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OBSERVATIONS ON THE OXIDOREDUCTION OF THE TWO CYTOCHROMES *b* IN CYTOCHROME *c*-DEFICIENT MITOCHONDRIA AND SUBMITOCHONDRIAL PARTICLES

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Summary

1. In cytochrome *c* depleted mitochondria cytochrome b_T is reduced rapidly upon addition of ATP or slowly during state 4 respiration, but cytochrome b_K is effectively reduced in such mitochondria respiring upon glutamate plus malate in all energy states. In mitochondria or in submitochondrial particles oxidized NADH or succinate, cytochromes b_K and b_T were always reduced and oxidized independently.

2. Difference spectra for the two *b* cytochromes were obtained in the presence of respiratory chain inhibitors. Reduced cytochrome b_K in the presence of cyanide can be reoxidized by CoQ_2. Cytochrome b_T reduced in the presence of antimycin can be reoxidized by O_2 if rotenone is added to an NADH-reduced system or malonate to a succinate-reduced system. There is no evidence for electron transfer between the two *b* cytochromes.

3. It is suggested that there is no electron transfer from cytochrome b_T to cytochrome b_K, but that a cytochrome $b_K b_T$ dimer accepts electrons from the CoQ pool jointly with cytochrome c_1 and another acceptor, perhaps the FeS centre. The major steady state species is $b_K^{2+} b_T^{3+}$, and a Q-loop occurs with reduction of CoQ by the fully reduced species $b_K^{2+} b_T^{2+}$. All proposed interactions between CoQ and Complex III are 2-electron processes and the change from 2-electrons to 1-electron transfer occurs within Complex III itself.

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Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; MOPS, 4-morpholinopropanesulphonic acid; BAL, 2,3-dimercaptopropanol; MCCP, carbonylcyanide *m*-chlorophenylhydrazone.

Introduction

It is proven that two *b*-type cytochromes, b_K and b_T *, occur in the eukaryotic respiratory chain [1,2], and their individual chemistry and catalytic behavior has been studied extensively [3—5], but understanding of the interaction of the two cytochromes *b* in the inner mitochondrial membrane is incomplete. The proposal of Slater et al. [6] that the *b* cytochromes occur as a dimer rather than as a pair of independent electron carriers has recently again been revived [7]. However, the evidence that only one of these cytochromes (b_T) is involved in energy transduction [1], and the idea of a proton-motive Q cycle [8], still favours the concept of a linear sequence involving the two cytochromes *b*.

Ubiquinone (CoQ), which can act as a redox pool between the dehydrogenases and the cytochromes [9], is probably available to both flavin-FeS dehydrogenases and to the hydrophobic cytochromes *b* [10]. Mitchell's 'proton-motive Q cycle' [8] assigns CoQ as redox carrier to cytochrome b_T and cytochrome c_1, but this postulate, involving vectorial movement of CoQ and $CoQH_2$ across the membrane, remains unproven.

Although the proton-motive Q-cycle predicts a role for CoQ in the catalytic operation of the quinol-cytochrome *c* reductase system, other studies suggest that electrons introduced at the cytochrome *b* level reach cytochrome *c* without CoQ involvement [11—13]. Purified complex III contains very little CoQ, but when incorporated into liposomes catalyses energized electron translocation to O_2 from CoQ_2 [14]; the latter quinol must therefore be capable of functioning like CoQ_{10} in vivo.

In submitochondrial systems, $CoQH_2$ addition results in the reduction of cytochrome b_K, while cytochrome b_T remains oxidized *. The energization response of the cytochrome b_T, initially thought of as an intrinsic molecular change [1,3], is now regarded as involving reversed electron transfer [15,16]. Although the responses of cytochromes b_T and b_K [17,18] have been analyzed in terms of electron transfer between b_T and b_K, just as electron transfer from ferrous cytochrome *a* to ferric cytochrome a_3 has never been shown [19], electron transfer in which cytochrome b_T is oxidized and cytochrome b_K reduced has also not been reported [2].

The present paper describes the behaviour of the two cytochromes *b* in coupled and uncoupled systems. We focus upon the responses of these carriers to each other, to NADH and succinate as donors, and to CoQ_2, oxygen and cytochrome *c* as acceptors. The simplest explanation of our and other observations is that the cytochrome *b* system behaves as a $b_K b_T$ dimer capable of existence in four states: fully oxidized, fully reduced, and two half-reduced forms ($b_K^{2+}b_T^{3+}$ and $b_K^{3+}b_T^{2+}$). Each state probably has its own characteristic chemistry and under most conditions rapid electron transfer between the two

* We have retained the terms cytochrome b_K to refer to the high potential component with α-maximum at 561 nm and cytochrome b_T to refer to the low potential component with α-maximum at 565 nm and shoulder at 558 nm because we feel this to be less confusing than terminology based upon α-peaks; use of the terminology is simply for convenience and the subscripts do not have the significance attributed to them by the original authors.

b cytochromes does not occur. We propose a modified version of the protonmotive Q cycle in which the two cytochromes *b* accept electrons separately from the $CoQH_2$ pool, perhaps jointly with cytochrome c_1 (cytochrome b_T) and another acceptor in Complex III (cytochrome b_K). The fully reduced dimer is a stronger reductant than $CoQH_2$ and is also kinetically capable of returning its reducing equivalents to the CoQ pool. Thus, $b_K^{3+}b_T^{3+}$ accepts electrons, $b_K^{2+}b_T^{3+}$ is the major steady state species, and the species that returns electrons to the CoQ pool is $b_K^{2+}b_T^{2+}$.

