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The oxidation-reduction kinetics of cytochromes *b*, c_1 and *c* in initially fully reduced mitochondrial membranes are in agreement with the Q-cycle hypothesis

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Stopped-flow experiments were performed to distinguish between two hypotheses, the Q-cycle and the SQ-cycle, each describing the pathway of electron transfer in the QH_2:cytochrome *c* oxidoreductases. It was observed that, when mitochondrial membranes from the yeast *Saccharomyces cerevisiae* were poised at a low redox potential with appropriate amounts of sodium dithionite to completely reduce cytochrome *b*, the kinetics of oxidation of cytochrome *b* showed a lag period of maximally 100 ms. Under the same experimental conditions, the oxidation-reduction kinetics of cytochromes $c + c_1$ showed transient behaviour. These results do not support the presence of a mobile species of semiquinone in the QH_2:cytochrome *c* oxidoreductases, as envisaged in the SQ-cycle, but are consistent with a Q-cycle mechanism in which the two quinone-binding domains do not exchange electrons directly on the timescale of turnover of the enzyme.

Introduction

The QH_2 : cytochrome *c* oxidoreductases play a central role in the process of membrane-bound oxidative phosphorylation taking place in mitochondria, aerobic bacteria, photosynthetic bacteria, chloroplasts and cyanobacteria. These enzymes catalyse the transfer of reducing equivalents of protons across the 'energy-transducing' membrane. In all these organisms, the enzyme is structurally similar, i.e., it consists of a cytochrome *b* polypeptide carrying two hemes, a [2Fe-2S] protein and a *c*-type cytochrome (c_1 or *f*). The similarity in structure and function of the enzyme, as present in various organisms, strongly suggests that the molecular mechanism of electron transfer coupled to proton translocation is similar, if not identical, in the QH_2 : cytochrome *c* oxidoreductases from different organisms.

Two important hypotheses describing how electron transfer is coupled to proton translocation in the QH_2 : cytochrome *c* oxidoreductases have been put forward: the Q-cycle [1,2] and the SQ-cycle [3,4]. Both these models are extensions of the original proposal by Wikström and Berden [5], in which for the first time an important role was designated to the semiquinone as an intermediate of the reaction. Today, there is overwhelming experimental evidence supporting the basic postulates of the original Q-cycle hypothesis [6–23]. The double-turnover Q-cycle [24,25] and the dou-

Abbreviations: Q, (ubi)quinone; SQ, (ubi)semiquinone (anion); QH_2, (ubi)quinol; HMHQQ, 7-(*n*-heptadecyl)mercapto-6-hydroxy-5,8-quinolinequinone; UHDBT, 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Mes, 4-morpholineethanesulphonic acid.

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ble Q-cycle [26–29] may be considered to be extensions of the original proposal by Mitchell. According to both these models, a complete catalytic cycle comprises the oxidation of two molecules of quinol (and the (re)reduction of one quinone) sequentially in a monomeric enzyme [24], or simultaneously in a dimeric enzyme [26,27], so that the enzyme functions as a self-contained oxidoreductase (see Ref. 2). These latter two features, i.e., two turn-overs and the self-containedness, are inherent to the proposal of the SQ-cycle.

Almost all experimental results referred to above do not distinguish between the Q-cycle and the SQ-cycle hypotheses; rather, they support both models. The distinction between the two models is small and can be summarized as follows. According to the SQ-cycle, the QH_2:cytochrome *c* oxidoreductase contains a single quinone-binding pocket to which semiquinone or quinol may also bind. During steady-state oxidation of quinol, the enzyme is alternately in a state in which the semiquinone is the reductant (SQ_{out}) to cytochrome *b*, and one in which it is the oxidant (SQ_{in}). The transition from one state to another is proposed to be accomplished by a 'flip-flop' of the semiquinone and occurs as fast as the turnover, or faster. According to the original Q-cycle [2], the enzyme contains two separate quinone-binding domains, located on opposite sites of the membrane. The transition from SQ_{out} to SQ_{in} (or vice versa) occurs only as the result of electron transfer via the two *b*-type hemes, and any other pathway yielding the same net result is excluded from occurring by the timescale of turnover [2]. Evidence has been presented indicating that QH_2:cytochrome *c* oxidoreductase contains two independent domains to which the inhibitory quinone analogues HMHQQ or UHDBT bind [17,30], strongly suggesting that the enzyme contains two Q-binding domains.

