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# The effect of ring substituents on the mechanism of interaction of exogenous quinones with the mitochondrial respiratory chain

Min Chen [a], Bel-li Liu [b], Lian-quan Gu [b] and Qin-shi Zhu [a],*

[a] *Institute of Biophysics, Academia Sinica, Beijing and* [b] *Department of Chemistry, Central China Normal University, Wuhan (China)*

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**In uncoupled pig-heart mitochondria the rate of the reduction of duroquinone by succinate in the presence of cyanide is inhibited by about 50% by antimycin. This inhibition approaches completion when myxothiazol is also added or British anti-Lewisite-treated (BAL-treated) mitochondria are used. If mitochondria are replaced by isolated succinate:cytochrome *c* oxidoreductase, the inhibition by antimycin alone is complete. The reduction of a plastoquinone homologue with an isoprenoid side chain (plastoquinone-2) is strongly inhibited by antimycin with either mitochondria or succinate:cytochrome *c* reductase. The reduction by succinate of plastoquinone analogues with an *n*-alkyl side chain in the presence of mitochondria is inhibited neither by antimycin nor by myxothiazol, but is sensitive to the combined use of these two inhibitors. On the other hand, the reduction of the ubiquinone homologues $Q_2$, $Q_4$, $Q_6$ and $Q_{10}$ and an analogue, 2,3-dimethoxyl-5-*n*-decyl-6-methyl-1,4-benzoquinone, is not sensitive to any inhibitor of $QH_2$:cytochrome *c* reductase tested or their combined use, either in normal or BAL-treated mitochondria or in isolated succinate:cytochrome *c* reductase. It is concluded that quinones with a ubiquinone ring can be reduced directly by succinate:Q reductase, whereas those with a plastoquinone ring can not. Reduction of the latter compounds requires participation of either center i or center o (Mitchell, P. (1975) FEBS Lett. 56, 1–6) or both, of $QH_2$:cytochrome *c* oxidoreductase. It is proposed that a saturated side chain promotes, while an isoprenoid side chain prevents reduction of these compounds at center o.**

## Introduction

Since the discovery of quinones in living organisms [1,2] and the establishment of their roles in respiratory and photosynthetic electron-transfer processes [3–5], a lot of quinone homologues and analogues have been synthesized and their interaction with the electron-transfer chains tested. Many have been found to be capable of redox interacting with the electron-transfer chains. For example, $Q_2$ and DBQ are as active as $Q_{10}$ in mitochondrial electron-transfer processes. Duroquinone (tetramethyl-1,4-benzoquinone) can be reduced by either succinate or NADH in the presence of mitochondria and can also react in chloroplasts [6]. Reduced trimethyl- [7], tetramethyl-1,4-benzoquinone and plastoquinone-2 (Chen, M. and Zhu, Q.S., unpublished results) can serve as an electron donor of mitochondrial $bc_1$

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* Present address: Department of Biochemistry, West Virginia University School of Medicine, Morgantown, WV 26506, U.S.A.

Abbreviations: $Q_n$, ubiquinone-*n*, where *n* is the number of isoprenoid units; $PQ_2$, plastoquinone-2; $PQ_0(CH_2)_nH$, plastoquinone analogue with a *n*-alkyl side chain of *n* $-CH_2$ units; BAL, British anti-Lewisite, 2,3-dimercaptopropanol; DBQ, 2,3-dimethoxyl-5-*n*-decyl-6-methyl-1,4-benzoquinone; DQ, duroquinone; $bc_1$ complex, mitochondrial $QH_2$:cytochrome *c* oxidoreductase.

Correspondence address: B.C.P. Jansen Instituut, Universiteit van Amsterdam, postbus 20151, 1000 HD Amsterdam, The Netherlands.

complex. Isolated plastoquinol:plastocyanin reductase can also use ubiquinol as substrate [8]. The electron-transfer processes with these homologues and analogues are in many cases similarly affected by inhibitors of the enzymes involved [9,10]. From these results, it seems that these homologues and analogues react with the electron-transfer chains by the same mechanism as the natural quinones, whether or not the benzene ring is substituted in the 2,3-positions by methoxy-groups (like ubiquinone) or methyl-groups (like plastoquinone and duroquinone), or whether the side chain is polyisoprenoid (as in both ubiquinone and plastoquinone) or a fully saturated *n*-alkyl chain (like DBQ).