Methods and Materials

Mitochondria were prepared from beef heart as previously described [20]. Nonphosphorylating Keilin-Hartree (K-H) particles were prepared by the Waring blendor method [21,22]. Sonic submitochondrial particles were prepared as described [20] with 2.5 μmol cytochrome *c* per ml mitochondrial suspension present during sonication to replace cytochrome *c* lost during preparation. The resulting pellet was washed with 0.25 M sucrose/0.01 M Tris (pH 7.4) medium and recentrifuged to remove untrapped cytochrome *c*. Cytochrome *c*-deficient mitochondria were prepared according to Jacobs and Sanadi [23]. Submitochondrial particles were treated with 1% deoxycholate as described by Smith and Camerino [24].

Oxidation-reduction studies of respiratory components utilized Aminco DW-2 and Cary-14 spectrophotometers. Changes involving both cytochromes *b* were monitored at 563—575 nm, cytochrome b_T was monitored at 566—575 nm, and cytochrome b_K was monitored at 561—568 nm. Oxygen uptakes were measured by the method of Strickland et al. [25].

NADH (Type III), rotenone, cytochrome *c* (horse heart type VI) and morpholinopropanesulfonic acid (MOPS) were from Sigma Chemical Co. Phenazine methosulphate and MCCP were from California Biochemicals. Antimycin A (Wisconsin Alumni Research Foundation) was dissolved in 95% ethanol. Coenzyme Q_2 was the generous gift of Dr. H. Morimoto, Takeda Chemical Industries, Osaka, Japan. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) was the gift of Dr. P. Heytler of Dupont Chemical Co., Wilmington, DE. Other reagents were either products of J.T. Baker Co. or BDH Chemicals.

Results

Cytochrome *c*-depleted beef heart mitochondria show the spectra of reduced cytochromes b_K and c_1 in the initial steady state with glutamate plus malate as electron donor system (Fig. 1). A fraction of cytochrome *b* with an α-band in the 561—563 region goes reduced more slowly as the mitochondria slowly become energized (cf. Eisenbach and Gutman [26]). Cytochrome b_K, identified by its α-peak at 561 nm, is fully reduced upon anaerobiosis (trace d). The presence of uncoupler during the steady state diminishes the steady state reduction of cytochrome *b*, while ATP addition induces reduction of a cytochrome *b* with absorption peak at 565 nm, identified as cytochrome b_T. Comparison between the steady states in presence of ATP and MCCP (trace e)

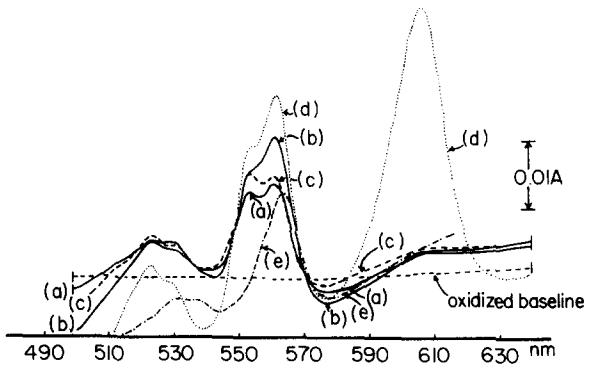

Fig. 1. Spectra of cytochrome *c* depleted beef heart mitochondria reduced with glutamate and malate. (a) Initial steady-state induced by 7 mM glutamate plus 14 mM malate (difference spectrum). (b) same as (a), final steady-state. (c) same as (a), plus 1.8 μM MCCP. (d) anaerobic plus 1.8 μM MCCP (difference spectrum). (e) 7 mM glutamate + 14 mM malate + 0.5 mM ATP steady-state vs. glutamate + malate + 1.8 μM MCCP steady-state. Medium: 225 mM mannitol, 75 mM sucrose, 10 mM sodium morpholinopropanesulphonate buffer (pH 7.4), 1 mM EDTA. Mitochondrial concentration equivalent to 1.5 μM cytochrome aa_3 (approx. 4 mg/ml protein).

shows that only cytochrome b_T is more reduced in the former case; these reagents had no effect on the reduction state of cytochrome b_K. As in these mitochondria respiration rates are cytochrome *c* limited and no reduction of cytochrome *a* is observed in any steady state (cf. traces a—c with trace d) the energization effects must be thermodynamic rather than kinetic in nature (cf. Boveris et al. [27]). Electron transfer between cytochromes b_T and b_K does not take place at a significant rate.