As a consequence of this difference between the two models, one may argue [4,31] that according to the Q-cycle, the kinetics of oxidation of cytochrome *b*, initially fully reduced, would show a lag period after a pulse of oxidant was given to the system. The SQ-cycle predicts that in such an experiment oxidation of cytochrome *b* starts immediately; a short lag period reflecting the time needed to oxidize cytochromes $c + c_1$ and one molecule of quinol by the [2Fe-2S] cluster may, however, be observed (cf. Ref. 32). Such an experiment has recently been performed by Rich and Wikström [33]. These authors did not find any sign of a lag period and their results thus favour an SQ-cycle. Similarly, the absence of a lag in the kinetics of oxidation of cytochrome *b* in photosynthetic systems poised at a very low redox potential, is difficult to reconcile with the basic principles of the original Q-cycle hypothesis, as discussed in detail in Refs. 34–36. However, De Vries and Dutton [30] have reported experiments with the so-called hybrid system that are difficult to explain in terms of the SQ-cycle. It was found that at redox potentials at which the Q-pool was less than half reduced, the kinetics of cytochrome *c* (re)reduction and cytochrome *b* oxidation matched, whereas at lower redox potentials, the rate of oxidation of cytochrome *b* greatly decreased, while the rate of cytochrome *c* reduction was unchanged. These results are consistent with the Q-cycle but not with the SQ-cycle, since according to the latter, the kinetics of cytochrome *b* and cytochromes $c + c_1$ should match at any redox potential.

In order to resolve further the question of which of the two models most closely describes the pathway of electron transfer in the QH_2:cytochrome *c* oxidoreductases, we have studied, by means of the stopped-flow technique, the oxidation-reduction kinetics of cytochrome *b* and cytochromes $c + c_1$ in mitochondrial membranes from yeast. We observed that at very low redox potentials, obtained by bringing the system to equilibrium with sodium dithionite, the kinetics of oxidation of cytochrome *b* showed a lag period of about 80–100 ms. Under the same experimental conditions, the kinetics of cytochromes $c + c_1$ showed transient behaviour, i.e., after the initial rapid oxidation, their reduction level increased, more-or-less simultaneously with the decrease in reduction level of cytochrome *b*. This finding suggests that during the lag period of about 100 ms the enzyme does not turn over. These results are in perfect agreement with the original Q-cycle hypothesis. Some of the results presented here have been published previously [37].

Materials and Methods

Yeast mitochondria were prepared from commercially grown *Saccharomyces cerevisiae* by breaking the cells with glass beads in a Dyno-Mill apparatus as in Ref. 37. By means of differential centrifugation, crude mitochondrial membranes were obtained at 35 000 × *g*. The membranes were washed, suspended in a buffer of 50 mM Hepes-KOH/250 mM KCl/1 mM EDTA (pH 7), and subsequently sonicated (four times for 10 s on ice). By this procedure cytochrome *c* is lost. The inner membrane retains the right-side-out conformation after sonication [45], in contrast to bovine heart mitochondria. After a low-speed run, the sonicated mitochondria were collected at 35 000 × *g*, washed once and suspended in 25 mM Mes-KOH/125 mM KCl/0.5 mM EDTA (pH 6.2).

Kinetic experiments were performed with a home-built stopped-flow apparatus (dead-time 2 ms, optical pathlength 2 mm) connected to an Aminco DW-2 spectrophotometer, a transient recorder and a computer allowing averaging and further manipulation of the traces. The traces shown are averages of 7–10 traces. Reaction temperature was 28–29 °C. The two reactants were mixed in a 1:1 ratio. Kinetics of cytochrome *b* were monitored at 560–575 nm, at which wavelength the contribution from cytochromes $c + c_1$ is nil. Cytochromes $c + c_1$ were monitored at 549.3–534 nm.

Sonicated mitochondria were diluted in the buffer at pH 6.2 to 20 mg/ml of protein (i.e., 2.5 μM in QH_2 : cytochrome *c* oxidoreductase). Subsequently, cytochrome *c* (from *S. cerevisiae*, purchased from Sigma) was added to a final concentration of 12 μM followed by sodium succinate (20 mM). Oxidation was initiated by mixing with a buffer (25 mM Mes-KOH/125 mM KCl/0.5 mM EDTA, pH 6.2) saturated with pure oxygen. In the experiments in which sodium dithionite was added to completely reduce cytochrome *b*, small amounts from a freshly prepared sodium dithionite stock solution (0.65 M in 1 M KOH kept at 0 °C under nitrogen) were added to a suspension of sonicated mitochondria made anaerobic with succinate (6.7 mM). Subsequently, this mixture was transferred to the stopped-flow apparatus and incubated for 10 min. A pH value of 6.2 was chosen to ascertain that the oxidation of cytochrome *c* by oxygen, via cytochrome *c* oxidase, was non-rate-limiting.

