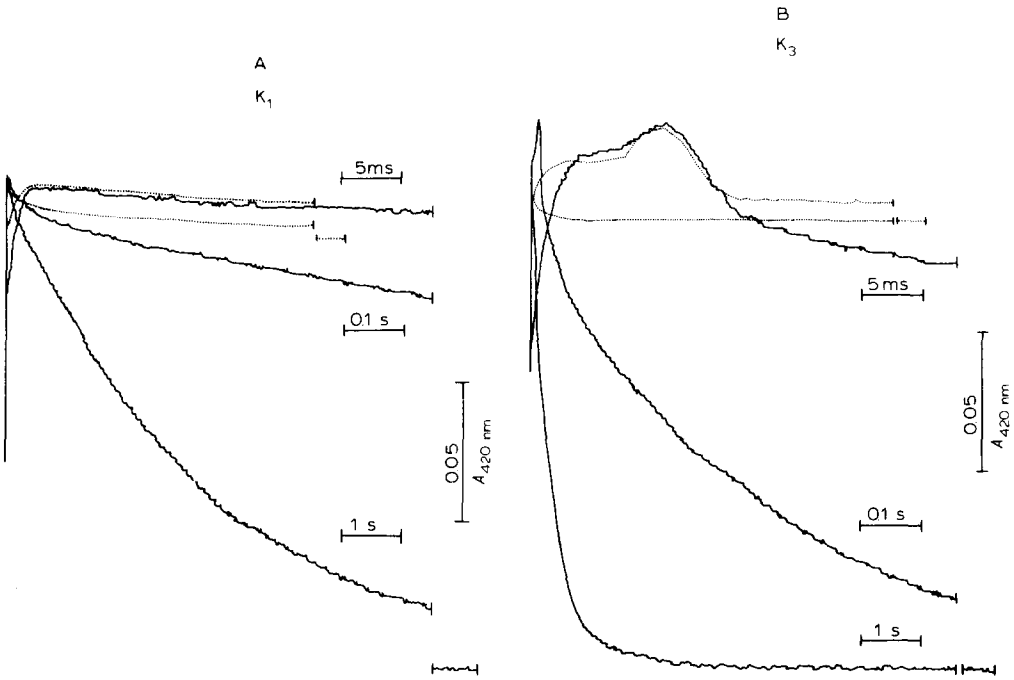


Fig. 8. Transient absorption changes during quinone-catalyzed ferricyanide reduction in liposomes by external dithionite. Liposomes contained 65 nmol K-1 (A) or K-3 (B). The conditions for measurements and the organization of the figure are analog to Fig. 7. Only two dotted traces are shown, however, which corresponds to the 5 ms bar (top trace) and the 1 s bar (bottom trace).

We think that the formation of neutral plasto- and ubisemiquinone, for which absorption spectra are known [16,17], can be excluded, because we did not find corresponding absorption changes. But semiquinone forms of higher molecularity could exist in quinone domains, which might only be detectable by ESR. Evidence for the formation of a $Q^{\cdot -}$-$Q^{\cdot -}$ pair of ubiquinone in the mitochondrial membrane has recently been reported [14].

It is feasible to assume that the isoprene side chain confines the head group of a quinone to the more hydrophobic regions of a membrane. Independent evidence for this has been discussed in the preceding paper [1]. But what effect of the side chain accelerates the oxidation of the quinol by ferricyanide? It is not clear, at the present experimental stage, why access to quinol should be facilitated for ferricyanide, but access to quinone should be impeded for dithionite, by an effect of the isoprene side chain. Of course, one could argue that the quinol favors a more hydrophilic environment, and therefore is located closer to the membrane surface. But this argument should hold for quinones lacking a long side chain as well, and it is the differential observation which is not understood. We would like, however, to suggest the following: oxidation of neutral quinols, lacking an isoprene chain, by ferricyanide is very slow, and oxidation of the quinol anion at physiological pH is limited by little dissociation, but can become very fast at high pH (see Figs. 4 and 5 of the preceding paper [1]). In case the quinol carries an isoprene side chain it is oxidized fast

by ferricyanide also in its neutral form. If our idea that the presence of the isoprene chain leads to clustering of the quinone in the membrane, quinol-quinone complexes could exist in such domains which might be oxidized fast by ferricyanide.

Semiquinone intermediates of isoprenoid quinones have been detected in biological electron transport systems [7—14], which is in apparent contradiction to the results with our model system reported here. The quinone complement of biological membranes is heterogeneous, however, part of it being certainly bound to protein [22,23], and the model system might be relevant for the mobile part of the quinone pool in biological membranes only. Of course, this relevance is further limited by the fact that reduction and oxidation of this mobile pool in nature are enzymatic processes, which are only poorly substituted for by our chemical redox compounds. This is already obvious from the notion that oxidation of the quinone pool is the rate-limiting step in biological electron transport [7,24], while the reduction is limiting in our model system. Nevertheless, the results presented here and in the preceding papers [1—3] suggest that the isoprenoid side chain, present in physiological benzo- and naphthoquinones, might play a mechanistic role in the redox process which goes beyond causing substrate specificity for the dehydrogenases [23].

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