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MINI-REVIEW

The Three-Subunit Cytochrome bc_1 Complex of *Paracoccus denitrificans*. Its Physiological Function, Structure, and Mechanism of Electron Transfer and Energy Transduction

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Abstract

The cytochrome bc_1 complex purified from *P. denitrificans* has the same electron-transfer and energy-transducing activities, is sensitive to the same electron-transfer inhibitors, and contains cytochromes b, c_1, iron-sulfur protein, and thermodynamically stable ubisemiquinone identical to the counterpart complexes from mitochondria. However, the bacterial bc_1 complex consists of only three proteins, the obligate electron-transfer proteins, while the mitochondrial complexes contain six or more supernumerary polypeptides, which have no obvious electron-transfer function. The *P. denitrificans* complex is a paradigm for the bc_1 complexes of all gram-negative bacteria. In addition, because of its simple polypeptide composition and apparently minimal damage during isolation, the *P. denitrificans* bc_1 complex is an ideal system in which to study structure–function relationships requisite to energy transduction linked to electron transfer.

Key words: *P. denitrificans*; bc_1 complex; protonmotive Q cycle.

Introduction

The cytochrome bc_1 complex of *Paracoccus denitrificans* has the smallest number of subunits of any bc_1 complex purified to date (Yang and Trumpower, 1986), appears to suffer less damage during its purification than do the bc_1 complexes from other species (Yang and Trumpower, 1986; Meinhardt *et al.*,

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1987), and uses the same pathway of electron transfer and mechanism of energy transduction as the bc_1 complexes of higher organisms (Yang and Trumpower, 1988). In addition, plasmids and protocols for genetically manipulating *P. denitrificans* have recently been developed (Van Spanning *et al.*, 1990). For these reasons the cytochrome bc_1 complex of this gram-negative bacterium appears to be ideally suited to investigating structure–function relationships relevant to electron transfer and energy transduction, utilizing the combined approaches of biochemistry, biophysics, and molecular genetics.

Physiological Function of the bc_1 Complex

P. denitrificans is a nonfermenting, denitrifying soil bacterium capable of autotrophic growth on H_2 and CO_2, or heterotrophic growth on a variety of carbon sources. The cytochrome bc_1 complex serves a central, although not obligatory, function in the electron transfer pathways which recovery the free energy from these nutrients. The bc_1 complex is localized in the plasma membrane of the bacterium, and the genes encoding the three subunits of the complex are expressed constitutively from an operon, as discussed by B. Ludwig elsewhere in this issue.

The scheme in Fig. 1 summarizes the relationship of the bc_1 complex to other electron-transfer complexes in *P. denitrificans*. This relationship of the bc_1 complex to the other electron-transfer complexes is largely based on the

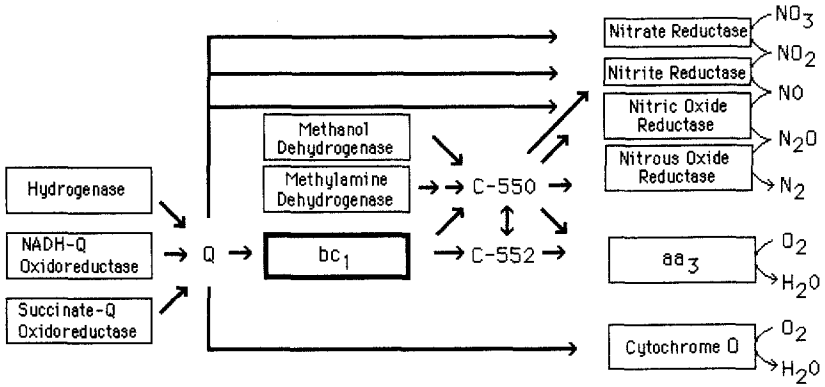

Fig. 1. Role of the cytochrome bc_1 complex in aerobic respiration and anerobic denitrification and its relationship to the alternate ubiquinol oxidase pathway in *P. denitrificans*. Ubiquinone is a common electron acceptor pool for the low-potential hydrogenase and oxidoreductases, while the *c*-cytochromes are a common acceptor pool for the higher-potential methanol and methylamine dehydrogenases, which bypass the cytochrome bc_1 complex. *Paracoccus denitrificans* contains numerous cytochromes *c* (see Van Spanning *et al.*, 1990) whose functions are poorly understood but which appear to have substitutive or overlapping functions in some instances. Only two of the cytochromes *c* are shown.

work of Dadak and Ferguson and their coworkers, and similar schemes have been proposed by them (Carr *et al.*, 1989; Ferguson, 1982; Ferguson *et al.*, 1987; Kucera *et al.*, 1984a,b; Page *et al.*, 1989).