Control experiments with the stopped-flow apparatus indicated that at the highest concentrations of sodium dithionite used (800 μM), cytochromes $c + c_1$ were reduced with a half-time of 95 ms, a value fully consistent with the results obtained in Ref. 39. Under the same conditions, only 40% of cytochrome *b* was reduced with a half-time of 900 ms. At a concentration of 400 μM sodium dithionite, no reduction of cytochrome *b* could be observed, whereas half-reduction of cytochromes $c + c_1$ was obtained after 150 ms.

The Q_2H_2 : cytochrome *c* oxidoreductase activity of mitochondria were measured in the suspension buffer using 100 μM ubiquinol-2 and 40 μM cytochrome *c*; the turnover for quinol was determined as 230 s^{-1}.

Results

In Fig. 1, stopped-flow traces are presented showing the kinetics of oxidation of cytochrome *b* following a pulse of oxygen. In the presence of succinate, cytochrome *b*-562, initially reduced for 80–85%, was rapidly oxidized, albeit after a transient reduction phase of about 10–20 ms (cf. Refs. 40,41). When, in addition, 300 μM sodium dithionite was added (all cytochrome *b*-562 having been initially reduced and cytochrome *b*-566 largely oxidized), the oxidation of cytochrome *b* was still rapid, following a lag period or transient phase of about 20 ms. Upon increasing the concentration of dithionite (400–500 μM), a clear lag period was seen which lasted for about 60–100 ms. After this lag period, cytochrome *b* was apparently slowly oxidized. Upon increasing the concentration from 300 μM to 550 μM dithionite, the extent of oxidation of cytochrome *b* increased until the maximal level was reached, as judged from experiments in the presence of antimycin. This was taken as evidence that at the higher concentrations of dithionite, cytochrome *b*-566 was also reduced before mixing. When a still higher concentration of dithionite was used (700 μM and 800 μM), the lag period unexpectedly disappeared, while the extent of oxidation of cytochrome *b* remained the same. Even though it is known that

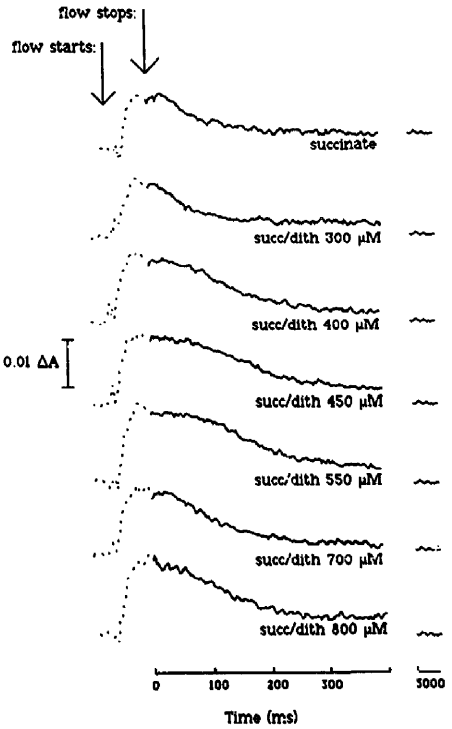

Fig. 1. Kinetics of oxidation of cytochrome *b* in sonicated mitochondria from yeast measured by stopped-flow (pH 6.2). See Materials and Methods for details. In the upper trace, 20 mM sodium succinate was added, in the others 6.7 mM. After anaerobiosis was obtained, the indicated amount of sodium dithionite was added. Traces are an average of seven to ten measurements. The increase in absorbance (in the dotted part of the traces) occurring at the mark 'flow starts' is due to filling of the optical chamber with reduced mitochondria. Owing to the abrupt stop of the flow (time 0) a spike in the trace is occasionally observed. The redox level shown at the right side of the traces is that obtained after 5 s.

the reducing power of solutions of dithionite decrease upon increasing their concentration [42], this cannot explain the disappearance of the lag, since the extent of oxidation of cytochrome *b* was not affected, indicating that the redox potential of the system before mixing was sufficiently low to reduce all cytochrome *b*. An alternative explanation for the disappearance of the lag is that, upon mixing with oxygen, an oxidant is formed capable of directly oxidizing some cytochrome *b* and/or QH_2. Possible oxidants are hydrogen peroxide and/or (bi)sulphite, the two products formed from dithionite after oxidation by oxygen. In order to test whether these compounds affect the kinetics of oxidation of cytochrome *b*, the following experiments were performed.