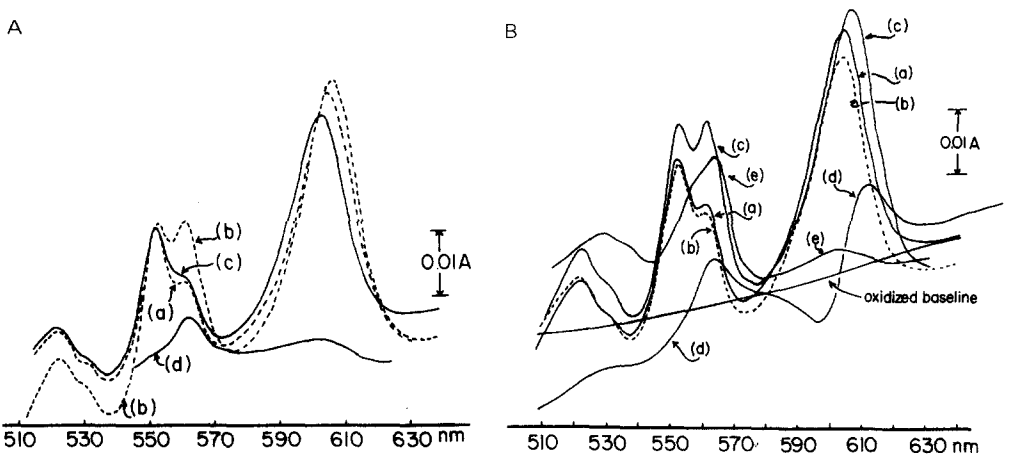

Fig. 2. (A) Difference spectra of K-H submitochondrial particles. 67 mM phosphate buffer (pH 7.4); showing slow oxidoreduction of b_K induced by NADH (1.35 mM) in presence of azide (5.3 mM). (a) initial anaerobic, (b) final anaerobic, (c) steady state aerobic, (d) re-aerated ([NADH] → 0). (B) Spectra of sonic submitochondrial particles. 22 mM phosphate buffer (pH 7.4) and 5.3 mM azide, (a) NADH plus azide steady state vs. oxidized + azide, (b) same as (a) plus MCCP in sample cuvette, (c) 'b' after anaerobiosis, (d) NADH (anaerobic) vs. NADH + azide (aerobic), (e) dithionite vs. NADH (anaerobic).

Nonphosphorylating submitochondrial (K-H) particles with largely inverted membrane configuration do not react readily with NAD-linked substrates but oxidize NADH directly [28]. In the presence of azide, cytochrome *b* is reduced in three stages (Fig. 2A). Reduction of cytochrome *b* increases only slowly upon anaerobiosis (cf. traces a and c); ATP and MCCP have no effects on these spectra (not shown). in sonic particles (Fig. 2B) anaerobiosis in the presence of NADH and azide results in two changes (Fig. 2B, traces b and c): a shift in the α-peak of cytochrome *a*, and an increased reduction of a *b*-type cytochrome, which is reoxidized upon aeration (trace d). This O_2-sensitive species is not identical with cytochrome b_T; full development of the 565 nm band requires the addition of dithionite to these particles (trace e). Cytochrome b_T is also present in deoxycholate-treated particles as addition of antimycin in the steady state induces reduction of a species with α-peak at 565 nm (Fig. 3). The absence of an appreciable 558-nm shoulder in this spectrum is due to the antimycin-induced shift of the α-peak of cytochrome b_K, as seen in Fig. 3 trace d [17,29]. Uncoupler addition had no effects on these systems, contrary to previous reports [30,31], as shown in Fig. 2B (trace b) and in Fig. 3 (trace b).

Separation of the cytochrome b_T and cytochrome b_K (antimycin) spectra is possible in the presence of oxygen plus malonate (Fig. 4). Succinate reduction of submitochondrial particles in the presence of antimycin and air gives rise to a reduced cytochrome *b* peak with α maximum at 563.5 nm (trace B). Aerobic addition of malonate removes the longer wavelength cytochrome b_T component and reveals the cytochrome b_K spectrum with antimycin-shifted α-peak at 562.5 nm (trace C). When both cuvettes contain succinate, antimycin, and O_2, and malonate is added to the reference cuvette, the resulting cytochrome b_T spectrum (trace D) shows no contributions from cytochrome b_K nor the latter's antimycin-induced shift.

Similar effects can be seen in the presence of antimycin and NADH on addition of rotenone (Fig. 5). After reduction in the presence of cyanide, cytochromes *c* and c_1 appear at 551 nm, cytochrome b_K at 561 nm, and cytochrome *a* at 605–606 nm; CoQ_2 in the presence of rotenone oxidizes cyto-

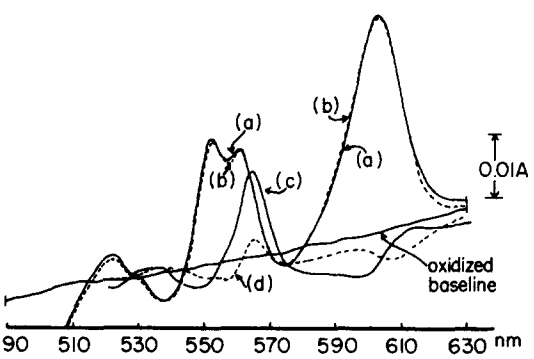

Fig. 3. Effect of antimycin on the two cytochromes *b* in deoxycholate-treated K-H particles, 67 mM phosphate buffer (pH 7.4), 30°C. (a) 18 mM succinate plus 5.3 mM azide vs. oxidized. (b) same as (a) plus 1.8 μM MCCP in sample cuvette, (c) succinate + azide + antimycin (18 μg/ml) vs. succinate plus azide, (d) dithionite plus antimycin vs. dithionite.

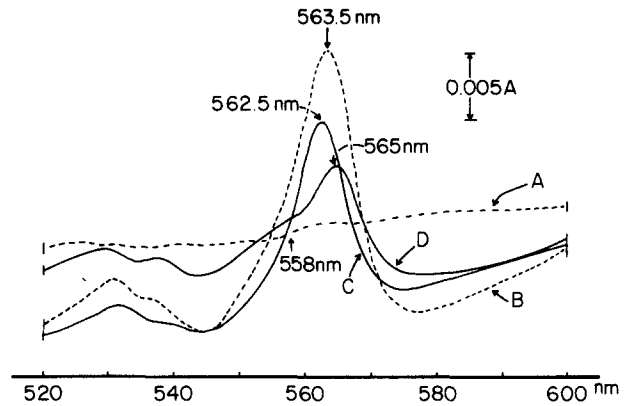

Fig. 4. Effect of malonate on the antimycin-induced spectra of submitochondrial particles. 1.25 mg protein/ml K-H submitochondrial particles in 0.1 M phosphate buffer (pH 7.4). Note cytochromes b_T (565 nm with 558 nm shoulder, Trace D) and b_K (562.5 nm, Trace C). - - - - - -, (A) oxidized vs. oxidized baseline. - - - - - -, (B) +2 mM succinate + 1.2 μM antimycin vs. oxidized. ———, (C) 2 mM succinate + 1.2 μM antimycin + 15 mM malonate vs. oxidized. ———, (D) succinate + antimycin vs. succinate + antimycin + 15 mM malonate (aerobic system; other conditions as in Fig. 3).