The relationship of the bc_1 complex to other electron-transfer complexes in *P. denitrificans* is, in many respects, a paradigm for nonphotosynthetic, facultative anaerobes which contain a bc_1 complex. Flavin or NAD linked dehydrogenases reduce ubiquinone to ubiquinol, as does hydrogenase (Fig. 1). A key feature of these bacterial electron transfer systems is that ubiquinol is then oxidized by one or more alternative routes. Under anaerobic, denitrifying conditions ubiquinol is the low-potential electron donor for nitrate reductase (Craske and Ferguson, 1986; Kucera *et al.*, 1984b, Parsonage *et al.*, 1986), bypassing the bc_1 complex and *c*-type cytochromes in an antimycin (John and Whatley, 1970) and strobilurin (Kucera *et al.*, 1984a, Parsonage *et al.*, 1986) insensitive reaction. Further reduction of the nitrogen acceptor, via nitrite and nitrous oxide reductase, involves the bc_1 complex and the *c* cytochromes, as indicated by sensitivity of these reactions to bc_1 complex inhibitors (Boogerd *et al.*, 1980; Kucera *et al.*, 1984a, 1988; see, however, Ferguson *et al.*, 1987).

The existence of alternate pathways from ubiquinol to oxygen, bypassing the bc_1 complex, appears to be universal to bacteria which contain a bc_1 complex. Although utilization of these alternate pathways sacrifices the extra energy transduction resulting from proton translocation by the bc_1 complex, these bypasses do provide such bacteria with a protection against competing microorganisms which produce bc_1-specific toxins such as myxothiazol and antimycin (Trumpower, 1990b).

This branching of electron transfer pathways must occur at the level of ubiquinol oxidation, and not within the cytochrome bc_1 complex. There is no electron transfer from one bc_1 complex to another on a physiologically relevant time scale. This can be inferred from the fact that in experiments examining effects of inhibitors on presteady state reduction of the cytochromes, inhibition of cytochrome c_1 reduction by myxothiazol (Von Jagow *et al.*, 1984) and inhibition of cytochrome *b* reduction by antimycin in the iron-sulfur protein-depleted complex (Edwards *et al.*, 1982) titrate as linear curves. If electron transfer from one complex to another were possible, one would expect that a fractional equivalent of inhibitor would fail to block electron transfer through the complex, since the lateral electron transfer from one complex to another would effectively bypass the inhibitor. This in turn would give rise to a "lag" in the titration curves. Such is not the case.

When grown aerobically *Paracoccus* elaborates a respiratory chain superficially so similar to that of mitochondria that this organism is frequently described as resembling a "free-living mitochondrion" (John and Whatley, 1970). Under these growth conditions ubiquinol is oxidized by the cytochrome

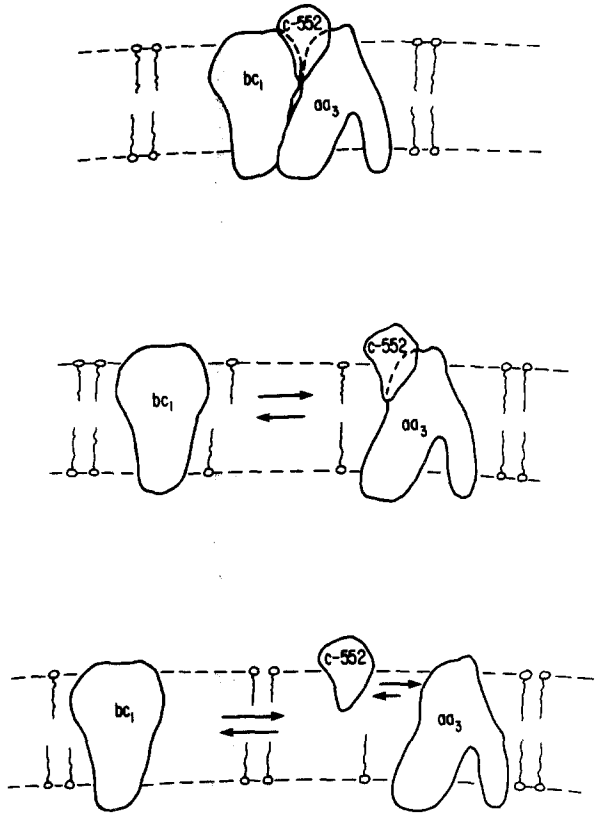

Fig. 2. Reversible formation of a ternary "super complex" between the cytochrome bc_1 and cytochrome aa_3 complexes and the membranous cytochrome *c*-552. It is postulated that the ternary complex exists for multiple electron-transfer events, but reversibly associates. Cytochrome *c*-552 appears to have a higher association constant for the cytochrome aa_3 complex, and this binary complex may be longer lived than the ternary complex, and may service auxiliary electron pathways which bypass the bc_1 complex (see Trumpower, 1990b, for a review).

