The Cytochrome bc₁ and Cytochrome c Oxidase Complexes Associate to Form a Single Supracomplex in Yeast Mitochondria*

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The mitochondrial electron transport chain complexes are large multisubunit complexes embedded in the inner membrane. We report here that in the yeast Saccharomyces cerevisiae, the cytochrome bc₁ and cytochrome c oxidase complexes co-exist as a larger complex of ~1000 kDa in the mitochondrial membrane. Following solubilization with a mild detergent, the cytochrome bc₁-cytochrome c oxidase complex remains stable. It was analyzed using the techniques of gel filtration and blue native-polyacrylamide gel electrophoresis. Direct physical association of subunits of the cytochrome bc₁ complex with those of the cytochrome c oxidase complex was verified by co-immunoprecipitation analysis. Our data indicate that the cytochrome bc₁ complex is exclusively in association with the cytochrome c oxidase complex in yeast mitochondria. We term this complex the cytochrome bc₁-cytochrome c oxidase supracomplex.

The mitochondrial electron transport chain complex is composed of four complexes, complex I (NADH-ubiquinone oxidoreductase), complex II (succinate-ubiquinone oxidoreductase), complex III (ubiquinone-cytochrome c reductase or cytochrome bc₁ complex), and complex IV (cytochrome c oxidase) (for recent review see Ref. 1). In contrast to most eukaryotes, the yeast Saccharomyces cerevisiae does not possess a complex I but rather contains two NADH dehydrogenases associated with the inner membrane (2, 3).

The electron transport chain complexes are large multisubunit complexes embedded in the mitochondrial inner membrane. They are electronically linked to each other by smaller components, which may be located in the membrane (e.g. quinone) or in the aqueous phase (e.g. cytochrome c). A number of studies exist that address the molecular organization of these complexes in the membrane, and two models have been proposed (see Ref. 4 for complete discussion). According to the popular “liquid state” model, one or all of the components of the electron transport chain are randomly arranged in the membrane and are free to diffuse in a lateral manner. The rate of electron transfer between the complexes would be determined by the diffusion process. The second model, the “solid state” model, involves the ordered association of the electron chain components with each other. Indeed a number of earlier reports provide evidence that the stoichiometric association of mitochondrial electron transport chain complexes could occur (4–6). Furthermore, using inhibitor titration-based experiments, evidence was recently provided to suggest that both quinone and cytochrome c do not diffuse freely through or along the membrane (7). From these findings it was concluded that, at least in yeast mitochondria, the respiratory chain may act as one functional unit (7). Experimental evidence, however, to demonstrate the physical interaction of one respiratory complex with another in yeast mitochondria has been lacking to date.

We have recently initiated a study of the assembly of the cytochrome bc₁ complex in S. cerevisiae and were investigating the role of the Bcs1p protein, a molecular chaperone, in the assembly process (8). In the course of this work, we observed that the cytochrome bc₁ complex could be isolated as a supracomplex, significantly larger than the estimated size of the previously described dimeric cytochrome bc₁ complex (8). In this present study, we have further analyzed the cytochrome bc₁ supracomplex. We present evidence here that this supracomplex represents the association of the cytochrome bc₁ complex with the cytochrome c oxidase complex. By using a co-immunoprecipitation approach, we demonstrate a physical interaction of subunits of the cytochrome bc₁ complex with those of the cytochrome c oxidase complex. We conclude the cytochrome bc₁ complex does not exist alone as a dimer in the mitochondrial inner membrane but rather is located exclusively in a large complex with the cytochrome c oxidase complex. We term this complex the cytochrome bc₁-cytochrome c oxidase supracomplex.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains—** All yeast strains used in this analysis were constructed in the same genetic background of W303-1A and are summarized in Table I. The Δshy1 (W303-1A shy1::HIS3) strain was constructed by replacing the entire open reading frame encoding the Shy1p (the SHY1 gene) with the HIS3 gene, as described previously (9, 10). All strains were grown at 30 °C in YPGal (2% peptone, 1% yeast extract, 2% galactose) supplemented with 0.5% lactate. All cells were harvested at an A_{578} nm of ~1–1.5. Mitochondria were isolated according to published procedures (11).