chrome b_K while leaving the *c* and *a* cytochromes unaffected (trace A). With antimycin as respiratory inhibitor instead of cyanide, the presence of CoQ_2 and rotenone results in the appearance of the (antimycin-shifted) cytochrome b_K peak at 562.5 nm (trace B). In the difference spectrum with NADH and antimycin in both cuvettes, but rotenone and CoQ_2 in the reference cuvette, cytochrome b_T is seen, with peak at 565 nm and shoulder at 558 nm, the only component reduced in the presence of NADH and antimycin that is reoxidized in the presence of rotenone (Fig. 5). These results again indicate completely

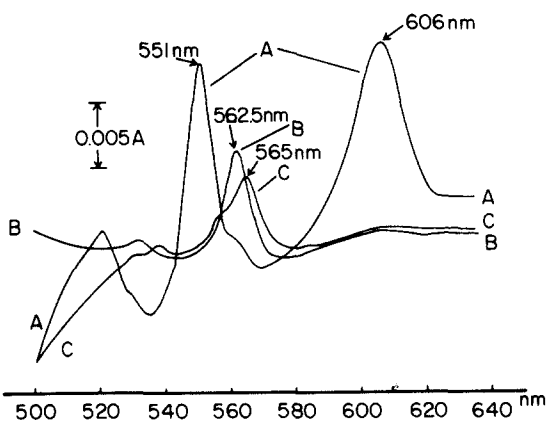

Fig. 5. Effect of rotenone and coenzyme Q_2 on the spectra of beef heart sonic submitochondrial particles. 0.9 mg protein/ml sonic submitochondrial particles in presence and absence of antimycin, showing cytochrome b_T (Note: α-peak at 565 nm and shoulder at 558 nm, Trace C) and b_K (α-peak at 562.5 nm in presence of antimycin, Trace B). Trace A: NADH + cyanide + rotenone + CoQ_2 vs. oxidized. Trace B: NADH + antimycin + rotenone + CoQ_2 vs. oxidized. Trace C: NADH + antimycin vs. NADH + antimycin + rotenone + CoQ_2. 75 mM sucrose, 225 mM mannitol, 10 mM $MgCl_2$, 10 mM potassium phosphate (pH 7.4), 10 mM MOPS, 1 mM EDTA, 30°C.

independent redox changes of the two *b* cytochromes, and are in essential agreement with the concepts advanced by Wikström [2] and by Boveris et al. [27].

The kinetics of these spectral changes are shown in Fig. 6. In sonic particles (Fig. 6A), more cytochrome *b* is reduced by NADH in presence of antimycin (trace b) than in presence of cyanide (trace a). Part of the antimycin-reducible *b* is reoxidized upon addition of rotenone; the remaining cytochrome b^{2+} cannot be reoxidized by CoQ_2 addition. However, the portion of cytochrome *b*