aa_3 type oxidase via the bc_1 complex. Electron transfer between the bc_1 and aa_3 complexes preferentially uses a membrane-bound cytochrome *c*-552 (Berry and Trumpower, 1985), although at least partial redox equilibration with the periplasmic cytochrome *c*-550 does occur. This preferential utilization of *c*-552 appears to be due to the transient association of the cytochrome bc_1 and cytochrome aa_3 complexes, along with cytochrome *c*-552, to form a ubiquinol oxidase "super complex" (Berry and Trumpower, 1985).

A diagram illustrating this ternary super complex is shown in Fig. 2. The ternary complex consists of one each of cytochrome bc_1 and aa_3 complexes, along with a novel cytochrome *c*-552, discovered by Ed Berry (Berry and

Trumpower, 1985). A similar association of electron-transfer complexes occurs in the gram-positive thermophile, PS3 (Sone *et al.*, 1987). This ternary complex is presumed to reversibly form and associate in the membrane. The membranous cytochrome *c*-552 appears to associate more tightly with the cytochrome *c* oxidase than with the bc_1 complex, based on its distribution when the ternary complex was subfractionated (Berry and Trumpower, 1985). It is possible that the membranous association of *c*-552 offers a kinetic advantage for electron transfer to the oxidase, and that a binary *c*-552/oxidase complex serves as a common oxidant for the heterogenous pool of *c*-type cytochromes which exist in the periplasmic space of *P. denitrificans*.

Although the bc_1 and aa_3 complexes can be isolated separately from each other, it seems likely that the ternary complex remains associated for multiple electron turnovers. This would be analogous to the situation in photosynthetic bacteria, where there is kinetic evidence for the formation of a super complex between the cytochrome bc_1 complex, cytochrome c_2, and the photosynthetic reaction center in *Rh. sphaeroides* (Joliot *et al.*, 1989). Such ternary complexes may be more commonly involved in bacterial electron transfer chains than is currently appreciated, and their possible physiological significance bears further investigation.

Attempts to demonstrate the formation of a comparable ubiquinol oxidase complex in mitochondria were unsuccessful (Berry and Trumpower, 1985). The apparent lack of any high-affinity association between the bc_1 and aa_3 complexes in mitocondria is consistent with the view that these electron-transfer complexes are independently laterally mobile in the inner mitochondrial membrane, and that the rate of respiration is 'diffusion controlled" (Gupte and Hackenbrock, 1988).

The extent to which an *o*-type cytochrome acts as terminal acceptor under aerobic conditions is not known, and probably varies with growth conditions and different strains of *Paracoccus* (Parsonage *et al.*, 1986; Scholes and Smith, 1968; Van Verseveld *et al.*, 1983). In addition to allowing the bacteria to survive exposure to bc_1 complex toxins, the alternate quinol oxidase pathway probably provides a "protonmotive release valve." As the electrochemical proton potential becomes higher with diminished ATP consumption, the membrane potential and pH gradient components of the protonmotive force would retard electron flow through the bc_1 complex. Under these conditions reducing equivalents which would otherwise accumulate in ubiquinol would be diverted through the pathway of lower protonmotive resistance to the *o*-type cytochrome.

In addition to the bifurcating ubiquinol oxidase pathway, *Paracoccus* has a periplasmic methanol dehydrogenase (Fig. 1), which utilizes a bound pyrrolo-quinoline quinone cofactor (Duine *et al.*, 1980), rather than NAD or

flavin, and reduces cytochrome *c*-550 directly (Alefounder and Ferguson, 1981). Deposition of protons from methanol oxidation into the periplasmic space contributes to the protonmotive force, but this route of electrons to the terminal oxidase also occurs at the expense of energy transduction by the bc_1 complex, thus accounting for lower growth rates on methanol. Likewise, methylamine dehydrogenase is located in the periplasmic space and apparently bypasses the bc_1 complex, transferring electrons to cytochrome *c*-550 via the blue copper protein, amicyanin, and cytochrome *c*-551 (Van Spanning *et al.*, 1990).

Purification and Properties of the bc_1 Complex

The cytochrome bc_1 complex was first purified from plasma membranes of aerobically grown *P. denitrificans* (Yang and Trumpower, 1986). Subsequently, an apparently identical complex was purified by the same procedure from bacteria grown anaerobically on nitrate. The subunit compositions of the membranes and of the bc_1 complexes from *P. denitrificans* grown on oxygen and nitrate are shown in Fig. 3.