**Detergent Solubilization of the Cytochrome bc₁ and Cytochrome c Oxidase Complexes—** Isolated mitochondria (200 μg of protein) were lysed in 40 μl of digitonin buffer (1% (w/v) digitonin, 50 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 0.1 μg/ml aprotinin, 1 μg/ml leupeptin) for 30 min on ice (8). Following solubilization, a clarifying spin (30 min, 226,000 × g, TLA45 rotor, Beckman TL-100 ultracentrifuge) was performed. SDS-PAGE1 and Western blot analysis of the resulting pellet and supernatant fractions indicated that approx...
imimately 90% of the total cytochrome bc1 complex and 85% of the cytochrome c oxidase complex had been solubilized by the digitonin extraction procedure.

Blue Native-Polyacrylamide Gel Electrophoresis (BN-PAGE)—Mitochondria (200 μg of protein) were solubilized with digitonin and subjected to a clarifying spin, as described above. The samples (40 μl) were supplemented with 4 μl of sample buffer (5% (w/v) Serva Blue G in 500 mM aminocaproic acid) prior to electrophoresis. Samples were then analyzed by BN-PAGE (12) using either 4–8 or 5–10% polyacrylamide gradient gels, as indicated. Following electrophoresis, Western blotting was performed, and the protein complexes were detected by immunoblotting. The calibration standards used in the BN-PAGE and gel filtration analysis (see below) are as follows: Hsp60 (840 kDa), bovine cytochrome c, horse spleen apoferritin (443 kDa), potato β-amylose (200 kDa), yeast alcohol dehydrogenase (150 kDa), bovine serum albumin monomer (66 kDa), and bovine erythrocyte anhydrase (29 kDa).

Gel Filtration Analysis—Isolated mitochondria (1 mg of protein) were solubilized in digitonin buffer (lacking 10% glycerol), as described previously (8). Following a clarifying spin, the detergent extract was applied to a Superose 6 fast protein liquid chromatography gel filtration column (Amersham Pharmacia Biotech, 25 ml column volume), which was previously equilibrated with the digitonin buffer. Fractions (0.5 ml) were collected, precipitated with trichloroacetic acid, and analyzed by SDS-PAGE and Western blotting. Subunits of the cytochrome bc1 and cytochrome c oxidase complexes were detected in the eluate fractions by immunoblotting.

Co-immunoprecipitation—Antibodies against Core1, Cox4p, and respective preimmune IgG were covalently bound to protein A-Sepharose with the cross-linker dimethyl pimelimidate, as described previously (8). Isolated mitochondria were lysed in digitonin buffer and following a clarifying spin were incubated under gentle shaking for 2 h at 4 °C either with anti-Core1, anti-Cox4p, or respective preimmune IgG coupled to the protein A-Sepharose, as described previously (8). Co-immunoprecipitates were washed three times with the digitonin buffer and analyzed by SDS-PAGE and immunoblotting.

Measurement of Respiratory Chain Activities—NADH-cytochrome c reductase and cytochrome c oxidase activities of wild-type mitochondria (10 μg) were measured at 23 °C, in a UVICON 930-Spectrophotometer (Kontron), essentially as described previously (13). When indicated, mitochondrial proteins were solubilized with detergent (1% (w/v) digitonin or 0.1% (w/v) lauryl maltoside or 0.5% (w/v) deoxycholate) prior to measurement of the enzyme activities.

Miscellaneous—Protein determinations and SDS-PAGE were performed according to the published methods of Bradford (14) and Laemmli (15), respectively. The detection of proteins after Western blotting on nitrocellulose was performed using the ECL detection system according to the supplier’s instructions (Amersham Pharmacia Biotech).