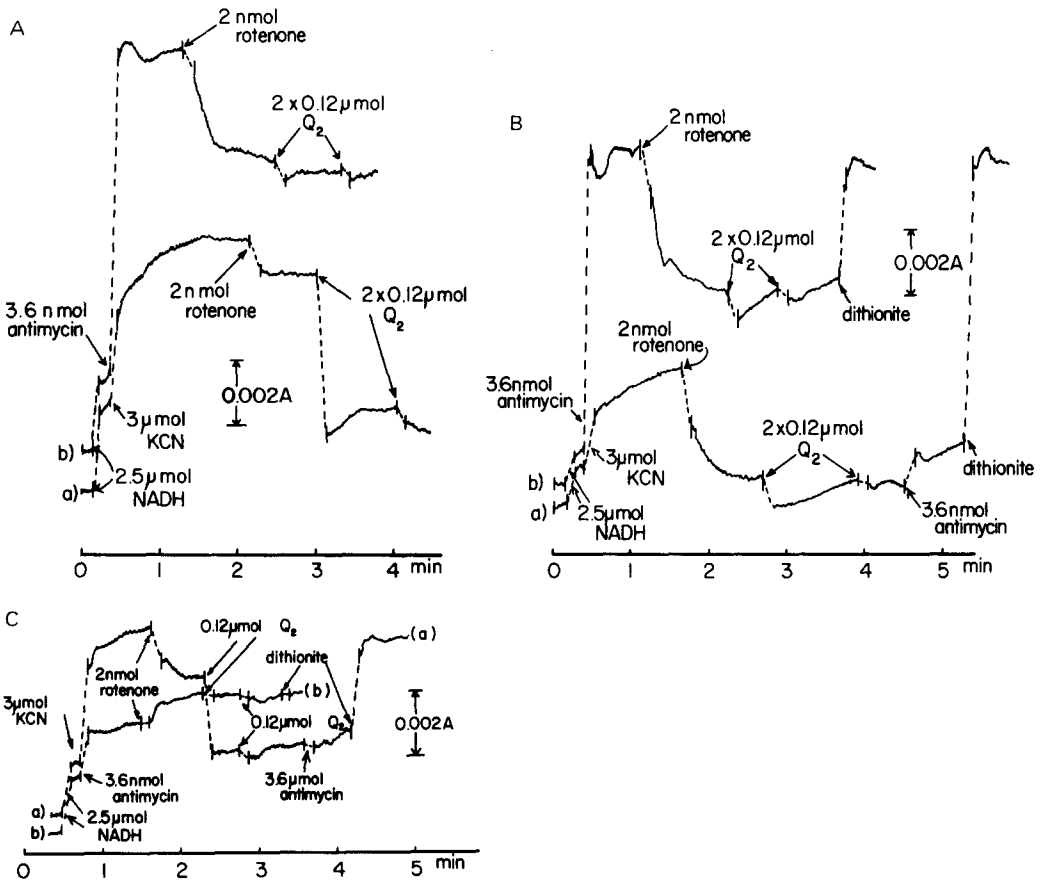

Fig. 6. Effects of antimycin and cyanide on cytochrome *b* oxidoreduction in sonic beef heart submitochondrial particles. (A) Kinetics measured in the 563—575 nm region, (a) cyanide-treated, (b) antimycin-treated. Note that reduction of cytochrome *b* by NADH in presence of antimycin (trace b) is greater than in presence of cyanide. The effects of rotenone and CoQ_2 additions are indicated. 0.9 mg protein/ml sonic particles in 2.7 ml medium as in Fig. 5. (B) Kinetics measured in the 566—575 nm region. (a) cyanide-treated system, (b) antimycin-treated system. Other experimental conditions are similar to those in Fig. 6A. Note: There is a greater effect of rotenone at this wavelength upon the reoxidation of cytochrome *b* reduced in presence of antimycin by NADH; the smaller amount of *b* reduced in presence of cyanide (trace (a)) is reoxidized in presence of rotenone. (C) Kinetics monitored in the 561—568 nm region. (a) cyanide-treated system, (b) antimycin-treated system. Experimental conditions are similar to those in Fig. 6A. Note: The relative effect of antimycin and cyanide on reduction are now reversed (compare Figs. 6A and B), because the antimycin induced spectral shift of cytochrome b_K from 561 to 562.5 nm ensures that the extinction measured at 561—568 nm is lower in the system. The effects of rotenone and CoQ additions are indicated, as well as the 100% reduction by dithionite.

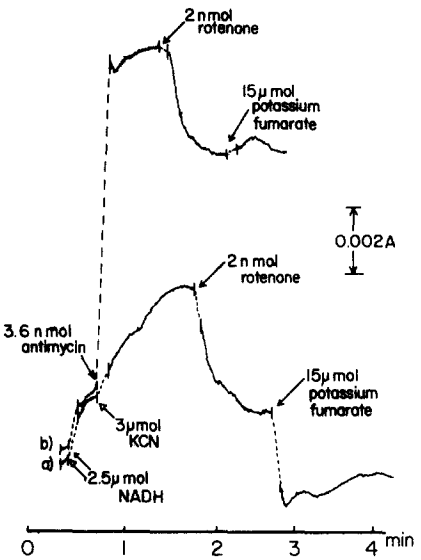

Fig. 7. Effects of rotenone and fumarate addition on cytochrome *b* reduced by NADH in presence of respiratory inhibitors. (a) cyanide-inhibited; (b) antimycin-inhibited. Effects seen in 563—575 nm region (cf. Fig. 6A). 0.9 mg protein/ml beef heart (K-H type) particles (as in Fig. 6) plus 1 μM cytochrome *c*. Rotenone addition in presence of antimycin or cyanide causes partial reoxidation; fumarate induces reoxidation of the remaining cytochrome *b* in the cyanide-inhibited but not the antimycin-inhibited system.

reduced in presence of cyanide, and unaffected by rotenone addition, is almost completely reoxidized upon addition of CoQ_2. Antimycin, which blocks NADH oxidation, also blocks the interaction of one of the cytochromes (b_K?) with coenzyme Q in these particles. If the 566—575 nm wavelength pair is selected (Fig. 6B), a much greater effect of rotenone addition upon the antimycin-reduced cytochrome *b* is seen; the smaller amount of cyanide-induced reduction is totally rotenone sensitive and very little CoQ-oxidizable material is present (trace a). Dithionite addition secures full reduction of all the cytochrome *b* (traces a and b). At 561—568 nm, the relative effects of antimycin and cyanide are reversed (Fig. 6C); the antimycin-induced spectral shift of the cytochrome b_K peak from 561 to 562.5 nm causes a lower measured extinction at 561—568 nm in the antimycin than in the cyanide inhibited system. Rotenone has opposite effects with the two inhibitors (trace a shows a slight oxidation in the presence of cyanide, while trace b indicates an increased reduction in the presence of antimycin). CoQ_2 oxidizes cytochrome *b* reduced in the presence of cyanide, but not that reduced in presence of antimycin. Dithionite is required for full reduction in the HCN-blocked particles, but not in the antimycin-inhibited systems (trace b).

The behaviour of the antimycin- and cyanide-blocked systems towards fumarate following reduction by NADH resembles their behaviour towards CoQ_2 (Fig. 7). If Figs. 6A and 7 are compared it can be seen that fumarate-induced reoxidation of cytochrome b_K is blocked by antimycin (trace b,

Fig. 7), while it proceeds in the presence of cyanide (trace a, Fig. 7), as with CoQ_2 and sonic particles (Fig. 6A). The major difference between the K-H particles (Fig. 7) and the sonic particles (Fig. 6) is that the *b* component reduced in the latter system in presence of cyanide is less autoxidizable than the corresponding component reduced in the former system (cf. traces a, Figs. 6A and 7).