However, Von Jagow and Bohrer [11] found that the reduction of duroquinone by succinate in the presence of mitochondria is antimycin sensitive. This is contrary to the classical concept of the respiratory chain [12,13], in which the reduction of quinone is considered to be the function of dehydrogenases, and therefore to be insensitive to inhibitors of $bc_1$ complex. Later, Weiss and Wingfield [14] and Zhu et al. [9] demonstrated that the continuous oxidation of durohydroquinone by $bc_1$ complex requires endogenous $Q_{10}$. These results show that the change of ring substituents and side chain does affect the mechanism of the interaction of quinone molecules with the mitochondrial respiratory chain.

In order to study further the influence of ring substituents and side-chain structure on the reactivity of quinone molecules toward the different Q-binding and reacting sites, we used the method for monitoring Q redox changes reported in the previous paper [15] to observe the reduction of various quinone homologues and analogues by succinate in the presence of mitochondria or succinate:cytochrome *c* reductase and the sensitivity of these reactions to the inhibitors of $bc_1$ complex. It was found that succinate:Q reductase by itself can reduce quinones with a UQ-type ring, whereas the reduction of quinones with a PQ-type ring requires the participation of the Q-reacting sites of the $bc_1$ complex. Flexible *n*-alkyl side-chain promotes the reduction of PQ-type quinones at center o by succinate dehydrogenase, whereas isoprene side chain prevents it.

## Materials and Methods

The preparation of pig-heart mitochondria, the activation of succinate dehydrogenase, BAL + $O_2$ treatment, the buffer used and the measurement of the reduction of quinones are the same as described in the previous paper [15], except for monitoring the reduction of duroquinone, $PQ_2$ and other plastoquinone analogues, in which the wavelength pair 263–290 nm was used.

$PQ_2$ and plastoquinone analogues were prepared by means of a free radical condensation between peroxides and 2,3-dimethyl-1,4-benzoquinone which was synthesized by direct oxidation of 2,3-dimethylaniline, using Silicon Gel-$Na_2Cr_2O_3$ as the oxidant. The detailed synthetic procedures will be published separately. $Q_2$, DBQ and myxothiazol were the kind gifts from Prof. E.C. Slater, Dr. J.A. Berden and Dr. S.P.J. Albracht. $Q_4$, $Q_6$ were kindly supplied by Prof. T.E. King. The source of the other reagents and other experimental conditions were the same as in the previous paper.

## Results

### *The sensitivity of duroquinone reduction by succinate to inhibitors of $bc_1$ complex*

According to Von Jagow and Bohrer [11], the inhibition of duroquinone reduction by antimycin is almost complete. Although we also observed this inhibitory effect of antimycin, but, in our hands, it was partial, around 50% at all concentrations of duroquinone between 4 $\mu$M and 100 $\mu$M (Fig. 1), in agreement with the result of Zhu et al. with submitochondrial particles [9].

When BAL-treated mitochondria were used, the inhibition by antimycin became almost complete (Fig. 2), showing that center 0 (cf. Refs. 16 and 17). Which cannot receive electrons when the Fe-S cluster is destroyed by BAL + $O_2$ [18], plays a role in duroquinone reduction and that succinate:Q reductase by itself cannot reduce duroquinone. Myxothiazol, a specific inhibitor of center o [19,20], had the same effect on duroquinone reduction as BAL treatment when both combined with the use of antimycin (Fig. 1). When used alone, myxothiazol had no inhibitory effect, rather it stimulated the reaction (Fig. 1), which seems