RESULTS

The Cytochrome bc1 Complex Exists as a Supracomplex in the Mitochondrial Inner Membrane—In order to analyze the oligomeric state of the cytochrome bc1 complex in yeast mitochondria, the complex was solubilized from the mitochondrial membranes using the mild detergent, digitonin. Measurement of the enzyme activities of the cytochrome bc1 complex (also the cytochrome c oxidase complex) indicated that it was solubilized as an active enzyme by the digitonin (Table II). Indeed the levels of activities measured following digitonin solubilization were similar to those achieved following solubilization by lauryl maltoside or deoxycholate (Table II).

The native molecular mass of the digitonin-solubilized cytochrome bc1 complex was estimated using the technique of BN-PAGE, followed by Western blotting and immune decoration with antibodies specific for subunits of the cytochrome bc1 complex (Fig. 1). The cytochrome bc1 complex was solubilized from wild-type mitochondria and parallel to mitochondria isolated from various strains bearing deletions in individual genes encoding subunits of the cytochrome bc1 complex. Immune decoration of the resulting blots with antisera against both the Rieske FeS protein and cytochrome b indicated that the predominant form of the wild-type cytochrome bc1 complex had an apparent molecular mass of ~1000 kDa. We designate this larger complex as the cytochrome bc1 supracomplex. The size estimation of the 1000-kDa complex was in good agreement with our previous gel filtration analysis, following detergent solubilization under similar conditions (8). A minor amount of the cytochrome bc1 complex had an apparent size of ~850 kDa.

The presence of Qcr10p (16) is apparently not essential to form this supracomplex, as the size of the cytochrome bc1 complex in the QCR10 null mutant, Δqcr10, was similar to that observed in wild-type mitochondria (Fig. 1). Furthermore, the absence of Qcr6p did not have a significant effect on the formation or stability of the supracomplex. The cytochrome bc1 supracomplex observed in the Δqcr6 mutant, the QCR6 null mutant, was slightly smaller than that observed in wild-type mitochondria. Qcr6p is a highly negatively charged protein (pI 8.7) (17), and its loss from the cytochrome bc1 complex would alter the net charge of the complex and may also alter the conformation of the complex. Both changes would likely have an effect on the mobility of the complex under BN-PAGE analysis.

Analysis of the cytochrome bc1 complex in mitochondria deficient in either Qcr6p or the Rieske FeS protein revealed that these subunits are essential for the formation of the cytochrome bc1 supracomplex (Fig. 1). In the Δrip1 and Δqcr9 mitochondria, two forms of the cytochrome bc1 complex smaller than the supracomplex were observed. The predominant form

| Strain | Genotype | Source/Ref. |
|--------|----------|-------------|
| W303−1A | ade2−1 his3−1,15 leu2−3,112 trp1−1 ura3−1 | This work |
| W303Δrip1 (Δrip1) | ade2−1 his3−1,15 leu2−3,112 trp1−1, rip1−1, HIS3 | 8 |
| W303Δctyt1 (Δc1) | ade2−1 his3−1,15, leu2−3,112 trp1−1, ura3−1, cyt1−1, HIS3 | 8 |
| W303Δqcr10 (Δqcr10) | ade2−1 his3−1,15, leu2−3,112 trp1−1, ura3−1, qcr10−1, LEU2 | 16 |
| Δrip1 | ade2−1 his3−1,15, leu2−3,112 trp1−1, ura3−1, qcr6−1, LEU2 | 17 |
| Δqcr9 | ade2−1 his3−1,15, leu2−3,112 trp1−1, ura3−1, qcr9−1, URAS | 19 |
| W303Δcbs3 (Δcbs3) | ade2−1 his3−1,15, leu2−3,112 trp1−1, chp3−1, LEU2 | 22 |
| W303Δcox4 (Δcox4) | ade2−1 his3−1,15, leu2−3,112 trp1−1, ura3−1, cox4−1, TRP1 | 29 |
| W303Δqcr9 (Δqcr9) | ade2−1 his3−1,15, leu2−3,112 trp1−1, ura3−1, qcr9−1, URAS | 30 |
| W303Δcor1 (Δcor1) | ade2−1 his3−1,15, leu2−3,112 trp1−1, ura3−1, cor1−1, HIS3 | 31 |
| W303Δshy1 (Δshy1) | ade2−1 his3−1,15, leu2−3,112 trp1−1, ura3−1, shy1−1, HIS3 | 31 |