When sonicated mitochondria were incubated with 500 μM dithionite, in the presence or absence of succinate, a lag in the oxidation of cytochrome *b* was seen of about 60–100 ms (Fig. 2). When hydrogen peroxide was added to the syringe containing oxygen, the length of the lag period was hardly affected. When (bi)sulphite was added, the lag period became much shorter, and when both hydrogen peroxide and (bi)sulphite were present in the syringe containing oxygen, the lag disappeared almost completely. These findings clearly indicate that the products formed after oxidation of dithionite are responsible for the disappearance

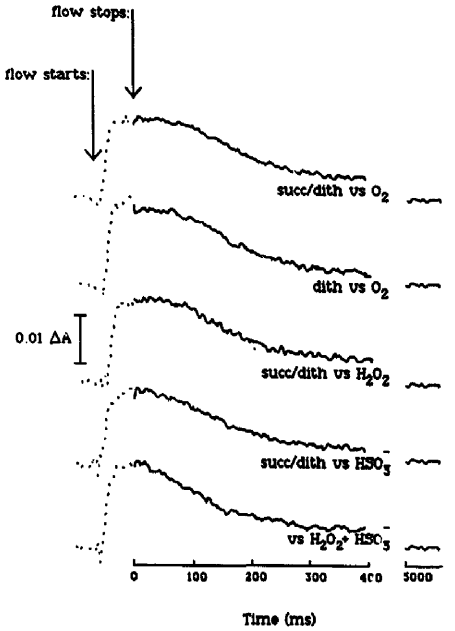

Fig. 2. The effect of hydrogen peroxide and/or (bi)sulphite on the kinetics of oxidation of cytochrome *b*. Samples were prepared as in Fig. 1. Sodium dithionite concentration was 500 μM. Where indicated, hydrogen peroxide (1 mM) and/or potassium sulphite (1 mM) was added to the syringe containing oxygen.

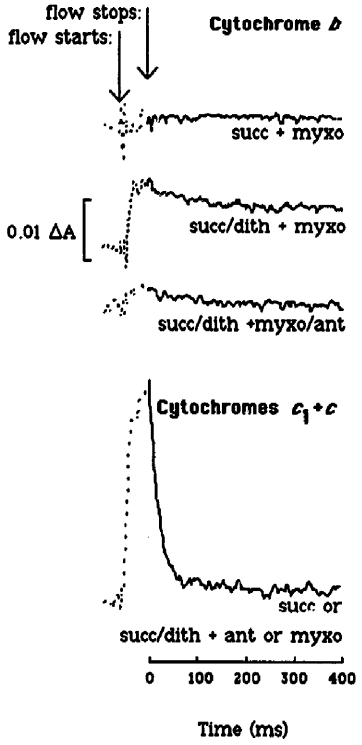

Fig. 3. Kinetics of oxidation of cytochrome *b* and cytochromes $c + c_1$ and the effect of the inhibitors antimycin and myxothiazol (pH 6.2). Upper three traces: kinetics of cytochrome *b*. Upper: sonicated mitochondria reduced by 20 mM sodium succinate in the presence of myxothiazol. Middle: as upper, but 6.7 nM sodium succinate and 550 μM sodium dithionite were added as reductants. Lower: as middle, but antimycin was also present. Bottom trace: kinetics of cytochrome $c + c_1$ corresponding to the conditions of the middle trace of cytochrome *b*. The traces corresponding to conditions of the upper and lower traces of cytochrome *b* are identical (not shown).

of the lag in the traces recorded with the higher concentrations of dithionite (Fig. 1).