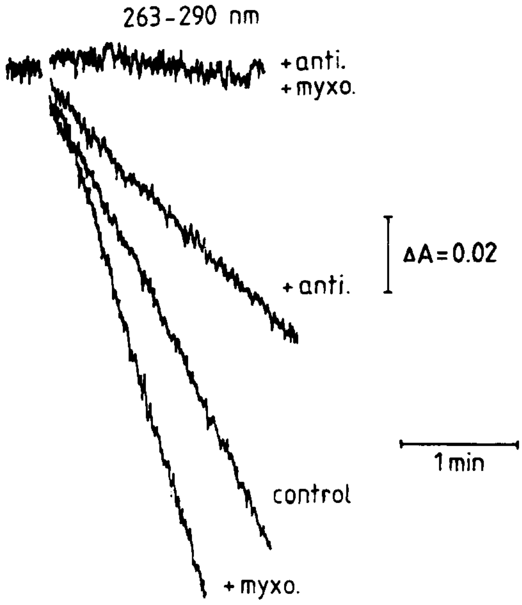


Fig. 1. Inhibition of duroquinone reduction by antimycin (anti.) and myxothiazol (mixo.). The reaction was started by the addition of succinate (2 mM) together with KCN (1.6 mM) after 5 min preincubation of mitochondira ($c_1$ concentration, 0.29 $\mu$M) with duroquinone (60 $\mu$M) and, where indicated, 1.7 $\mu$M antimycin and/or 1.7 $\mu$M myxothiazol.

surprising, since antimycin alone inhibits only by 50%. Similarly, BAL treatment did not lower the ability of mitochondria to catalyze the reduction of duroquinone by succinate (data not shown).

When duroquinone reduction by succinate was catalyzed by isolated succinate:cytochrome *c* reductase, antimycin alone could inhibit almost all the reduction (Fig. 2B).

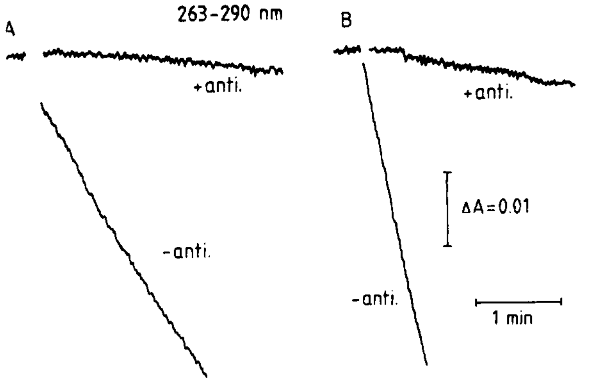


Fig. 2. Duroquinone reduction by succinate catalyzed by BAL-treated mitochndria (A); $c_1$ concentration, 0.26 $\mu$M) or untreated succinate:cytochrome *c* reductase (B; $c_1$ concentration, 0.3 $\mu$M) and its sensitivity to antimycin (anti.). Other experimental conditions were the same as in Fig. 1, except that the concentration of duroquinone was 20 $\mu$M.

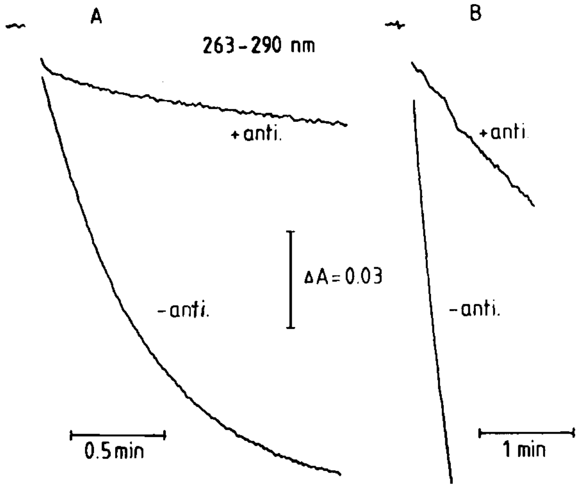


Fig. 3. Plastoquinone-2 reduction by succinate in the presence of mitochondria (A; $c_1$ concentration, 0.1 $\mu$m) or succinate:cytochrome *c* reductase (B; $c_1$ concentration, 0.16 $\mu$M) and its sensitivity to antimycin (anti.) (0.8 $\mu$m). The concentration of plastoquinone-2 was 21 $\mu$M. Other experimental conditions were the same as in Fig. 1. Since the detergent in the succinate: *c* reductase preparation improved the dispersion of $pQ_2$ molecules, more $pQ_2$ can be rapidly reduced.