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The cytochrome bc\textsubscript{1} complex exists in a supracomplex in the mitochondrial inner membrane. Mitochondria from wild-type (WT), \(\Delta qcr9\), \(\Delta qcr6\), and \(\Delta qcr10\) strains were solubilized with digitonin and analyzed by BN-PAGE. A 4–8\% polyacrylamide gel was used. The protein complexes were detected by Western blotting and immune decoration with antisera specific for cytochrome b (\(\alpha\)-Cyt b), Rieske FeS (\(\alpha\)-FeS), and Cox5a of the cytochrome c oxidase complex (\(\alpha\)-Cox5a). The abbreviations used are as follows: s, supracomplex; p1 and p2, partial assembly forms of the supracomplex. See “Experimental Procedures” for details of the calibration standards.

Fig. 1. The cytochrome bc\textsubscript{1} complex exists in a supracomplex in the mitochondrial inner membrane. Mitochondria from wild-type (WT), \(\Delta qcr9\), \(\Delta qcr6\), and \(\Delta qcr10\) strains were solubilized with digitonin and analyzed by BN-PAGE. A 4–8\% polyacrylamide gel was used. The protein complexes were detected by Western blotting and immune decoration with antisera specific for cytochrome b (\(\alpha\)-Cyt b), Rieske FeS (\(\alpha\)-FeS), and Cox5a of the cytochrome c oxidase complex (\(\alpha\)-Cox5a). The abbreviations used are as follows: s, supracomplex; p1 and p2, partial assembly forms of the supracomplex. See “Experimental Procedures” for details of the calibration standards.

The cytochrome bc\textsubscript{1} complex for its assembly into the 1000-kDa supracomplex is addressed in more detail later (see Fig. 3).

Formation of the Cytochrome bc\textsubscript{1} Supracomplex Requires the Presence of the Cytochrome c Oxidase Complex—In order to gain further insight into the possible physical interaction of the cytochrome bc\textsubscript{1} complex with the cytochrome c oxidase complex, we analyzed the assembly state of the cytochrome bc\textsubscript{1} complex in yeast strains that do not contain an assembled functional cytochrome c oxidase complex. The cytochrome c oxidase-deficient mitochondria used in this analysis were isolated from the \(\Delta imp1\) strain (deficient in Imp1p peptidase, necessary for the maturation of cytochrome c oxidase subunit II) (19), \(\Delta cox4\) (deficient in subunit 4, an essential subunit of the cytochrome c oxidase complex) (20), and \(\Delta shy1\) (deficient in Shy1p, a protein involved in the assembly of the cytochrome c oxidase complex) (21). Mitochondria were isolated from each of these mutant strains and, in parallel to wild-type mitochondria, were solubilized with digitonin. Co-migration of Rieske FeS and cytochrome b proteins upon the BN-PAGE analysis indicated that the cytochrome bc\textsubscript{1} complex was functionally assembled in these cytochrome c oxidase-deficient mitochondria. In each case, however, the cytochrome bc\textsubscript{1} complex had an apparent molecular mass of \(\sim 670\) kDa, in contrast to the 1000-kDa supracomplex observed in wild-type mitochondria (Fig. 2).

Thus we conclude that in the absence of the cytochrome c oxidase complex, the cytochrome bc\textsubscript{1} complex fails to form a supracomplex but assembles into a complex whose native molecular mass corresponds closer to that described previously for the dimeric cytochrome bc\textsubscript{1} complex.