A further way of preparing a system containing reduced cytochrome b_K but no reduced cytochrome b_T involves the use of BAL (2,3-dimercaptopropanol), as shown by Slater [32] and by Deul and Thorn [33]. Approx. 3 ml of K-H particles in 0.1 M phosphate buffer (pH 7.4) were shaken aerobically in a manometer flask at 37°C for 30 min in the presence of BAL. The resulting product was dialyzed at 25°C against 0.01 M phosphate buffer for 1 h to remove excess BAL. Fig. 8 shows the spectra of the particles treated in this way. Addition of succinate (trace B) reduced only a cytochrome *b* component absorbing at 561 nm. Subsequent addition of antimycin shifted the peak to 562 nm while inducing only slight reduction. Dithionite reduced three further components of the system (trace D), cytochrome $c_1(+c)$ at 552.5 nm, cytochrome $a(a_3)$ at 605 nm, and the remaining cytochrome $b(b_T?)$ at 564 nm. The difference spectrum obtained when dithionite is present in both cuvettes, and antimycin is added to the sample cuvette, is shown in Trace E; the characteristic 'antimycin shift' occurs with maximum at 564 nm and minimum at 558 nm,

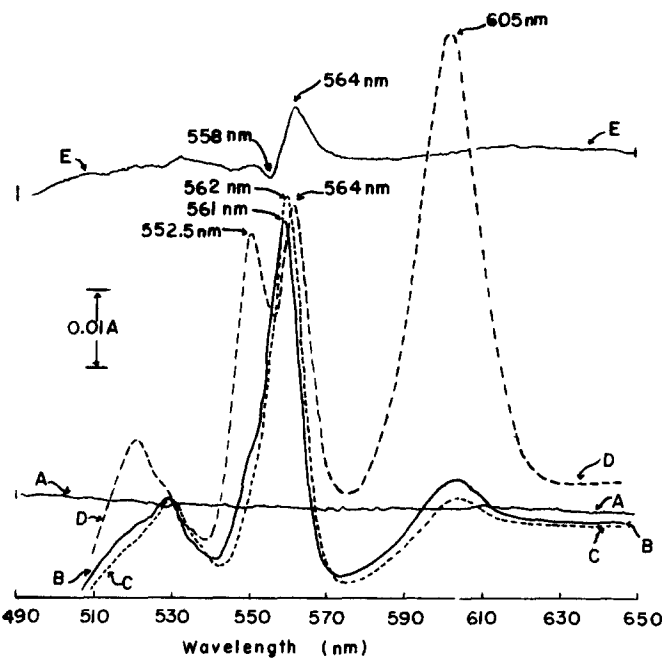

Fig. 8. Difference spectra of BAL-treated submitochondrial particles. Approx. 4.0 mg protein/ml non-phosphorylating submitochondrial particles treated with 20 mM BAL aerobically and dialysed as described in text, were reduced as follows: Trace A: oxidized vs. oxidized baseline. Trace B: + 17.5 mM succinate to sample cuvette. Trace C: as B, + 9.0 μg/ml antimycin to sample cuvette. Trace D: as C, + dithionite to sample and succinate to reference cuvette. Trace E: as D, + dithionite to reference cuvette. 2-ml samples dialysed against 0.01 M phosphate were diluted to 6.5 ml with 0.55 M sucrose, 0.125 M phosphate, 0.062 M borate, 0.01 M EDTA medium, pH 7.5.

as reported by Deul and Thorn [33]. We conclude that BAL-treatment does not affect the spectrum of either cytochrome b_K or b_T; nor does it affect the binding of antimycin and resulting shift in the absorption peak of cytochrome b_K. But after incubation with BAL the cytochrome b_T in the preparation loses its ability to be reduced by succinate in presence of antimycin; only dithionite is then capable of producing the fully reduced $b_K b_T$ species.

Discussion

There are two cytochromes *b* in Complex III, in mitochondria and submitochondrial particles [2,3,13]. Cytochrome b_K (with α-peak at 561 nm) is normally about 75% reduced in the aerobic steady state with either NADH or succinate; when respiration is slowed by malonate in the case of succinate, or by rotenone in the case of NADH, the reduction of cytochrome b_K diminishes accordingly. When oxygen runs out, cytochrome b_K is 100% reduced. Cytochrome b_T has its α-peak at 565 nm (30°C) and a shoulder at 558 nm; although some workers have suggested that these two peaks belong to separate species, in our experiments the two bands were always found to appear and disappear synchronously. The stoichiometry of cytochrome composition in Complex III suggests that only two separate cytochromes *b* can be involved in the activity of this system; a recent MCD study supports the idea that cytochrome b_T is correctly identified as a cytochrome like cytochrome b_5 [34], with a split alpha peak due to an asymmetric environment at the haem iron.

Cytochrome b_T in nonphosphorylating submitochondrial particles is reduced only by dithionite or substrate plus antimycin. In the uninhibited steady state, even in presence of a terminal inhibitor such as cyanide, not more than 20% b_T reduction is seen. At anaerobiosis, it remains largely oxidized. Reduced in the presence of antimycin and NADH, cytochrome b_T can be reoxidized upon addition of oxygen and rotenone (Fig. 6B). In such a case cytochrome b_K remains fully reduced and with red-shifted α-peak (Fig. 5). However, cytochrome b_K reduced with NADH and cyanide can be reoxidized upon the addition of rotenone by either fumarate (Fig. 7) or CoQ (Fig. 6C). These results clearly indicate a lack of direct electron transfer between cytochromes b_K and b_T, contrary to the usual formulation [8].