The purification of the bc_1 complex from *P. denitrificans* used extraction with dodecyl maltoside followed by ion-exchange chromatography (Yang and Trumpower, 1986). The use of this detergent appears to be critical to the purification, since cholate, deoxycholate, Triton, and detergents of the polyoxyethylene alkyl ether series were all ineffective at extracting a stably active complex. During purification the complex appears to aggregate, possibly due to partially delipidation. Ubiquinol–cytochrome *c* reductase activity of the purified complex requires addition of phospholipid to the complex or addition of dodecyl maltoside to the assay. When thus reactivated the purified *Paracoccus* complex has a turnover number in excess of 500 s^{-1}, which is one of the highest reported for a purified bc_1 complex to date.

The method for purifying the three-subunit bc_1 complex differs only slightly from the method for purifying a six-subunit ubiquinol oxidase "super complex" (Berry and Trumpower, 1985). In purifying the ternary ubiquinol oxidase complex the dodecyl maltoside-dispersed membranes were chromatographed on a longer DEAE cellulose column, and the detergent extract was not stirred with salt prior to the chromatography. As a result of these minor differences in purification procedures, it appears that the cytochrome bc_1 and cytochrome aa_3 complexes and cytochrome *c*-552 are delipidated while still associated on the ion-exchange column, and thus not resolvable thereafter (see Fig. 8 in Yang and Trumpower, 1986). Attempts to split an active bc_1 complex from the ternary ubiquinol oxidase complex consistently damaged the bc_1 complex (Berry and Trumpower, 1985).

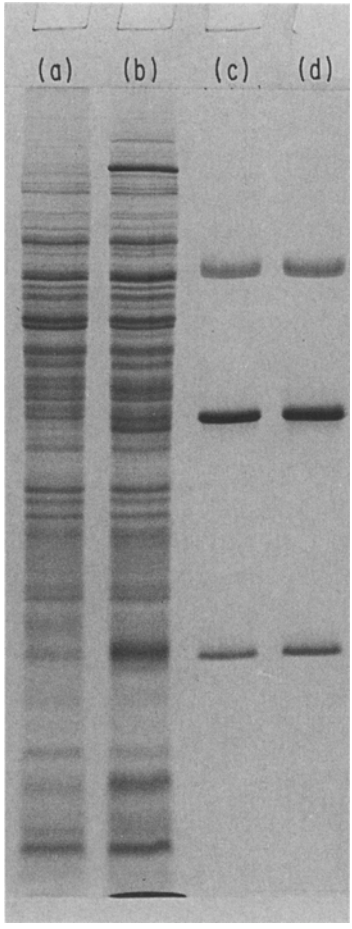

Fig. 3. PAGE-SDS showing the protein composition of plasma membranes and of the purified cytochrome bc_1 complexes from aerobically and anaerobically grown *P. denitrificans*. Lanes (a) and (b) contain plasma membranes from bacteria growth aerobically and anaerobically on nitrate, respectively. Lanes (c) and (d) show the subunit compositions of the cytochrome bc_1 complexes from bacteria grown aerobically and anaerobically on nitrate, respectively. The bc_1 complexes from bacteria grown under the two conditions were purified by extraction with dodecyl maltoside and ion exchange chromatography (Yang and Trumpower, 1986).

This fusion of cytochrome bc_1 and cytochrome aa_3 complexes, along with cytochrome *c*-552, apparently represents their physiological status (see above), since the resulting ternary complex retained full ubiquinol oxidase activity without addition of exogenous cytochrome *c* (Berry and Trumpower, 1985). Indeed, almost 1000-fold as much added cytochrome *c* was required

to double the oxidase rate above that obtained with the intrinsic, membranous cytochrome *c*-552.

The most striking feature of the bc_1 complex from *P. denitrificans* is that it contains only three protein subunits. These three subunits, in order of increasing electrophoretic mobility on SDS-PAGE (Fig. 3), were identified as cytochrome c_1, cytochrome *b*, and iron-sulfur protein. Cytochrome c_1 is readily identifiable as the only subunit containing covalently attached heme, detectable by tetramethylbenzidine staining of the electrophoresis gel (Yang and Trumpower, 1986). Cytochrome *b* was identified from the optical spectrum of the single subunit extracted from the three-subunit complex into Triton X-114 (Payne and Trumpower, 1987). Iron-sulfur protein was identified by cross-reactivity of polyclonal antibodies raised against the smallest subunit of the complex. These antibodies cross reacted with the iron-sulfur protein of the purified beef heart bc_1 complex, which in turn had been previously identified by its purification from that complex (Trumpower and Edwards, 1979).