The Assembly of the Cytochrome c Oxidase Supracomplex Requires the Presence of the Assembled Cytochrome bc\textsubscript{1} Complex—The native molecular mass of the yeast cytochrome c oxidase complex was also estimated by a second independent technique, gel filtration chromatography, following solubilization of mitochondrial membrane proteins by digitonin. Consistent with the BN-PAGE results, the subunits of the cytochrome bc\textsubscript{1} and cytochrome c oxidase complexes of wild-type mitochondria co-migrated together under these conditions, with an estimated molecular mass in the range of 1000 kDa (Fig. 3A). A small fraction of the cytochrome c oxidase subunit 4 (Cox4p) proteins eluted in a low molecular weight fraction which most likely represents a fraction of Cox4p dissociated from the cytochrome c oxidase complex.

BN-PAGE analysis of the cytochrome c oxidase complex of mitochondria isolated from a number of yeast mutant strains deficient in an assembled cytochrome bc\textsubscript{1} complex was then performed (Fig. 3B). The cytochrome c oxidase supracomplex in
The presence of the cytochrome bc₁ complex is required for the formation of the cytochrome c oxidase supracomplex. A, gel filtration analysis of the cytochrome bc₁ and cytochrome c oxidase supracomplex. Wild-type mitochondria were solubilized in digitonin, and the molecular mass of the cytochrome bc₁ and cytochrome c oxidase complex was estimated by gel filtration analysis. Eluate fractions were collected and analyzed by SDS-PAGE and Western blotting. The proteins cytochrome c₁ (Cyt c₁), Rieske FeS (FeS₁), Cox4p (Cox4), and Cox2p (Cox2) were detected in the eluate fractions following immunodetection with specific antisera and quantified as described under “Experimental Procedures.” B, BN-PAGE analysis of the cytochrome c oxidase complex of wild-type (WT), Δbc₃, Δcbp3, and Δc₁ strains was performed. A 5–10% polyacrylamide gradient gel was used. The cytochrome c oxidase complex was detected following Western blotting and immune detection with antisera specific for Cox5a (α-Cox5a). The abbreviations used are as follows: s, cytochrome c oxidase supracomplex; p₁, p₂, partial assembly forms of the supracomplex. See “Experimental Procedures” for details of the calibration standards.

In a parallel analysis, antibodies specific for cytochrome c oxidase subunit 4 were used for the co-immunoprecipitation procedure (Fig. 4B). Analysis of the resulting immunoprecipitate revealed that in addition to Cox2p, subunits of the cytochrome bc₁ complex, Core1 and Core2, had been co-immunoprecipitated with Cox4p. Similar levels of the both cytochrome c oxidase and cytochrome bc₁ complex subunits (ranging from 45 to 60% of the total protein in mitochondria) were recovered in the Cox4p immunoprecipitate. Thus, as had been observed in

the Δqcr6 mitochondria was found to be slightly smaller than in wild-type mitochondria. This size difference is similar to that observed previously for the cytochrome bc₁ supracomplex, an observation consistent with the notion that the supracomplex involves an interaction of the cytochrome bc₁ and cytochrome c oxidase complexes. Furthermore, the assembly of the cytochrome c oxidase supracomplex was severely affected in mitochondria of two other mutants of the cytochrome bc₁ complex, Δcbp3 and Δc₁. Both of these strains do not contain an assembled cytochrome bc₁ complex; Cbp3p is a factor required for the assembly of the cytochrome bc₁ complex, and cytochrome c₁ is an essential subunit of the cytochrome bc₁ complex (8, 22, 23). In these mutant strains, two distinct forms of the cytochrome c oxidase complex were observed as follows: a less abundant form of ~670 kDa, and a smaller, more abundant form of ~500 kDa. Thus the formation of the cytochrome c oxidase supracomplex required the presence of an assembled cytochrome bc₁ complex.

