Cytochrome c₂-Independent Respiratory Growth of Rhodobacter capsulatus

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To assess the role of cytochrome c₂ as a respiratory electron carrier, we obtained a double mutant of Rhodobacter capsulatus defective in cytochrome c₂ and in the quinol oxidase₆₃₀. This mutant was able to grow chemoheterotrophically, indicating that an electron pathway independent of cytochrome c₂ was functional between the ubiquinol-cytochrome c₂ oxidoreductase and the cytochrome oxidase₄₁₀.

The respiratory electron pathway of the purple, nonsulfur, photosynthetic bacterium Rhodobacter capsulatus has two branches (Fig. 1) (9, 11, 21). The “main” branch consists of two major membrane-bound, energy-conserving complexes, the ubiquinol:cytochrome c₂ oxidoreductase (also called the cyt bc₁ complex) and the cytochrome oxidase₄₃₀, and of at least one periplasmic electron carrier between these complexes, cytochrome c₂ (cyt c₂). The “alternate” branch of the respiratory pathway is less well defined, and it contains a quinol oxidase (22). R. capsulatus mutants affecting these terminal oxidases (i.e., C ox₄₁₀ and Q ox₆₃₀ mutants) have been described previously (11). The presence of one of the two oxidases appears sufficient for aerobic growth, since only double mutants lacking both of the oxidases (C ox₄₁₀ Q ox₆₃₀) are unable to grow chemoheterotrophically (11). The main respiratory branch can also be inactivated by mutations affecting the ubiquinol:cytochrome c₂ oxidoreductase (bc₁ mutants) (4). Although both the bc₁ and the C ox₄₁₀ mutants can grow chemoheterotrophically via the alternate respiratory branch (Fig. 1), the “oxidase-negative” mutants are proficient in photosynthesis but are unable to catalyze the Nadi (α-naphthol + dimethyl-p-phenylene-diamine + O₂ → indophenol blue + H₂O) reaction (11). Conversely, the “oxidoreductase-negative” mutants cannot grow by photosynthesis but can catalyze the Nadi reaction (4).

Earlier, Daldal et al. discovered that the photosynthetic growth of R. capsulatus was not drastically impaired by the absence of cyt c₂ (3). The nearly wild-type photosynthetic growth rate of a cyt c₂-negative (c₅⁻) mutant was mediated by direct electron transfer from the cyt bc₁ complex to the photochemical reaction center via cytochrome c₁ (cyt c₁) (14) (Fig. 1, broken vertical arrow). Further, Prince and Daldal recently showed that in the absence of both cyt c₁ and cyt c₂, electron transfer between these two complexes was completely abolished, leading to the loss of the photosynthetic growth ability (13). These studies established that, at least in this bacterium, the presence of cyt c₁ but not that of cyt c₂ is obligatory for anoxygenic photosynthetic growth (Fig. 1) (4). The question then arises as to whether the absence of cyt c₂ has any effect on chemoheterotrophic (aerobic, dark) growth. The study of this question is complicated in a wild-type strain of R. capsulatus because of the branching of the respiratory pathway (Fig. 1). However, with a mutant defective in quinol oxidase₆₃₀, aerobic, dark growth can be limited solely to the main branch. In this background the role of cyt c₂ in chemoheterotrophic growth can then be assessed by deletion of the corresponding structural gene.

R. capsulatus strains were grown on either MPYE or RCV media (10, 18). For Escherichia coli strains Luria broth or M9 medium was used (12). All media were supplemented with required antibiotics as described earlier (4). Photosynthetic growth (anaerobic, with a light intensity of approximately 12 J/m² per s) was monitored with a Klett-Summerson colorimeter, and for chemoheterotrophic growth the A₆₃₀ was measured. Gene transfer agent (GTA)-mediated crosses were performed as described earlier (4, 17) with either R121 or its derivatives, containing appropriate plasmids, as GTA-overproducing strains (19). Chromatophore supernatants were prepared and analyzed by absorption spectroscopy as described earlier (14) with a Hewlett-Packard diode array spectrophotometer (model 8452A).