Fig. 3 shows the effect of the inhibitors myxothiazol and antimycin on the kinetics of oxidation of cytochrome *b*. As expected, the redox level of cytochrome *b* (viz. *b*-562), as obtained by reduction with succinate in the presence of myxothiazol, did not change upon mixing with an oxygen-containing buffer. However, when dithionite was also present, part of cytochrome *b* was slowly oxidized. The extent of oxidation corresponded to the extent of cytochrome *b*-566 at the wavelength used. With the additional presence of antimycin some oxidation was still observed (Fig. 3), which, by definition, is non-enzymatic and is possibly caused by direct interaction of the oxidants hydrogen peroxide and/or (bi)sulphite with cytochrome *b*. At higher concentrations of dithionite (800 μM) the non-enzymatic partial oxidation of cytochrome *b* upon addition of oxygen progressed even faster.

In Fig. 4, the oxidation-reduction kinetics of cytochromes $c + c_1$ are shown. Following a pulse of oxygen, these cytochromes were very rapidly oxidized. Although the traces do not follow a single exponential, the decay was monotonic when succinate was used as the reductant, irrespective of whether myxothiazol, antimycin, or both these inhibitors, were added (Figs. 3, 4). The same result was obtained when succinate plus dithionite (550 μM) was used as reductant, but only in the presence of either, or both inhibitors. In the absence

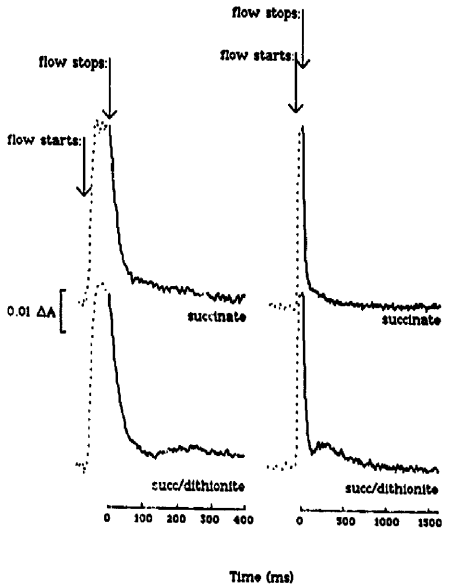

Fig. 4. Kinetics of oxidation of cytochrome $c + c_1$. The traces were recorded on two different timescales to show the transient phase more clearly. Upper traces show mitochondria reduced with 20 mM sodium succinate; in the lower traces 6.7 mM sodium succinate and 550 μM sodium dithionite were added.

of inhibitors, a transient reduction phase of the cytochromes $c + c_1$ was seen (Fig. 4, lower traces). In these traces, cytochromes $c + c_1$ were first oxidized almost completely, but became more reduced after 100–125 ms. After approx. 250 ms, the redox level of cytochrome $sc + c_1$ started to decrease again and this oxidation phase ended after approx. 800 ms, at which time, the same steady-state redox level (determined by the rate of oxidation of succinate) was obtained as in the upper traces of Fig. 4.

Discussion

The experiments reported in this paper were intended to distinguish between the Q-cycle and the SQ-cycle, using the rationale detailed in Refs. 4 and 31. These authors argued that, if the QH_2: cytochrome *c* oxidoreductases were functioning according to a Q-cycle mechanism, the kinetics of oxidation of cytochrome *b*, initially fully reduced, would show a lag period following a pulse of oxidant. However, no lag period (or a very short one, in which cytochromes $c + c_1$ are first oxidized by an oxidant and subsequently one molecule of quinol is oxidized by the Rieske iron-sulphur centre) would be observed should the SQ-cycle give the correct description of the pathway of electron transfer in the QH_2: cytochrome *c* oxidoreductases. Fig. 5 shows the sequence of events occurring after a pulse of oxidant is given to a fully reduced enzyme. The Q-cycle scheme predicts that, after the oxidation of cytochromes $c + c_1$ and the oxidation of $Q_{out}H_2$ to SQ_{out} by the Rieske iron-sulphur cluster, the enzyme is 'trapped' (state 3), and is not able to perform a catalytic cycle because no oxidant is present to oxidize cytochrome *b*-562. It seemed rather that the SQ_{out} formed serves as the reductant to cytochrome *b*-566. According to the SQ-cycle, however, SQ_{out} is transpositioned into SQ_{in} under these conditions, in this way providing an intermediate capable of oxidizing cytochrome *b*-562 (state 4 of the SQ-cycle, in Fig. 5).