In summary, these data demonstrate that both the cytochrome bc₁ complex and the cytochrome c oxidase complexes form a larger oligomeric supracomplex of ~1000 kDa in the mitochondrial inner membrane. The cytochrome bc₁ and the cytochrome c oxidase complexes display a co-dependence for the formation of their respective supracomplex forms. Taken together, these data imply that the cytochrome bc₁ and cytochrome c oxidase complexes exist together in one supracomplex in the mitochondrial inner membrane.

Subunits of the Cytochrome bc₁ and Cytochrome c Oxidase Complex Can Be Co-immunoprecipitated—In order to demonstrate a physical association of subunits of the cytochrome bc₁ complex with those of the cytochrome c oxidase complex, a co-immunoprecipitation approach was adopted. Mitochondria were solubilized with digitonin, and immunoprecipitation of the cytochrome bc₁ complex was performed using antibodies specific for the Core1 subunit (Fig. 4). The cytochrome bc₁ complex had remained intact under these immunoprecipitation conditions, as indicated by the co-immunoprecipitation of the Rieske FeS protein with Core1. Probing the Core1 immunoprecipitate with antibodies specific for subunits of the cytochrome c oxidase complex, Cox2p, Cox4p, and Cox5ap, indicated that the cytochrome c oxidase complex was physically associated with the cytochrome bc₁ complex under these solubilization conditions. Quantitation analysis indicated that approximately 50% of the total Core1 protein and 45–60% of total Cox4p and Cox5ap proteins were immunoprecipitated with the Core1 antibodies. Thus the levels of the cytochrome c oxidase subunits in the Core1 immunoprecipitate were similar to those of the cytochrome bc₁ complex subunits. The cytochrome c oxidase subunits were not detected when preimmune serum was used in the immunoprecipitation analysis. The specificity of the immunoprecipitation of the cytochrome c oxidase subunits with the Core1 antiserum was further demonstrated when mitochondria isolated from the Δorf₇ null mutant were used for the immunoprecipitation analysis. In the absence of Core1, the subunits of the cytochrome c oxidase complex were not found in the immunoprecipitate, thus ruling out the unexpected possibility of cross-reactivity of the Core1 antiserum with the cytochrome c oxidase subunits (Fig. 4A).
the Core1 immunoprecipitate described above, the efficient co-immunoprecipitation of the supracomplex by Cox4p antibodies is achieved under these conditions. Finally, the specificity of the Cox4p co-immunoprecipitation of components of the cytochrome c oxidase complex and of cytochrome bc1 complex with Cox4-specific antibodies (α-Cox4). The co-immunoprecipitations using isolated mitochondria from wild-type (WT) and Δcox4 strains were performed as described in A. Western blots were decorated with antisera specific for Core1, Rieske FeS (FeS), subunits 2, 4, and 5a of the cytochrome c oxidase complex (Cox2, Cox4, and Cox5a). B, co-immunoprecipitation of components of the cytochrome c oxidase complex and of cytochrome bc1 complex with Cox4-specific antibodies (α-Cox4). The co-immunoprecipitations using isolated mitochondria from wild-type (WT) and Δcox4 strains were performed as described in A. Western blots were decorated with antisera specific for Core1, Core2, Cox2p (Cox2), and Cox4p (Cox4).

In summary, these results indicate that the cytochrome bc1 complex physically interacts with the cytochrome c oxidase complex to form a supracomplex in the mitochondrial inner membrane. This supracomplex can be efficiently immunoprecipitated with antibodies specific for either the cytochrome bc1 or the cytochrome c oxidase complexes.