### *The reduction of plastoquinone homologue and analogues*

The reduction of plastoquinone-2 by succinate was strongly inhibited by antimycin both with

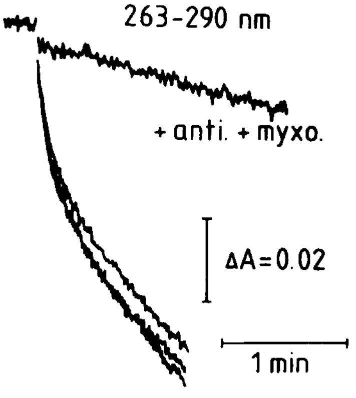


Fig. 4. The reduction of a plastoquinone analogue $PQ_0(CH_2)_{11}H$ (12.5 $\mu$M) by succinate in the presence of mitochondria ($c_1$ cocnentration 0.29 $\mu$M) and the effect of antimycin (anti.) and myxothiazol (mixo.). The three curves at the left side of the figure are, from left to right: with 1.7 $\mu$M myxothiazol, 1.7 $\mu$M antimycin and control, respectively.Other experimental conditions were the same as in Fig. 1.

mitochondria (Fig. 3A) and succinate:cytochrome *c* reductase (Fig. 3B). When the isoprene side chain of plastoquinone is replaced by a *n*-alkyl chain, the sensitivity to antimycin disappears (Fig. 4). In this case, both antimycin and myxothiazol stimulate the activity slightly. However, when antimycin and myxothiazol were used together, the activity was largely inhibited.

### *The reduction of ubiquinone homologues and analogues*

Consistent with the classical concept of the respiratory chain and differently from plastoquinones, the reduction of $Q_2$, $Q_4$, $Q_6$, $Q_{10}$ and DBQ by succinate was not sensitive to the above representative inhibitors of center o and center i of $bc_1$ complex or their combined use, either in normal or BAL-treated mitochondria or in isolated succinate:cytochrome *c* reductase (Fig. 5) indicating that quinones substituted with methoxy-groups as in ubiquinone can be reduced directly by succinate:Q reductase.

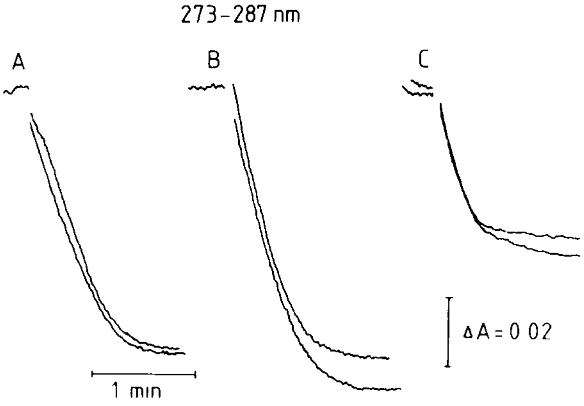


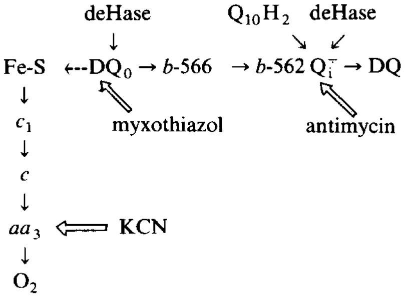
Fig. 5. $Q_2$ reduction by succinate in the presence of mitochondria (A; $c_1$ concentration, 0.1 $\mu$M), BAL-treated mitochondria (B; $c_1$ concentration, 0.1 $\mu$M) or succinate:cytochrome *c* reductase (C; $c_1$ concentration, 0.03 $\mu$M). $Q_2$ concentration was 18 $\mu$M in A, 20 $\mu$M in B and 8.8 $\mu$M in C. The right side line in all three parts was obtained in the presence of 1.7 $\mu$M antimycin. Other experimental conditions were the same as in Fig. 1. The effect of myxothiazol and the combined use of antimycin and myxothiazol are not shown.