The oxidation-reduction kinetics of cytochrome *b*, as well as those of cytochromes $c + c_1$ observed in Figs. 1 and 4, both favour a Q-cycle mechanism. With respect to cytochrome *b*, a clear lag period was observed when the enzyme was initially highly reduced (400–500 μM dithionite). The duration of the lag under these experimental conditions was maximally 100 ms, a period equivalent to more than 20 turnover-times of the enzyme. Concerning the kinetics of cytochromes $c + c_1$, after an initial very rapid oxidation, their reduction level decreased relatively slowly to a steady-state level when the enzyme was initially reduced by succinate, but a transient was observed when, in addition to succinate, dithionite (400–550 μM) was also present to obtain full reduction of cytochrome *b*. This transient phase started after approx. 100–125 ms, i.e., immediately after the disappearance of the lag period observed in the traces of cytochrome *b*. This observation is completely consistent with a Q-cycle mechanism. During the first 100 ms the enzyme is trapped. Thereafter, the enzyme becomes catalytically active as shown by the oxidation of cytochrome *b*. The rate of oxidation of quinol is then so high that the electron-transferring capacity of the cytochrome *c*/cytochrome *c* oxidase system becomes somewhat limiting, leading to an increase in the reduction level of cytochromes $c + c_1$. When the quinol pool is largely oxidized, the rate of turnover of the enzyme decreases, resulting in a decrease in the reduction level of cytochromes $c + c_1$. Finally, their reduction level is given by the relatively slow rate

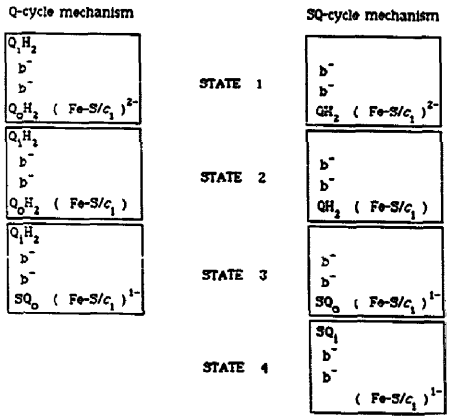

Fig. 5. Series of events occurring in the QH_2: cytochrome *c* oxidoreductases after a pulse of oxidant is given to an initially fully reduced enzyme (state 1). A pulse of oxidant keeps the [Fe-S] cluster and cytochromes $c + c_1$ oxidized.

of electron input furnished by the succinate dehydrogenase system. Both the long duration of the lag period seen in the traces of cytochrome *b*, and the transient behaviour of the cytochromes $c + c_1$ are difficult to interpret in terms of the SQ-cycle.

The Fig. 1 traces show that the lag period present in the traces of cytochrome *b*, recorded at concentrations of dithionite between 400–550 μM (and also the transient reduction phase of cytochromes $c + c_1$, data not shown), was absent when the mitochondria were incubated at higher concentrations of dithionite. In the control experiments of Fig. 2, we showed that when the mitochondria were incubated with 500 μM dithionite and mixed with an oxygen-containing buffer to which (bi)sulphite (with or without hydrogen peroxide) was added, the lag period in the kinetics of cytochrome *b* was shortened, or even disappeared. Furthermore, in the presence of myxothiazol, no oxidation of cytochrome *b* was observed when the mitochondria were reduced by succinate, but some oxidation of cytochrome *b* (mainly cytochrome *b*-566) was seen when they were reduced by succinate in the presence of dithionite (Fig. 3). This oxidation was partially sensitive to the additional presence of antimycin. We conclude therefore that (bi)sulphite (and, probably to a lesser extent, hydrogen peroxide) is capable of oxidizing cytochrome *b* indirectly, i.e., by oxidizing ubiquinol from the pool, and also directly, i.e., bypassing the Q-binding domain(s). At higher concentrations of dithionite the non-enzymatic oxidation of cytochrome *b* upon addition of oxygen (myxothiazol and/or antimycin present) progressed faster.

The observations listed above may (partly) explain why Rich and Wikström [33] did not observe a lag in the kinetics of oxidation of cytochrome *b* in the experiments in which 1.9 mM or 0.67 mM dithionite (and succinate) were used to reduce rat-liver mitochondrial membranes, and ferricyanide served as the oxidant. The high concentration of (bi)sulphite formed under these conditions, may oxidize some quinol and/or cytochrome *b* non-enzymatically which, subsequently, leads to a rapid enzymatic oxidation of these components.