The finding that this bacterial bc_1 complex has only three subunits, corresponding to the three obligate electron transfer proteins, raised obvious questions about the existence and possible function of the numerous subunits lacking prosthetic groups which are found in the bc_1 complexes of mitochondria. I have referred to these as "supernumerary subunits," to emphasize that they are numerically in excess of the subunits found in the *P. denitrificans* complex. The demonstration that the three-subunit *P. denitrificans* bc_1 complex has the same electron transfer pathway and the same proton translocation activity as the eukaryotic complexes (Yang and Trumpower, 1988; see below) has led to speculation that the supernumerary subunits are in same manner regulatory in the mitochondrial complexes. In this regard it should be emphasized that counterparts of these subunits may be present in the bacterial complex, but loosely associated and lost during purification of the complex. Alternatively, if the supernumerary polypeptides are auxiliary to assembly of the complex, it is possible that they are short-lived in bacteria, and degraded after the respiratory complex is assembled. In any event, the purified three-subunit bc_1 complex from *P. denitrificans* is functionally identical to the purified complexes from mitochondria by any critera currently known.

An additional important property of the purified bc_1 complex from *P. denitrificans* is that the optical and thermodynamic properties of the redox components are more clearly defined than are the corresponding parameters of the mitochondrial complexes. This, combined with the high activity and minimal number of subunits, suggests that this bacterial complex has suffered less damage during purification, and is thus more suitable for structure–function studies than the purified mitochondrial complexes.

The optical spectrum of the purified bc_1 complex of *P. denitrificans* shows quite clearly absorption contributions from two cytochrome *b* hemes, resolvable by differential reduction of the purified complex. When the spectrum of cytochrome c_1 was subtracted, the remaining cytochrome *b* spectrum could be resolved into two optically distinct components. The lower-potential portion of the cytochrome *b* had a split α band, with absorption maxima at 566.1 and 558.5 nm. This portion of the *b* spectrum titrated as a single component with a midpoint potential of -95 mV.[2]

The high-potential portion of the cytochrome *b* had an absorption maximum at 559.5 nm, and titrated as two components, with midpoint potentials of $+30$ and $+120$ mV.[2] This biphasic potentiometric behavior is attributable to a single *b*-560 heme group, the midpoint potential of which is affected by a proximal association with ubiquinone (Salerno *et al.*, 1989). The purified *Paracoccus* bc_1 complex contained 3.5 molecules of ubiquinone per complex (Yang and Trumpower, 1986), which is 3–4-fold as much ubiquinone as typically found in the purified complexes from bovine heart (Berden and Opperdoes, 1972).

The endogenous ubiquinone in the *Paracoccus* bc_1 complex forms a thermodynamically stable ubisemiquinone anion, detectable by EPR spectroscopy during potentionmetric titrations of the complex (Meinhardt *et al.*, 1987). This ubisemiquinone radical signal obtained a maximum spin concentration of 0.57 per complex. This corresponds to a stability constant of 0.22, assuming three molecules of ubiquinone per complex. This stability constant is higher than that observed for the ubisemiquinone anion in cytochrome *c* reductase purified from bovine heart (Ohnishi and Trumpower, 1980), and is further indicative of minimal damage to the complex during purification. Whether this ubisemiquinone is exchange coupled with the high potential *b*-560, as shown to be the case with the yeast mitochondrial bc_1 complex (De la Rosa and Palmer, 1983), has not been examined.

The EPR spectrum of the Rieske iron-sulfur protein is also more clearly defined in the purified *Paracoccus* bc_1 complex than in the purified mitochondrial complexes. The line shape of the EPR spectrum arising from the paramagnetic iron-sulfur cluster changes coincidently with changes in the redox status of ubiquinone, and the resonance frequencies are shifted by UHDBT.[3] The latter also increases the midpoint potential of the iron-sulfur protein at pH 7.5 from $+265$ to $+320$ mV, indicating that the hydroxyquinone analogue of ubiquinone binds almost 10-fold more tightly to the complex when the iron-sulfur cluster is reduced.

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[2] E. A. Berry and B. L. Trumpower, unpublished observations.

[3] 5-*n*-Undecyl-6-hydroxy-4,7-dioxobenzothiazole.