R. capsulatus strains M6 (Q ox₆₃₀) and M7 (C ox₄₁₀) (Table 1), chosen as appropriate background strains to test the role of cyt c₂ in chemoheterotrophic growth, were isolated earlier by Marrs and Gest as spontaneous revertants of the respiratory-deficient strain M5 (11). Although the exact nature of the genetic lesion in these mutants is unknown, biochemical analyses have indicated that M6 and M7 are defective in the terminal oxidases of the alternate and main branches of the respiratory pathway, respectively (9, 21). To facilitate future spectroscopic studies, strains M6G and M7G, “green” derivatives of M6 and M7, were isolated by using the GTA obtained from R121 (a strain that carries a crtD mutation that leads to the accumulation of neurosporene derivatives instead of natural sphériodene and spheroidene) as a donor. M6G is therefore virtually identical to strain ZM6, previously described by Zannoni and Marrs (20). Table 1 lists the phenotypes of M6G and M7G with respect to their ability to catalyze the Nadi reaction (11) and to their sensitivity to myxothiazol, a potent inhibitor of the quinol oxidation site (Q₂) of the cyt bc₁ complex. Interestingly, inhibition of the respiratory growth of M6G by myxothiazol (2.5 µg/ml of MPYE) indicated that the cyt bc₁ complex became indispensable for growth when respiration was limited to the main branch (Fig. 1). Further, considering that only M7G was Nadi negative, catalysis of this reaction appeared to be related primarily to the availability of a functional cytochrome oxidase rather than to the presence of its electron donors, cyt c₁ and cyt c₂ (Table 1) (4).

The c₅⁻ derivatives of M6G and M7G were obtained by using a deletion-insertion allele of cycA (structural gene for

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FIG. 1. Electron transport pathways operating between various energy-transducing components involved in different growth modes of *R. capsulatus*. Ps, Photoheterotrophic growth; Res, chemoheterotrophic growth; DMSO, anaerobic dark growth in the presence of auxiliary electron acceptors like dimethyl sulfoxide; NADH and SDH, respiratory dehydrogenases; Q/QH2, ubiquinone/ubiquinol pool; R.C, photochemical reaction center; bc1, ubiquinol:cytochrome *c*2 oxidoreductase (cyt *bc*1 complex); cyt *c*2; C ox410 and Q ox260, respiratory terminal oxidases. Newly discovered cyt *c*2-independent pathways are indicated by broken arrows.

cyt *c*2 (3), originally constructed by replacing the heme-binding region of cyt *c*2 between amino acid residues 10 and 79 with a gene that encodes kanamycin resistance and that was derived from the transposon Tn5 (15). M6G and M7G were infected with GTA obtained from a derivative of M121 carrying this *cycA* allele on a plasmid, and kanamycin-resistant transductants were selected photosynthetically, a permissible growth condition for *c*2- mutants (3). The strains obtained, M6G-G4/S4 (*cycA qox-260*) and M7G-G4/S4 (*cycA Cox-410*) (Table 1), were analyzed for their cyt *c*2 content by optical spectroscopy (Fig. 2). Ascorbate-reduced minus ferricyanide-oxidized difference spectra obtained by using chromatophore supernatants clearly indicated that, like the original *c*2 mutant MT-G4/S4 (3), strains M6G-G4/S4 and M7G-G4/S4 were devoid of cyt *c*2 (Fig. 2).