The observation that at very low redox potentials the oxidation of cytochrome *b* in the b_6/f complex is rapid and accompanied by the slow phase of the carotenoid bandshift, is not easily explained in terms of the original Q-cycle [34–36]. It cannot, however, be completely be ruled out that in these experiments the various methods used to establish redox equilibrium between the mediators and cytochrome *b* (and the Q-pool) are inadequate. A lack of equilibrium of cytochrome *b* and the Q-pool with substrates like NADH or succinate is well-documented [43]. However, assuming equilibration, we think that the effect of NQNO on the kinetics of cytochrome *b*, or rather the almost complete lack of efficacy of this inhibitor at low redox potentials [35,36,44], is a more serious problem. In order to apply our experimental results and conclusions to other mitochondrial and photosynthetic QH_2 : cytochrome *c* oxidoreductases, we should like to give an alternative interpretation of the data presented in Refs. 34–36 in terms of the original Q-cycle.

We assume, similarly to Joliot and Joliot [35], that NQNO is bound to the enzyme at low redox potentials (all cytochrome *b* reduced) and inhibits oxidation of cytochrome *b* via the Q_{in}-binding domain. The series of events occurring after the flash (which actually was observed to be the same in the presence or absence of NQNO, except for a small effect on the rates) is schematically depicted in Fig. 6, and is essentially the same as given in Ref. 35, i.e., the SQ_{out} formed can serve as the

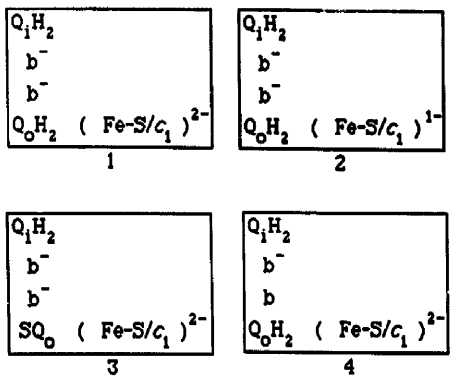

Fig. 6. Series of events occurring in the QH_2 : cytochrome *c* oxidoreductases after a single flash is given to a fully reduced enzyme. A Q-cycle model is shown.

oxidant of the fully reduced cytochrome *b*. The extent of oxidation of cytochrome *b* is given by the value of the equilibrium constant for this reaction and is assumed to favour partial oxidation of cytochrome *b* under these conditions. (Normally, e.g., during steady-state turnover, the degree of reduction of cytochrome *b* is so low that reduction of cytochrome *b* by SQ_{out} is favoured). So far, the original Q-cycle satisfactorily explains the oxidation of cytochrome *b*. However, the finding that the oxidation of cytochrome *b* is accompanied by a carotenoid bandshift is apparently not in agreement with he Q-cycle. Therefore, Joliot and Joliot have suggested that protons flow from the inside medium (mitochondrial orientation) directly to the Q_{out}-binding domain, bypassing the Q_{in}-binding domain. This bypass could be established by a small displacement of SQ_{out}. Inspection of the reactions shown in Fig. 6 indicates that the only difference between the initial and final states in such an experiment is the net oxidation of cytochrome *b* (low potential). This oxidation may very well be accompanied by the vectorial extrusion of one proton or more from the cytochrome *b* polypeptide into the outside medium [38], resulting in a carotenoid bandshift. In other words, it is not necessary to postulate a proton channel bypassing the Q_{in}-binding domain.

We propose that in order to observe a lag in the kinetics of oxidation of cytochrome b_6, a strong saturating flash creating sufficient 'double hits', or two (or three) closely spaced flashes, i.e., within the turnover-time of the enzyme, should be delivered to the system. In such an experiment, the high-potential electron carriers are sufficiently oxidized (similarly to the oxygen pulse experiments reported here) to promote or sustain an extensive reduction of cytochrome *b*. In other words, the oxidation of initially fully reduced cytochrome b_6 by SQ_{out} observed in a single flash experiment in the presence or absence of NQNO, is due to reductant-induced oxidation.

We conclude that the results obtained in this work are in agreement with the original Q-cycle hypothesis [1,2], and not with the SQ-cycle hypothesis [3,4]. Since we consider it unlikely that the pathway of electron transfer coupled to proton translocation is different for the respiratory and the photosynthetic QH_2:cytochrome *c* oxidoreductases, and/or that the catalytic mechanism is dependent on the redox state of the system, we have offered an alternative interpretation in terms of the original Q-cycle experimental data.

Acknowledgements

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