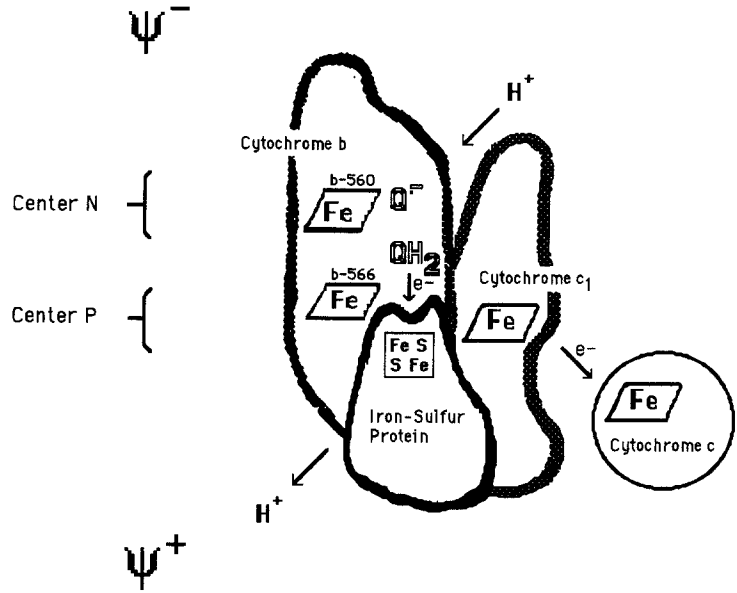

Fig. 4. Topographical arrangement of the three redox-containing subunits of the cytochrome bc_1 complex in the plasma membrane of *P. denitrificans*. The complex is asymmetric and spans the membrane, with the iron-sulfur protein and cytochrome c_1 located on the electropositive, periplasmic side of the membrane. Also depicted are the ubiquinol oxidase site (Q_p) at the iron-sulfur protein–cytochrome *b* interface, the ubisemiquinone/ubiquinone reductase site (Q_n) proximal to the *b*-560 heme group on cytochrome *b*, and proton uptake and release at these sites. For a review, see Trumpower, (1990b).

Taken together, the EPR spectra of ubisemiquinone and the iron-sulfur protein demonstrate that the *Paracoccus* bc_1 complex has a binding site for ubisemiquinone, and that there is a separate site at which ubiquinone or ubiquinol binds to the complex, near the iron-sulfur protein. Since the purified *Paracoccus* complex contains only the three-redox proteins, the binding sites which stabilize ubisemiquinone and which position ubiquinone or ubiquinol near the iron-sulfur cluster must be composed of domains contributed by the cytochromes and/or iron-sulfur protein (Meinhardt *et al.*, 1987).

In Fig. 4 I have depicted a postulated model for the arrangement of the three-electron transfer proteins of the bc_1 complex in the plasma membrane of *P. denitrificans*. This model is based largely on topographical studies conducted with mitochondrial bc_1 complexes, but has been modified to include only the three-redox subunits. Experimental findings supporting the postulated topographical arrangement in Fig. 4 have been summarized elsewhere (Trumpower, 1990b).

Mechanism of Electron Transfer and Energy Transduction

Prior to purification of the bc_1 complex from *P. denitrificans*, it had been established that electron transfer and proton translocation in the mitochondrial bc_1 complexes occur by a protonmotive Q cycle mechanism. After purifying the bc_1 complex from *P. denitrificans*, it was shown that the bacterial bc_1 complex likewise uses a protonmotive Q cycle mechanism (1988).

The protonmotive Q cycle, shown in Fig. 5, describes the pathway of electron transfer among the redox prosthetic groups of the cytochrome bc_1