Formation of the Cytochrome bc1-Cytochrome c Oxidase Supracomplex Is Not Required for the Stability of the Individual Respiratory Chain Complexes—Does the formation of the supracomplex enhance the stability of the subunits of the cytochrome bc1 and cytochrome c oxidase complexes? The following data would suggest this is not the case. Mitochondria isolated from mutants deficient in the cytochrome c oxidase complex contain similar levels of the cytochrome bc1 complex subunits as wild-type mitochondria (Fig. 5A). Thus these results indicate that the cytochrome bc1 complex does not depend on the formation of the supracomplex for its proteolytic stability in the membrane. Likewise the levels of Cox2p did not differ significantly from those of wild-type mitochondria when analyzed in a number of mutants defective in the cytochrome bc1 complex and an unrelated marker, Tim23p. B, equivalent amounts of mitochondria isolated from wild-type, Δqcr9, Δc1, Δqcr6, and Δcbp3 strains were analyzed as described in A for steady state levels of components of the cytochrome c oxidase complex and an unrelated marker protein, Tim23p.

**DISCUSSION**

In the present study we report the observation that the cytochrome bc1 complex (complex III) of yeast mitochondria exists as a supracomplex with the cytochrome c oxidase com-
plex (complex IV). This cytochrome bc_{1}-cytochrome c oxidase supercomplex was observed by techniques of BN-PAGE, gel filtration, and co-immunoprecipitation, following solubilization of mitochondrial membrane proteins with the mild detergent digitonin. Although in this size range it is difficult to determine accurately, we estimate the size of the supercomplex to be approximately 1000 kDa, consistent with a stoichiometry of two cytochrome bc_{1} complexes associated with two cytochrome c oxidase complexes (III_{2}-IV_{2}).

The possibility that the mitochondrial electron chain complexes may be found in physical association with each other has been discussed (4–7), but the isolation of such stable supercomplexes from yeast mitochondria has not been reported previously. In the prokaryotes Paracoccus denitrificans and Sulfolobus acidocaldarius and thermophilic bacterium PS3, complexes III and IV have been isolated together as supercomplexes (24–26). In yeast mitochondria, enzymatic data suggest that the electron carriers ubiquinone and cytochrome c do not display a pool type of behavior under physiological conditions that the electron carriers ubiquinone and cytochrome bc_{1} complexes III and IV have been isolated together as supercomplexes. The assembly of the individual cytochrome bc_{1} complex and the cytochrome c oxidase complexes are exclusively located in a stable supercomplex.

Is complex II associated with the digitonin-solubilized complex III-IV supercomplex? Our findings suggest this is not the case. Subunits of the succinate dehydrogenase complex (complex II) did not co-fractionate with the cytochrome bc_{1}-cytochrome c oxidase complex upon gel filtration analysis (results not shown). Furthermore, the formation or size of the cytochrome bc_{1}-cytochrome c oxidase supercomplex was not impaired in mitochondria isolated from yeast mutants deficient in an assembled complex II (results not shown).

The isolation of a stable complex between the cytochrome bc_{1} and cytochrome c oxidase complexes has been achieved here due to the mild nature of the detergent, digitonin, we used for the membrane solubilization. By using this mild detergent, we had succeeded in isolating and maintaining the ATP synthase complex as a larger, dimeric complex, in contrast to the more traditional detergents that resulted in the solubilization of the complex in its monomeric form (10, 27). Subunit analysis of the dimeric complex led to the identification of four novel subunits of the yeast ATP synthase complex (10, 27, 28). Assembly of the supracomplex requires the presence of the functionally assembled cytochrome bc_{1} or cytochrome c oxidase complexes to their respective dimeric forms appears not to be dependent on their ability to form the supercomplex.

The assembly of the individual cytochrome bc_{1} or cytochrome c oxidase complexes was not, however, adversely affected in the absence of the supercomplex. Physical association of these mitochondrial respiratory chain complexes may serve to enhance the flow of electrons between these complexes and to reduce the dependence on random diffusion of electron carriers quinone and cytochrome c. Indeed formation of a supercomplex between complexes III and IV in P. denitrificans has been demonstrated to enhance significantly electron transfer between these complexes (24). Such a tighter coupling of the electron transfer steps, as a result of physical association of mitochondrial electron chain complexes, would be compatible with the solid state model of electron transfer, as discussed by Rich (4).

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