The Occurrence and Role of Ubiquinone in Electron Transport to Oxygen and Nitrate in Aerobically, Anaerobically and Symbiotically Grown *Rhizobium japonicum*

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Ubiquinone was extracted from free-living *Rhizobium japonicum*, grown aerobically or anaerobically, and from the symbiotic bacteroid form; it was tentatively identified as the Q-10 homologue. The ubiquinone concentration was highest in symbiotically grown *R. japonicum* but the ratio ubiquinone:total cytochrome was about 1:5:1 in membrane particles from organisms grown under all three conditions. The ubiquinone was reduced 75% by NADH, completely oxidized by oxygen but not oxidized by nitrate. NADH oxidase activity and nitrate reductase activity in membrane particles from organisms grown under the different conditions were similar except that nitrate reductase activity was low in aerobically grown organisms. It is concluded that ubiquinone functions in electron transport to oxygen but not to nitrate.

INTRODUCTION

Work on the cytochromes of *Rhizobium japonicum* has outlined electron transport pathways to oxygen in this organism grown aerobically and symbiotically within root nodules (Appleby, 1969a, b; Appleby & Bergersen, 1958; Tuzimura & Watanabe, 1964). However there are no reports on the occurrence or role of any quinone in these pathways; nor is there any report concerning the components involved in the electron transport pathway to nitrate reduction, although the nitrate reductase from aerobically grown (Lowe & Evans, 1964), anaerobically grown (Daniel & Appleby, 1972; Daniel & Gray, 1976) and symbiotically grown *R. japonicum* (Evans, 1954; Cheniae & Evans, 1959, 1960; Kennedy et al., 1975) has been studied. For symbiotically grown *R. japonicum*, it has been shown that nitrate respiration generates sufficient ATP to support nitrogen fixation (Rigaud et al., 1973).

There is evidence that the symbiotic N₂-fixing form of *R. japonicum* exists in an environment of very low oxygen tension (Appleby, 1960), but obtains oxygen at high flux via leghaemoglobin (for a review, see Appleby, 1974). The large number of cytochromes and other haemoproteins present (Appleby, 1969a, b; Daniel & Appleby, 1972; Appleby & Daniel, 1973) make it uncertain whether the electron transport pathway in the symbiotic N₂-fixing form resembles that in anaerobically grown *R. japonicum* or whether it differs from both aerobically and anaerobically grown *R. japonicum*. A study of a non-haemoprotein component of the electron transport system may be helpful in this respect.

METHODS

*Maintenance and growth of Rhizobium japonicum.* *Rhizobium japonicum* strain CC705 (Wisconsin 505) was grown aerobically and anaerobically as described by Daniel & Appleby (1972). Bacteroids were isolated and...
purified from N₂-fixing soybean root nodules (Glycine max Merr., cv. Lincoln) inoculated with R. japonicum strain CC705 and grown as described by Appleby (1969a).

Membrane particles were prepared as described by Appleby (1969a).

**EXTRACTION, CHARACTERIZATION AND DETERMINATION OF REDOX STATE OF UBIQUINOANE.** Total lipid was extracted from whole cells of *R. japonicum* as described by Daniel (1970). The quinone was purified from this extract by repeated thin-layer chromatography (t.l.c.) using 2 and 10 ml petroleum ether (b.p. 40 to 60 °C) on alumina, and benzene/chloroform (2:3 and 3:2, v/v) on silica gel. The homologue was tentatively identified by reversed-phase t.l.c. of the purified quinone with known ubiquinone homologues on paraffin-treated silica gel with acetone/water (9:1, v/v) as the mobile phase.

The reaction mixture contained cell membrane particles (15 to 20 mg protein) in 0.1 M-phosphate buffer pH 6.8. All reactions were stopped by tipping the methanol/pyrogallol mixture at 70 °C followed by the immediate addition of 5 ml petroleum ether (b.p. 40 to 60 °C) at 0 °C. To determine the total percentage of oxidizable ubiquinone, the reaction mixture was vigorously agitated on a vortex mixer for 3 min before the reaction was stopped. To determine the total percentage of NADH-reducible ubiquinone it was necessary to use a Thunberg tube flushed with nitrogen. The reaction mixture contained 5 μmol NADH, and the reaction was stopped by tipping the methanol/pyrogallol mixture at −70 °C from the side-arm. This gave results in agreement with those obtained in the presence of 5 mM-KCN under an atmosphere of CO.