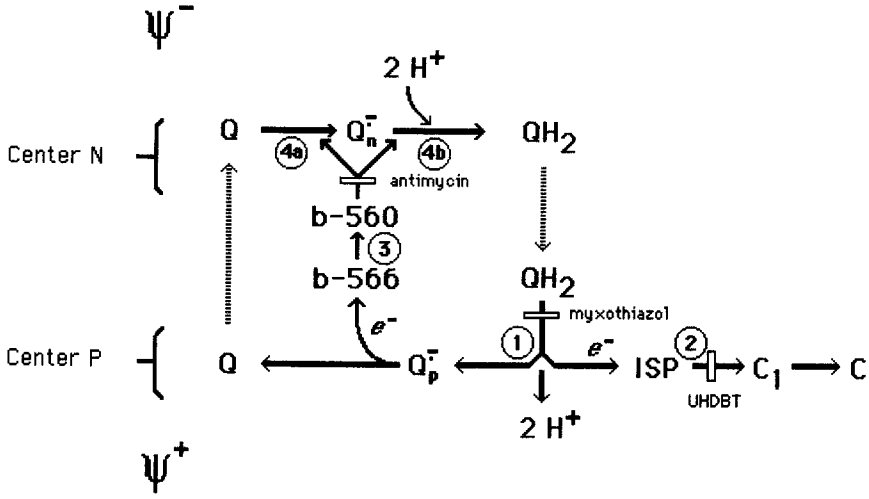

Fig. 5. Protonmotive Q cycle mechanism of electron transfer and proton translocation in the cytochrome bc_1 complex. The scheme shows the branched, cyclic pathway of electron transfer from ubiquinol (QH_2) to cytochrome *c* (C) through the four redox centers of the cytochrome bc_1 complex. The circled numbers designate electron transfer reactions. In step 1 ubiquinol is oxidized at center P in an essentially concerted reaction, in which one electron is transferred from ubiquinol to iron-sulfur protein (ISP), generating a low-potential ubisemiquinone anion (Q_P), which immediately reduces the *b*-566 heme group. Two protons are released at the P surface of the membrane coincident with oxidation of ubiquinol to ubisemiquinone anion. In step 2 the electron transferred to iron-sulfur protein is transferred to cytochrome c_1, and then to cytochrome *c*. In step 3 the electron transferred to the *b*-566 heme group is transferred against the membrane potential inward through the membrane from the *b*-566 to the *b*-560 heme (see Fig. 1). Steps 2 and 3 can, and probably do, occur simultaneously. In step 4a the *b*-560 heme reduces ubiquinone to the relatively stable ubisemiquinone anion (Q_n). When *b*-560 is rereduced by a repeat of the above series of reactions, *b*-560 reduces ubisemiquinone anion to ubiquinol (step 4b). Reduction of ubisemiquinone anion to ubiquinol at center N consumes two protons at the N surface of the membrane. The divergent oxidation of ubiquinol at center P (step 1) occurs twice during one complete Q cycle. One complete Q cycle thus deposits four protons on the positive side of the membrane, reduces two cytochrome *c* molecules, and consumes two protons from the negative side of the membrane. The open boxes show the sites at which myxothiazol, UHDBT, and antimycin inhibit electron transfer reactions within the complex. The effects of these inhibitors on pre-steady state reduction of the cytochromes have been used to substantiate the Q cycle mechanism, as described in the text.

complex and accounts for linkage of proton translocation to this electron transfer. In step 1 of the Q cycle ubiquinol (QH_2) is oxidized at center P in a concerted reaction in which one electron is transferred to iron-sulfur protein (ISP) to form a ubisemiquinone anion (Q_p), which immediately reduces the *b*-566 heme. This oxidation deposits two protons on the positive side of the membrane. One is released coincident with oxidation of ubiquinol to ubisemiquinone; the second is released essentially simultaneously with the first as ubisemiquinone ionizes to ubisemiquinone anion.

The two electrons from ubiquinol thus diverge at center P. One is transferred to iron-sulfur protein. In step 2 this electron is transferred to cytochrome c_1 and then to cytochrome *c*. The second electron from ubiquinol, transferred from Q_p to *b*-566, recycles through the bc_1 complex as it is transferred from the low-potential *b*-566 to the higher-potential *b*-560 (Fig. 5, step 3). Cytochrome *b*-560 then reduces ubiquinone (Q) to ubisemiquinone anion (Q_n) at center N (Fig. 5, step 4a).

At this point the Q cycle is only half complete, since only one electron from ubiquinol has been transferred to cytochrome *c*. This "half-cycle" deposits two protons on the P side of the membrane, while the second electron from ubiquinol transiently resides on the ubisemiquinone anion at center N (Q_n). As discussed below, Q_n is relatively stable.

A second molecule of ubiquinol is then oxidized by iron-sulfur protein, transferring one electron to cytochrome c_1 en route to reduce a second molecule of cytochrome *c*, again forming the ubisemiquinone anion, Q_p, and depositing two more protons on the P side of the membrane. Q_p reacts by the same pathway as previously, transferring an electron to *b*-566 and *b*-560. Cytochrome *b*-560 then reduces the previously formed stable ubisemiquinone anion (Q_n) to ubiquinol at center N, consuming two protons from the negative side of the membrane and completing the Q cycle (Fig. 5, step 4b).

During one complete Q cycle two molecules of ubiquinol are oxidized to ubiquinones, but one molecule of ubiquinol is regenerated by rereduction of one of these ubiquinones. One complete Q cycle requires that the iron-sulfur protein, cytochrome c_1, and the two hemes of cytochrome *b* undergo two "redox turnovers," duplicating steps 1, 2, and 3. In the course of one Q cycle cytochrome *b*-560 alternately reduces ubiquinone to ubisemiquinone anion (step 4a) and ubisemiquinone anion to ubiquinol (step 4b).