As antimycin binds to Complex III, cytochrome c_1 is oxidized and cytochromes b_T and b_K are reduced in the presence of either NADH or succinate. Although antimycin binding is clearly responsible for a shift in the cytochrome b_K α-peak, its binding stoichiometry is dependent on the amount of b_T present in the system [35,36]. From the dependence of respiration rate on (b_K^{2+}) in the absence of antimycin and on (b_T^{3+}) in its presence, we identify the species $b_K^{2+} b_T^{3+}$ as the major active species, and the one present in highest concentration in the deenergized state. Slater et al. [37,38] have shown that antimycin binds most tightly to the fully reduced form, $b_K^{2+} b_T^{2+}$, or to the fully oxidized form, $b_K^{3+} b_T^{3+}$, and much less tightly to the half-reduced steady state species, but interpret their results in a rather different way from that adopted here.

Under none of the conditions employed in these experiments is there evidence for electron exchange between the two *b* cytochromes as envisaged in

both the classical linear chain, and in the proton motive redox loop models of Mitchell and others [39,40]. Moreover, antimycin has complex effects on the two cytochromes *b* that are not explained by its binding between cytochrome b_T and cytochrome c_1, or between cytochrome b_K and CoQ [39]. Antimycin inhibition involves and is affected by both of the *b* components. BAL treatment [32,33], which also blocks electron transfer at the level of Complex III, prevents the reduction of cytochrome b_T without affecting the antimycin-binding site (Fig. 8). A similar conclusion was reached by Deul and Thorn [33], although they identified cytochrome b_T as 'modified cytochrome *b*', and did not demonstrate the presence of cytochrome b_T by addition of dithionite. Fig. 9 below, therefore, distinguishes between the BAL and the antimycin effects by placing the site of inhibition for the former reagent between $CoQH_2$ and the b_Tc_1 system and for the latter between the b_Tb_K system and CoQ.

The reciprocal behaviour of cytochrome b_T and c_1 was analysed by Wikström and Berden [41] in terms of the equilibrium in Eqn. 1:

$$QH_2 + b_T^{3+} + c_1^{3+} \rightleftharpoons Q + b_T^{2+} + c_1^{2+} + 2\,H^+ \qquad (1)$$

This behaviour, together with the inhibition by antimycin of CoQ dependent oxidation of the *b* cytochromes (Fig. 6) is most readily explained by a Q-loop electron transfer model in this region of the respiratory chain. However, our results suggest modification of earlier versions [39,40] of the proton-motive Q-loop in favor of the model shown in Fig. 9.

In this model a cytochrome b_Kb_T dimer accepts electrons from the CoQ pool jointly with cytochrome c_1 and another electron acceptor in the system, perhaps the Rieske Fe-S protein group. The major steady state species is the half-reduced form, but the form which returns electrons to the Q-pool is the fully reduced species of the dimer. It is this reaction which is blocked by antimycin and similar inhibitors.

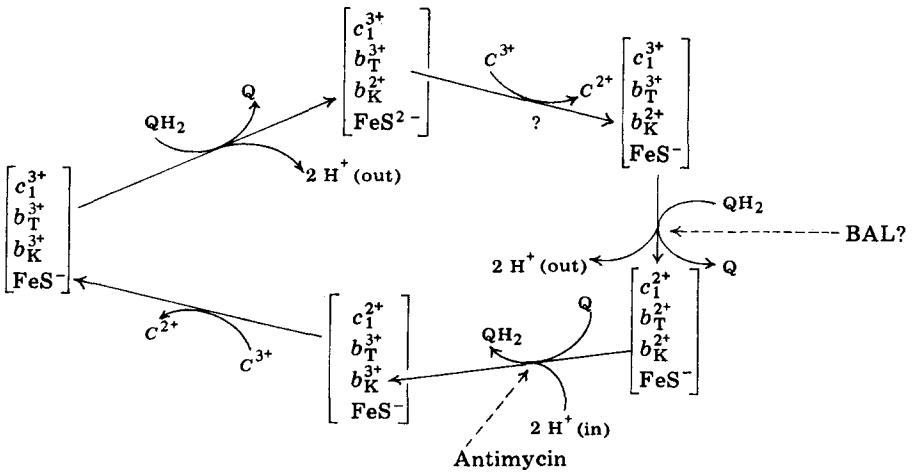

Fig. 9. A possible cycle for Complex III containing a cytochrome b_Kb_T dimer (see Discussion). ------→ indicates possible site of inhibition.

As the redox potentials of these components of Complex III are: b_T −30 mV; b_K +30 mV; c_1 +220 mV; Rieske Fe-S +260 mV; CoQ (2-electrons) +45 mV [3,4,17,42], the following combined E'_0 values would prevail for 2-electron reduction systems: $b_T + c_1$, + 95 mV; b_K + Fe-S, + 145 mV; $b_T + b_K$, 0 mV. Evidently the reduction of the first two couples and the oxidation of the cytochrome *b* dimer by the $CoQH_2/CoQ$ system would be thermodynamically favoured in the absence of any special kinetic pathways or haem/haem interaction. Indeed, at the present time there is no evidence for haem-haem interaction in the b_Tb_K system analogous to the affects on spectrum and redox potentials seen in the aa_3 system [19,43]. It may be noted that the scheme given in Fig. 9 does not require involvement of the CoQ semiquinone form. All the systems within the Complex III interacting with CoQ are postulated to function as 2-electron donors or acceptors. In this model it is Complex III rather than the CoQ system that acts as the 'transducer' between 2-electron transfer and 1-electron transfer.