To determine the role of cyt *c*2 in respiration we compared the growth of several mutants of *R. capsulatus* (Table 1) under various conditions with either MPYE-rich medium (Table 2) or RCV synthetic medium (data not shown). As expected, the photosynthetic growth rates of various strains were similar. Further, the chemoheterotrophic growth rates of mutants defective in various components of the main or alternate respiratory pathway were not drastically different from that of a wild-type strain. Perhaps more interestingly, respiratory growth also continued at an appreciable rate (170-min doubling time at 35°C on MPYE medium) even when cyt *c*2 and quinol oxidase260 were both absent (Table 2, M6G-G4/S4). Further, M6G-G4/S4 was sensitive to myxothiazol under these conditions and was Nadi positive, indicating that its growth was mediated via the main respiratory branch (Fig. 1). Therefore, a cyt *c*2-independent electron pathway between the ubiquinol:cytochrome *c*2 oxidoreductase and the cytochrome oxidase410 must be operational during chemoheterotrophic growth of *R. capsulatus*.

The role of cyt *c*2 in the respiratory electron transport chain of *R. capsulatus* has been briefly investigated in the past (1). It was shown with spheroplast preparations of M6 that succinate oxidation can be partially inhibited by the addition of polyclonal antibodies against cyt *c*2. Since a *c*2- derivative of M6 can still grow chemoheterotrophically, it is likely that the succinate oxidation insensitive to anti-cyt *c*2 antibodies corresponds to the cyt *c*2-independent pathway observed here.

How electrons are transferred during respiration from the cyt *bc*1 complex to the cytochrome oxidase in the absence of cyt *c*2 is not yet well known in *R. capsulatus* (Fig. 1, broken horizontal arrow). It is possible that besides cyt *c*2 various other *c*-type cytochromes also act as electron carriers between these membrane-bound complexes. The newly discovered membrane-bound or soluble *c*-type cytochromes, distinct from cyt *c*1 and cyt *c*2 (13), or the cytochrome oxidase410-associated cytochrome described earlier (5, 6).

### Table 1. *R. capsulatus* strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotypea</th>
<th>Phenotypeb</th>
<th>Origin or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT1131</td>
<td><em>crtD121</em></td>
<td>Nadi&quot;Myx&quot;</td>
<td>B. Marrs</td>
</tr>
<tr>
<td>R121</td>
<td><em>crtD121</em></td>
<td>Nadi&quot;Myx&quot;</td>
<td>B. Marrs</td>
</tr>
<tr>
<td>MT-G4/S4</td>
<td><em>crtD121 Δ(cycA:kan)1</em></td>
<td>Nadi&quot;Myx&quot;</td>
<td>3</td>
</tr>
<tr>
<td>M6</td>
<td><em>qox-260</em></td>
<td>Nadi&quot;Myx&quot;</td>
<td>11</td>
</tr>
<tr>
<td>M7</td>
<td><em>cox-410</em></td>
<td>Nadi&quot;Myx&quot;</td>
<td>11</td>
</tr>
<tr>
<td>M6G</td>
<td><em>crtD121 qox-260</em></td>
<td>Nadi&quot;Myx&quot;</td>
<td>20; This work</td>
</tr>
<tr>
<td>M7G</td>
<td><em>crtD121 Cox-410</em></td>
<td>Nadi&quot;Myx&quot;</td>
<td>This work</td>
</tr>
<tr>
<td>M6G-G4/S4</td>
<td><em>crtD121 Δ(cycA:kan)1 qox-260</em></td>
<td>Nadi&quot;Myx&quot;</td>
<td>4</td>
</tr>
<tr>
<td>M7G-G4/S4</td>
<td><em>crtD121 Δ(cycA:kan)1 Cox-410</em></td>
<td>Nadi&quot;Myx&quot;</td>
<td>This work</td>
</tr>
<tr>
<td>MT-CBC1</td>
<td><em>crtD121 ΔpetBC::spe18</em></td>
<td>Nadi&quot;Myx&quot;</td>
<td>13</td>
</tr>
<tr>
<td>M7G-CBC1</td>
<td><em>crtD121 Cox-410 ΔpetBC::spe18</em></td>
<td>Nadi&quot;Myx&quot;</td>
<td>This work</td>
</tr>
<tr>
<td>MT-GS18</td>
<td><em>crtD121 Δ(cycA:kan)1 ΔpetBC::spe18</em></td>
<td>Nadi&quot;Myx&quot;</td>
<td>This work</td>
</tr>
</tbody>
</table>

* a *cox-410 and *qox-260* are used to designate the genes mutated in strains M7 (*aer-412-S12x34*) and M6 (*aer-412-20-512*) that led to the absence of the cytochrome oxidase410 and quinol oxidase260 activities, respectively. All the other gene designations are as described previously (3, 4, 11).