## Discussion

Duroquinone resembles plastoquinone in that the benzene ring is substituted by methyl-groups and not by a methoxy-group as in ubiquinone. Our results demonstrated that the ability of a quinone molecule to react directly with succinate: Q reductase depends on the presence of these methoxy-groups. Plastoquinone analogues can hardly be reduced directly by succinate: Q reductase, but largely through center i or/and center o of $bc_1$ complex.

This effect of the benzene ring substituents on the interaction between quinone and succinate: Q reductase can be better understood from an evolutionary point of view. From the fact that ubiquinone exists in many bacteria as well as in mitochondria of both plants and animals, whereas plastoquinone, except for blue-green algae which contains two different photosystems like higher plants, is found only in eukaryotic photosynthetic organisms and always coexists with Photosystem II, possibly plastoquinone is later than ubiquinone in evolution. Since all hydroquinone dehydrogenases (ubiquinol:cytochrome *c* reductase in mitochondria, ubiquinol:cytochrome $c_2$ reductase in purple photosynthetic bacteria and plastoquinol:plastocyanin reductase in chloroplasts) very possibly have the same origin and are still strikingly alike both functionally and structurally [21,22], the same electron donor can serve for all three enzymes. On the other hand, succinate dehydrogenase is directly connected with the energy-conserving electron-transfer chain only in mitochondria and bacteria but not in chloroplasts. During the evolution of Photosystem II and chloroplasts, in adapting to the new environment and function, the methoxy groups of quinone have been replaced by methyl groups. (Possibly this change reflects the requirement of Photosystem II and the thylakoid membrane for the higher hydrophobicity or/and the smaller size of the ring part). While this change should not affect the reactivity of quinone molecules towards the plastoquinol: plastocyanin reductase, there is no need to care for succinate dehydrogenase which is not directly linked to this photosynthetic electron-transfer chain.

According to our experimental results, the re-



Scheme I

duction of duroquinone and plastoquinone take place essentially on $bc_1$ complex, not on succinate:quinone reductase. Through the previous reports [9,11,14] and our experimental results, a detailed mechanism by which duroquinone and plastoquinone interact with the $bc_1$ complex is gradually revealing itself.

As shown by Von Jagow and Bohrer [11], the electron transfer from $DQH_2$ to mitochondrial $Q_{10}$ is antimycin sensitive. This means that $DQH_2$ cannot reduce $Q_{10}$ molecules either when they are in free state (cf. Ref. 24) or bound to center o. From the dependence of $V_1$ and $V_2$ of $DQH_2$ oxidation on $DQH_2$ concentration, it can be derived that $Q_{10}$ is not involved in $DQH_2$ oxidation at center o [22]. Therefore, $DQH_2$ must be able to react directly at this center. In addition, $DQH_2$ oxidation in mitochondria at neutral pH's is largely antimycin sensitive, it must proceed via the Q-cycle, which can only be achieved if it is firmly bound to center o, although it has no side chain. Since the continuous oxidation of duroquinol by $bc_1$ complex requires $Q_{10}$ [9,14], it follows that duroquinone cannot be reduced by cytochrome $b$ directly at center i, but via $Q_{10}$.