Our conclusion is not dissimilar from that of von Jagow and Bohrer (with *Neurospora crassa* [44]); however, these authors like previous workers had assumed that b_Kb_T system was arranged as a linear electron transfer chain.

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References

1 Chance, B., Wilson, D.F., Dutton, P.L. and Erecińska, M. (1970) Proc. Natl. Acad. Sci. U.S.A. 66, 1175—1182
2 Wikström, M.K.F. (1973) Biochim. Biophys. Acta. 301, 155—193
3 Wilson, D.F. and Dutton, P.L. (1970) Biochem. Biophys. Res. Comm. 39, 59—64
4 Erecińska, M., Oshino, R., Oshino, N. and Chance, B. (1973) Arch. Biochem. Biophys. 157, 431—445
5 Berden, J.A., Opperdoes, F.R. and Slater, E.C. (1972) Biochim. Biophys. Acta 256, 594—599
6 Slater, E.C., Lee, C.P., Berden, J.A. and Wegdam, H.J. (1970) Biochim. Biophys. Acta 223, 354—364
7 Wainio, W.W. (1977) J. Bioenerg. Biomemb. 9, 321—335
8 Mitchell, P. (1976) J. Theor. Biol. 62, 327—367
9 Kroger, A. and Klingenberg, M. (1973) Eur. J. Biochem. 39, 313—323
10 Crane, F.L. (1977) Annu. Rev. Biochem. 46, 439—469
11 Gutman, M., Coles, C.J., Singer, T.P. and Casida, J.E. (1971) Biochemistry 10, 2036—2043
12 Ernster, L., Lee, I.Y., Norling, B. and Persson, B. (1969) Eur. J. Biochem. 9, 299—310
13 Guerrieri, F. and Nelson, B.D. (1975) FEBS Lett. 59, 339—342
14 Leung, K.H. and Hinkle, P.C. (1975) J. Biol. Chem. 250, 8467—8471
15 Caswell, A.H. (1971) Arch. Biochem. Biophys. 144, 445—447
16 Wikström, M.K.F. and Lambowitz, A.M. (1974) FEBS Lett. 40, 149—153
17 Erecińska, M. and Wilson, D.F. (1976) Arch. Biochem. Biophys. 174, 143—157
18 Rieske, J.S. (1971) Arch. Biochem. Biophys. 145, 179—193
19 Lemberg, M.R. (1969) Physiol. Rev. 49, 48—121
20 Malviya, A.N., Parsa, B. Yodaiken, R.E. and Elliott, W.B. (1968) Biochim. Biophys. Acta 162, 195—209
21 Nicholls, P. and Malviya, A.N. (1968) Biochemistry 7, 305—310
22 Malviya, A.N., Elliott, W.B., Nicholls, P. and Kimelberg, H.K. (1971) Ind. J. Biochem. Biophys. 9, 121—129
23 Jacobs, E.E. and Sanadi, D.R. (1960) J. Biol. Chem. 235, 531—534

24 Smith, L. and Camerino, P.W. (1963) Biochemistry 2, 1428—1432
25 Strickland, E.M., Ziegler, F.D. and Anthony, A. (1961) Nature 141, 969
26 Eisenbach, M. and Gutman, M. (1975) Eur. J. Biochem. 52, 107—116
27 Boveris, A., Erecińska, M. and Wagner, M. (1972) Biochim. Biophys. Acta 256, 223—242
28 Slater, E.C. (1950) Biochem. J. 46, 484—499
29 Pumphrey, A.M. (1962) J. Biol. Chem. 237, 2384—2390
30 Nicholls, P. (1969) Fed. Proc. 28, No. 2 (Abstract)
31 Malviya, A.N., Ozawa, T. and Yagi, K. (1976) Proc. X. Intl. Congr. Biochem. Hamburg, Germany, p. 325
32 Slater, E.C. (1949) Biochem. J. 45, 14—30
33 Deul, D.H. and Thorn, M.B. (1962) Biochim. Biophys. Acta 59, 426—436
34 Arutjunjan, A.M., Kamensky, Y.A., Milgrom, E., Surkov, S., Konstantinov, A.A. and Sharonov, Y.A. (1978) FEBS Lett. 95, 40—44
35 Berden, J.A. and Slater, E.C. (1970) Biochim. Biophys. Acta 216, 237—249
36 Von Jagow, G. and Klingenberg, M. (1972) FEBS Lett. 24, 278—282
37 Slater, E.C. (1973) Biochim. Biophys. Acta 301, 129—154
38 Berden, J.A. and Slater, E.C. (1972) Biochim. Biophys. Acta 256, 199—215
39 Mitchell, P. (1975) FEBS Lett. 56, 1—6
40 Lawford, H.G. and Garland, P.B. (1973) Biochem. J. 136, 711—720
41 Wikström, M.K.F. and Berden, J.A. (1972) Biochim. Biophys. Acta 283, 403—420
42 Wilson, D.F. and Erecińska, M. (1975) Arch. Biochem. Biophys. 167, 116—128
43 Nicholls, P. and Chance, B. (1974) in Molecular Mechanisms of Oxygen Activation (Hayaishi, O. ed.), pp. 479—534, Academic Press
44 Von Jagow, G. and Bohrer, C. (1975) Biochim. Biophys. Acta 387, 409—424