**RESULTS AND DISCUSSION.** Determination of cytochromes was carried out as described by Appleby (1969a, b). Oxygen uptake was measured using a Rank electrode. The reaction mixture consisted of 200 μmol KH₂PO₄/Na₂HPO₄ buffer pH 6.8, a suitable amount of cell membrane particle protein and 3 μmol NADH in a final volume of 2.5 ml.

Nitrate reductase activity was measured as described by Daniel & Gray (1976).
Table 1. Ubiquinone (UQ) concentrations in R. japonicum grown under different conditions

<table>
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<tr>
<th>Growth conditions</th>
<th>Intact organisms</th>
<th>Membrane particles</th>
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<tr>
<td></td>
<td>pmol UQ (g dry wt)^{-1}</td>
<td>pmol UQ (g protein)^{-1}</td>
</tr>
<tr>
<td>Aerobic</td>
<td>0.34</td>
<td>0.79</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>0.31</td>
<td>0.46</td>
</tr>
<tr>
<td>Symbiotic (bacteroid)</td>
<td>0.46</td>
<td>1.23</td>
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Table 2. NADH oxidase and nitrate reductase activities and ubiquinone reduction in cell membrane particles of R. japonicum

| Growth conditions | NADH oxidase activity [µmol O_2 min^{-1} (g protein)^{-1}] | Nitrate reductase activity [µmol min^{-1} (g protein)^{-1}] | Percentage reduction of ubiquinone in the presence of:*
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<tbody>
<tr>
<td>Aerobic</td>
<td>42</td>
<td>9</td>
<td>10 mm-KNO_3  NADH</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>46</td>
<td>37</td>
<td>55</td>
</tr>
<tr>
<td>Symbiotic (bacteroid)</td>
<td>48</td>
<td>41</td>
<td>60</td>
</tr>
</tbody>
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* Ubiquinone from aerobic suspensions of cell membrane particles in the absence of substrate was fully oxidized in all cases.

significantly different. The finding that 20 to 30% of the total ubiquinone was not reducible by substrate is not uncommon (e.g. Knowles & Redfearn, 1968). A time course of the reduction of ubiquinone after substrate addition showed that maximum reduction occurred as the oxygen tension reached zero. This substrate-reduced ubiquinone was completely reoxidized by air, but not by nitrate (within the range 100 µM to 100 mM). Even after incubation periods of up to 3 h, using twice-washed membrane particles derived from starved cells and 50 nmol NADH instead of 5 µmol, the ubiquinone was still 50 to 60% reduced. This was only apparent if the experiment was carried out under anaerobic conditions, since the ubiquinone was very easily reoxidized by traces of oxygen in the reaction mixture or the methanol/pyrogallol solution used to stop the reaction. The simplest explanation consistent with these results is that ubiquinone is a functional component of the electron transport system to oxygen, but not to nitrate, in R. japonicum grown aerobically, anaerobically or symbiotically. The lower percentage reduction of ubiquinone in the presence of nitrate compared with substrate reduction is probably due to a minor side pathway or reverse electron transport.

Other workers have concluded that in Escherichia coli (Itagaki, 1964; Enoch & Lester, 1974) and Klebsiella (Aerobacter) aerogenes (Kno"ok & Planta, 1971a, b) ubiquinone functions in the electron transport system to nitrate. These conclusions were based largely on evidence from extraction-reactivation, reconstitution and ultraviolet irradiation experiments, rather than on the reduction of ubiquinone. This type of work is difficult to interpret unequivocally; possible complications can include an induced requirement for lipids and cytochromes by solvent extraction (Crane et al., 1957; Lester & Fleischer, 1961), the non-specific effect of ultraviolet irradiation (Erickson & Parker, 1969; Kno"ok & Planta, 1971b), inhibition by residual solvent (Redfearn & Pumphrey, 1958), the non-specific enhancement, inhibition or reactivation of electron transport by ubiquinone and menaquinone (Knowles et al., 1967; Hollander, 1976), the action of ubiquinone on the basis of its redox potential rather than enzyme specificity to set up artificial pathways (Hollander, 1976) and the inter-substitution of ubiquinone and menaquinone when both are present (Newton et al., 1971; Hollander, 1976). It is therefore difficult to be completely certain
that the non-participation of ubiquinone in the nitrate reduction system of *R. japonicum* is unusual. However, two implications of this work are that despite the large difference in cytochromes known to occur in *R. japonicum* grown under different conditions the electron transport system to oxygen involves ubiquinone in all cases, and that the electron transport system to nitrate diverges from that to oxygen before ubiquinone.

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### REFERENCES