Our experiments show that duroquinone reduction by succinate is fully sensitive to antimycin in isolated succinate:cytochrome $c$ reductase. Since in this case both $Q_{10}H_2$ and duroquinone can still reach and react at center o as evidenced by the high succiante:cytochrome $c$ reductase activity and the fact that $DQH_2$ is still a good substrate for isolated succinate:cytochrome $c$ reductase, it seems that free $Q_{10}H_2$ can reduce neither free duroquinone molecules (cf. Ref. 24) nor the ones bound to center o. Under our experimental conditions, the Rieske iron-sulfur protein and cytochrome $c_1$ were fully reduced a few turnovers after succinate addition, so $Q_{10}H_2$ could not be oxidized at center o. It is difficult to imagine how $Q_{10}H_2$ can reduce DQ at center o in this situation. According to our results, the sensitivity to antimycin of duroquinone reduction in mitochondria is only 50%, so possibly the antimycin-insensitive reduction of duroquinone occur through the direct interaction of the bound DQ at center o with the bound $Q_{10}$ (possibly in its semiquinone form [25]) on succinate:Q reductase. For isolated succinate:cytochrome $c$ reductase, due to the procedure of isolation, the structure of the membrane, the mobility and the relative positions of the complexes may be changed to such an extent that this direct interaction is no longer possible. If this is the case, then the complete inhibition by antimycin of duroquinone reduction in mitochondira observed by Von Jagow and Bohrer [11] might be explained by the difference of the preparations in the relationship between succinate:Q reductase and center o.

On the other hand, no evidence exists which apposes the reduction of duroquinone at center i by $Q_{10}H_2$ from the pool. It is also possible that succinate:Q reductase has direct contact with center i as suggested by Mitchell and Trumpower in their version of Q-cycle models [26,27].

On the basis of above analyses and the phenomenon that myxothiazol is stimulatory when used alone but inhibitory when used together with antimycin, a mechanism of duroquinone reduction by succinate with mitochondria is proposed (Scheme I).

According to Scheme I, duroquinone can be reduced at both center o and center i. At center i, the Q reductase may reduce the bound $Q_{10}$, the semiquinone thus formed gives its electron to duroquinone. Free $Q_{10}H_2$ may also first reduce $b$ at center i, the resulting $Q_i^-$ and reduced $b$ then reduce DQ at $DQH_2$. At center o, bound duroquinone gets its electrons directly from the Q reductase. It should be pointed out that in these two cases, the 'direct' delivery of electrons from succinate:Q reductase is throgh the bound $Q_{10}$ of the Q reductase. In the absence of antimycin, the durosemiquinone formed at center o is oxidized immediately by cytochrome $b$ (which is in partially oxidized state due to the presence of duroquinone, cf. Ref. 15), the latter can also reduce the bound Q at center i and form $Q_i^{\overline{\cdot}}$. So in the absence of inhibitors, no $DQH_2$ is formed at center o, the main route of duroquinone reduction is the pathway through center i, therefore the addition of myxothiazol will not affect the speed of duroquinone reduction. In addition, myxothiazol greatly reduces the electron leak from $DQH_2$ to oxygen either through the 'cyanide leak' or through autooxidation of durosemiquinone [26], thence 'stimulated' the overall reduction. When antimycin is added, the reduction through center i becomes impossible and cytochrome *b* becomes reduced (cf. Ref. 15). The durosemiquinone formed at center o can now be further reduced to $DQH_2$, giving rise to the antimycin-insensitive reduction of duroquinone. When both myxothiazol and antimycin are used, both pathways to duroquinone are blocked, so duroquinone can no longer be reduced.

The higher sensitivity to antimycin of plastoquinone-2 reduction indicates that the reducing activity at center o hardly exists, suggesting that the isoprene side chain prevents the direct interaction of center o with succinate:Q reductase. On the other and, the absence of the sensitivity to antimycin for plastoquinone analogues with an *n*-alkyl side chain indicates that the required activity at center o is no less than that at center i, different from that of duroquinone, which has only 50% activity of that of center i.

Yu et al. [29] have recently reported that the replacement of the isoprene side chain of $Q_2$ by an *n*-decyl one has no effect on the electron-transfer activity in Q- and phospholipid-depleted succinate: *c* reductase. On the other hand, Beattie and Clejan [30] show that DB and $Q_2$ have different electron-transfer activities in Q-deficient yeast mitochondria. Our data show that in $Q_{10}$-containing mitochondria such a change in side chain does affect the reaction behavior of plastoquinone analogues at different Q-reaction sites in their reduction by succinate.

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