As a result of the *net* oxidation of one ubiquinol, two molecules of cytochrome *c* are reduced, four protons are deposited on the positive side of the membrane coincident with the two divergent center P oxidations, and two protons are consumed on the negative side of the membrane as *b*-560 rereduces a ubiquinone to ubiquinol. This stoichiometry of proton translocation implies that for each *one* molecule of cytochrome *c* reduced, a pH meter will detect deposition of *two* protons outside of the mitochondrial

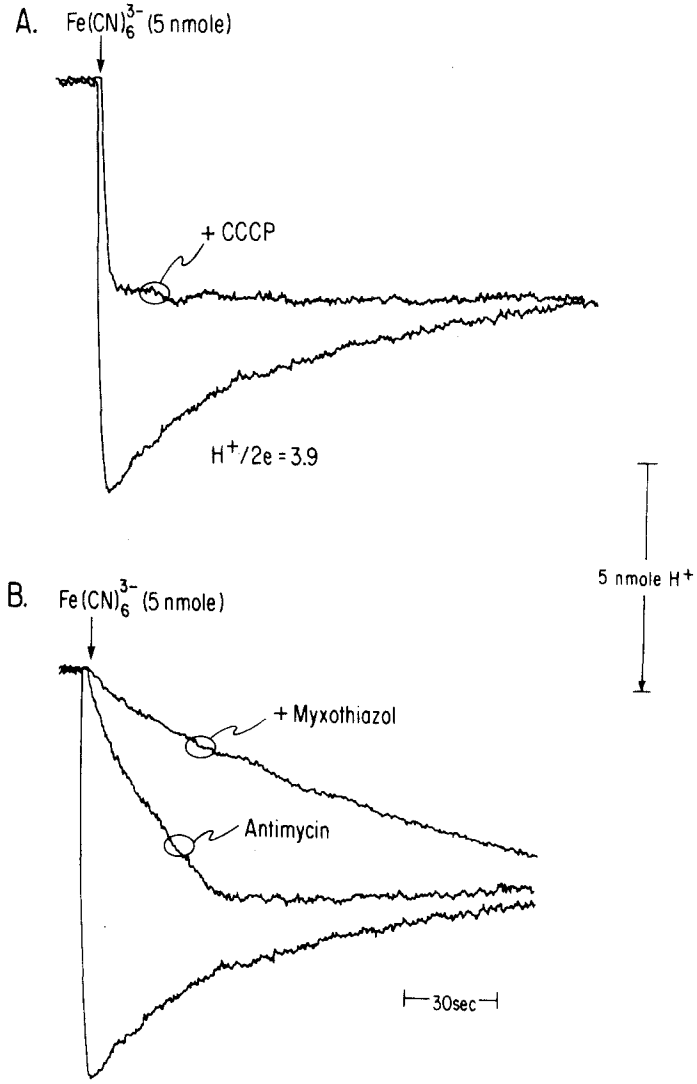

Fig. 6. Protonmotive energy transduction by the purified three-subunit bc_1 complex from *P. denitrificans*. The purified bc_1 complex was reconstituted into liposomes by incubating the purified complex with sonicated phospholipids in the presence of detergent, followed by recovery of the reconstituted proteoliposomes by density gradient centrifugation (Yang and Trumpower, 1988). To demonstrate proton translocation, the complex was reduced with a slight molar excess of ubiquinol analogue, after which valinomycin was added to dissipate any membrane potential, and one-half equivalent of cytochrome *c* was added as an electron transfer mediator. The reaction was then initiated by addition of ferricyanide.

membrane, while measurements of potassium uptake in the presence of valinomycin will detect only *one* compensating positive charge electrophoresed through the membrane. These stoichiometries have been confirmed with mitochondria and purified cytochrome bc_1 complexes reconstituted into liposomes (see Trumpower, 1990a for a review).

It was of special interest to establish whether the simple three-subunit bc_1 complex was capable of proton translocation consistent with a Q cycle mechanism. As shown by the tracings in Fig. 6 the three-subunit complex translocates protons with the same $4H^+/2e^-$ stoichiometry as others had demonstrated for the mitochondrial bc_1 complex (Leung and Hinkle, 1975).

Ubiquinol–cytochrome *c* reductase activity of the purified *Paracoccus* complex is sensitive to antimycin, myxothiazol, and UHDBT. These inhibitors have the same effects on presteady state reduction and reoxidation of the cytochromes in the *Paracoccus* complex as they do with the mitochondrial bc_1 complexes (Yang and Trumpower, 1988), and antimycin abolishes the EPR signal arising from ubisemiquinone (Meinhardt *et al.*, 1987), as it does in the mitochondrial complex. All of these results support the view that the purified bc_1 complex from *P. denitrificans* utilizes the protonmotive Q cycle mechanism of electron transfer and proton translocation, and that only three proteins, the three obligate electron-transfer proteins, are required for these activities.

Acknowledgments

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