* Only phenotypes related to the presence of the Nadi reaction (Nadi" or Nadi") (11) and to the resistance or sensitivity of chemoheterotrophic growth to myxothiazol (Myx" or Myx") are listed. With the exception of MT-CBC1 and MT-GS18, which cannot grow by photosynthesis, all the other strains are sensitive to myxothiazol (2.5 μg/ml) under photoheterotrophic growth conditions.
may be the likely candidates for this role. Alternatively, the electron donor from the cyt bc$_1$ complex to the cytochrome oxidase$_{410}$ may be the cyt c$_1$ of the cyt bc$_1$ complex via a direct interaction between the complexes. Interestingly, ubiquinol oxidase supercomplexes, composed of at least a cyt bc$_1$ complex and a cytochrome oxidase, have recently been isolated from Paracoccus denitrificans (2) and from the thermophilic bacterium PS3 (16). Finally, it should be noted that cyt c$_{1}$-independent electron transfer pathways operating between various membrane-bound energy-transducing complexes may also exist in bacterial species other than R. capsulatus, e.g., P. denitrificans (8) and Rhodopseudomonas viridis (7).

In conclusion, the isolation of a double mutant of R. capsulatus lacking both cyt c$_2$ and quinol oxidase$_{260}$ has revealed the existence of a cyt c$_{1}$-independent electron pathway between the cyt bc$_1$ complex and the cytochrome oxidase$_{410}$ (Fig. 1, broken horizontal arrow). Future genetic and spectroscopic analyses will hopefully better define the characteristics of the components involved in this newly discovered respiratory pathway.

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LITERATURE CITED


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**TABLE 2. Growth characteristics of various R. capsulatus strains tested**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant phenotype</th>
<th>Growth rate$^a$ (doubling time [min]) during:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Photosynthesis</td>
</tr>
<tr>
<td>MT1131</td>
<td>Wild type</td>
<td>126</td>
</tr>
<tr>
<td>MT-G4/S4</td>
<td>c$_{2}$</td>
<td>168</td>
</tr>
<tr>
<td>M6G</td>
<td>Q ox$_{260}$</td>
<td>130</td>
</tr>
<tr>
<td>M7G</td>
<td>C ox$_{110}$</td>
<td>162</td>
</tr>
<tr>
<td>M6G-G4/S4</td>
<td>c$<em>{2}$ Q ox$</em>{260}$</td>
<td>156</td>
</tr>
<tr>
<td>M7G-G4/S4</td>
<td>c$<em>{2}$ C ox$</em>{410}$</td>
<td>158</td>
</tr>
<tr>
<td>MT-CBC1</td>
<td>bc$_{1}$</td>
<td>NG</td>
</tr>
<tr>
<td>MT-GS18</td>
<td>c$<em>{1}$ bc$</em>{1}$</td>
<td>NG</td>
</tr>
</tbody>
</table>

$^a$ Growth rates were determined at 35°C on MPYE-rich medium as described in the text. NG, No growth.
essential for electron flow from the cytochrome bc, complex to
copies of genes coding for nitrogenase in Rhodopseudomonas
properties of a quinol oxidase super-complex composed of a bc1
complex and a cytochrome oxidase in the thermophilic bacte-
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sulata cells grown anaerobically in the dark on glucose and
position and function of the branched oxidase system in the wild
type and respiration deficient mutants of Rhodopseudomonas
resolution of cytochromes of b type and the nature of the
CO-sensitive oxidase present in the respiratory chain of Rhodo-